



# PROCEEDING BOOK

Full Texts and Abstracts

## 2nd International Congress of Veterinary Medicinal Plants and Traditional Medicine

*Atatürk University – Urmia University*

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# **2<sup>nd</sup> International Congress of Veterinary Medicinal Plants and Traditional Medicine**

**29-30 September 2025  
Atatürk University  
Türkiye**

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# Foreword



The "2nd International Congress of Veterinary Medicinal Plants and Traditional Medicine" is a product of the strong collaboration between the Faculty of Veterinary Medicine at Atatürk University and Urmia University. We are deeply honored to host this event, which will serve as a multidisciplinary platform to address key issues in veterinary medicine, particularly in the areas of herbal and traditional treatment methods. This congress aims to re-examine traditional knowledge through the lens of modern scientific research, while exploring the potential of natural resources in advancing veterinary medicine. With contributions from distinguished scholars and researchers, the congress will facilitate comprehensive discussions on cutting-edge approaches poised to shape the future of veterinary medicine. It will also highlight the significant role that herbal and traditional medicine plays in enhancing both animal and human health and will present the latest discoveries and innovative practices in the field. We are confident that this collaboration between Atatürk University and Urmia University will continue to grow, opening new horizons for regional and international research partnerships. We hope that the congress will provide all participants with a stimulating and rewarding experience, both scientifically and socially, and we sincerely thank you for your participation.

**Prof. Dr. Mustafa Sinan AKTAŞ**

Scientific Secretary (Türkiye) Dean of Atatürk University Faculty  
of Veterinary Medicine



# Foreword



It is with a profound sense of gratitude and accomplishment that I welcome you to the proceedings of the 2<sup>nd</sup> International Congress of Veterinary Medicinal Plants and Traditional Medicine. Serving as the Scientific Secretary from Iran, has been both an immense honor and a great responsibility, a journey that has allowed me to witness firsthand the power of shared knowledge and cross-cultural collaboration in the pursuit of scientific advancement. We stand at a pivotal moment in veterinary science. As the

challenges of antimicrobial resistance, chemical residues, and the demand for sustainable, organic animal husbandry continue to grow, the global scientific community is compelled to look back to look move forward. We are re-evaluating the ancient wisdom encapsulated in traditional medicine and subjecting it to the rigorous scrutiny of modern science. This congress was conceived with precisely this mission: To build a robust bridge between the rich, empirical knowledge of our ancestors and the innovative, evidence-based methodologies of the 21<sup>st</sup> century. The resounding success of this congress is a testament to a collective effort far greater than any individual. On behalf of the entire scientific community present here, I must first extend our deepest appreciation to Atatürk University. The university esteemed reputation as a center of academic excellence provided the perfect foundation for our gathering. We are particularly indebted to the President of Atatürk University, whose visionary leadership and unwavering support transformed the concept of this congress into a vibrant reality. Furthermore, our sincere thanks must go to the Dean and the entire Faculty of Veterinary Medicine. Your hospitality, the provision of exceptional facilities, and the scholarly environment you cultivate were instrumental in facilitating the rich exchanges that defined this event. The dedication of your institution to advancing veterinary science is truly commendable. The seamless



execution of a congress of this scale rests on the shoulders of its organizers. To the Members of the Organizing Committee, the Scientific Committee, and every individual who worked tirelessly behind the scenes—your meticulous planning, relentless dedication, and problem-solving prowess did not go unnoticed. To the distinguished speakers, presenting authors, and all the participants who travelled from near and far: You were the heart of this congress. Your groundbreaking research, insightful presentations, and passionate engagement in discussions had enriched our collective understanding and charted new courses for future investigation. The diversity of topics—from the phytochemistry of novel botanicals and their mechanisms of action to the validation of ethnoveterinary practices—has painted a comprehensive and hopeful picture about its potential. It has been the privilege of a lifetime to contribute to this monumental event. I am confident that the seeds planted here in Erzurum will bear fruit for many years to come, advancing the noble cause of integrating safe, effective, and sustainable plant-based solutions into veterinary practice for the health of animals, humans, and our planet.

With warmest regards,

**Prof. Dr. Masoud Maham**

Scientific Secretary (Iran) and Dean of Faculty of Veterinary  
Medicine Urmia University

# Congress Participation Summary

## General Submission Statistics

Total number of proceedings: 102

Proceedings from Türkiye: 42

Proceedings from foreign sources: 60

## Participating Countries

Türkiye

Iran

China

United States

Ukraine

Kyrgyzstan

Kazakhstan

Pakistan

**Highlights:** A total of eight different scholars participated in the conference. International participation accounts for approximately **59%** of the abstracts. Multinational participation highlights the diversity of work and strengthens its global communication. The wide geographical distribution of the presented papers highlights the global relevance of the conference themes and underscores the collaborative spirit of the scientific community. The presence of contributions from multiple continents enriches the academic environment and fosters interdisciplinary exchange.



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# SECTION 1

## Full Text Oral Papers



## THE ROLE OF PHENOLIC ACIDS IN THE TREATMENT OF VIRAL INFECTION

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### Abstract

Viral infections continue to pose significant global health challenges despite advances in modern medicine. Current antiviral therapies face several limitations, including high cost, the emergence of drug resistance, a restricted spectrum of activity and, most importantly, an insufficient number of available antiviral agents. These shortcomings highlight the urgent need for the development of novel therapeutic options. Among natural products, plant-derived phenolic acids have attracted increasing attention because of their diverse biological activities. Their antiviral properties are mediated through multiple mechanisms, such as the inhibition of viral entry and release, the suppression of viral enzymes, the disruption of replication processes and the modulation of host immune and inflammatory responses. The literature indicates that caffeic acid and its derivatives, chlorogenic acid, gallic acid and ellagic acid, are the most extensively studied phenolic acids in this context. Most available studies remain at the in vitro or preclinical stage, with limited clinical validation. In this review, the antiviral activities of phenolic acids are examined in relation to five clinically significant viruses, namely, influenza A virus, herpes simplex virus (HSV-1/2), hepatitis B virus (HBV), severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) and human immunodeficiency virus (HIV). Although no phenolic acid has yet received FDA approval as a standalone antiviral drug, emerging evidence suggests its potential as an adjuvant in combination therapies. This review emphasizes the importance of phenolic acids as promising antiviral candidates and aims to provide a foundation for future translational research in this field.

**Keywords:** Antiviral, Efficacy, Phenolic acid, Viral infection

### INTRODUCTION

Viral infections continue to represent a major global health threat despite the development of various types of vaccines, owing to their diversity and complex structures (Saifulazmi et al., 2022). Migration, rapid urbanization, the expansion of international trade and increased animal movement, combined with the inconsistent implementation of vaccination and antiviral treatment strategies across different regions and challenges in ensuring access, have positioned viral infections as critical issues in both veterinary medicine and public health (Denaro et al., 2020). In recent years, the emergence of novel viral diseases such as COVID-19 and Monkeypox has undermined public confidence in the possibility of completely eradicating infectious diseases (Saifulazmi et al., 2022). The high mutation capacity of viruses facilitates changes in surface molecules and contributes to the development of diverse invasion strategies (Denaro et al., 2020). The biological diversity arising from viral mutations, together with the variability of antiviral treatment requirements across viral species, necessitates the exploration of new sources for potential antiviral agents (Saifulazmi et al., 2022).

Vaccination has long been one of the fundamental strategies for combating viral infections; however, as vaccines are primarily used for preventive purposes, antivirals remain indispensable in the treatment of viral diseases (Bule et al., 2019). The development of antiviral drugs is a highly demanding and costly process,

encompassing target identification and screening, the selection and optimization of candidate compounds, the performance of clinical trials and final regulatory approval (Kausar et al., 2021). Since viruses rely on host cell metabolic processes, they exploit similar metabolic pathways. Consequently, newly developed antiviral agents must demonstrate virus-specific properties and exhibit low toxicity to avoid damaging cellular structures. Currently available antivirals are designed to target essential viral components or viral enzymes involved in replication, thereby preventing viral proliferation rather than eliminating the virus itself (Denaro et al., 2020).

The development and approval of idoxuridine in 1963 as the first antiviral drug marked the beginning of a new era in this field (Bule et al., 2019). In subsequent years, between 1963 and 2016, the number of antiviral drugs approved by the U.S. Food and Drug Administration (FDA) reached 90, and by 2022, this figure had increased to 106, with a large proportion targeting human immunodeficiency virus (HIV) infections (De Clercq & Li, 2016; Namasivayam et al., 2022). While some of the approved antivirals are effective against only a single viral species or even a specific genotype (e.g., telaprevir against hepatitis C virus genotype 1), others exhibit inhibitory activity against multiple viral species (e.g., lamivudine against HIV and hepatitis B virus [HBV]; ribavirin against HCV, respiratory syncytial virus [RSV] and influenza viruses) (De Clercq & Li, 2016). In veterinary medicine, however, the use of antivirals remains limited. High costs and challenges in selecting the appropriate antiviral due to limited rapid diagnostic options and difficulties in drug accessibility are among the major obstacles. Consequently, antivirals originally developed for human viral diseases are frequently employed in the treatment of animal viral infections (Bule et al., 2019; Hoppes & Payne, 2025). Nevertheless, the number of antivirals specifically approved for veterinary use remains very small. Recombinant feline interferon- $\omega$  is one of the few antivirals approved for the treatment of retroviral diseases in cats (Bule et al., 2019).

The development of an antiviral drug typically requires approximately 10 years and incurs costs reaching 1–2 billion USD. One of the major challenges in antiviral development is ensuring that the discovered molecule is specific to both the virus and the host species while avoiding disruption of essential cellular mechanisms. Moreover, because the targeted viral structure or mechanism may differ across viral species, a compound that is effective against one virus may not provide inhibitory activity against another (Namasivayam et al., 2022). An important methodological approach in antiviral research involves computer-aided modeling to assess the binding affinity between the viral target site and the candidate antiviral compound. The validity of these models was subsequently tested through animal experiments. The first example of this approach was the discovery of nelfinavir for HIV in 1990 (Parks & Smith, 2020). However, the ability of viruses to develop resistance to existing drugs and the failure to achieve complete eradication of infections have increased interest in exploring alternative plant-derived antiviral agents. Bioactive compounds extracted from plants—including essential oils, phenolic acids, flavonoids, terpenes, lignans, coumarins, alkaloids and proteins—have been reported to exhibit noteworthy antiviral effects (Denaro et al., 2019).

The discovery of antivirals remains a particularly complex process, as the preservation of cellular pathways must be prioritized. Consequently, the compounds identified to date have largely focused on virus-specific targets such as attachment proteins, viral enzymes and nucleic acid replication processes (Table 1).

## 1. Nucleoside/Nucleotide Analogs (Polymerase Inhibitors)



These agents are prodrugs that structurally resemble the natural substrates of viral polymerases. Once phosphorylated inside the host cell, they become active and disrupt RNA/DNA synthesis by inducing chain termination, delaying chain termination, or increasing error incorporation, also referred to as lethal mutagenesis (Abdullah Al Awadh, 2022; Kamzeeva et al., 2023; Karim et al., 2023). Viral specificity is achieved through high affinity for viral RNA-dependent RNA polymerases (RdRp) or DNA polymerases, which differ from host polymerases (Abdullah Al Awadh, 2022). For example, remdesivir has been employed against severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RdRp by inducing delayed chain termination, representing one of the broad-spectrum agents within this group (Karim et al., 2023). Molnupiravir, a ribonucleoside analog, increases mutagenesis by disrupting replication fidelity (Abdullah Al Awadh, 2022). Sofosbuvir, tenofovir derivatives and entecavir, on the other hand, target HCV and HBV DNA/RNA polymerases (Abdullah Al Awadh, 2022; Kamzeeva et al., 2023). In the veterinary field, the number of licensed antivirals remains limited. However, famciclovir—a purine analog that is metabolized to penciclovir—is widely used in the management of feline herpesvirus-1 (FHV-1). Dosage regimens and pharmacokinetic details are provided in veterinary references (Merck & Co., n.d.).

## 2. Viral Protease Inhibitors

Many RNA viruses synthesize polyproteins that must be processed by viral proteases into functional components of the replication complex. Protease inhibitors block this maturation step, thereby reducing viral infectivity (Borges et al., 2024; Karim et al., 2023). In severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), inhibitors targeting the 3CLpro/Mpro protease (e.g., nirmatrelvir) are among the most recent examples. Protease inhibitors are also widely used to treat HIV and HCV. Drugs such as nirmatrelvir (SARS-CoV-2) and grazoprevir (HCV) suppress viral replication by preventing proteolytic processing (Borges et al., 2024). In veterinary medicine, however, there are currently no protease inhibitors with specific veterinary approval. Their use has been limited to experimental applications of human-approved drugs in animal species rather than routine clinical practice (Merck & Co., n.d.).

## 3. Entry/Attachment/Fusion Inhibitors

Viral entry involves binding to host receptors, activation of viral proteins by host proteases (such as TMPRSS2 or cathepsin L) and subsequent membrane fusion. Small molecules, peptides, or monoclonal antibodies can block these critical steps (Chitsike et al., 2021; Jackson et al., 2022). For HIV treatment, CCR5 antagonists (e.g., maraviroc) and fusion inhibitors (e.g., enfuvirtide) are well documented examples (Jackson et al., 2022). In the case of SARS-CoV-2, the spike-ACE2 interaction and endosomal proteases are key targets; recent studies have shown that agents that suppress the cathepsin L/TMPRSS2 axis can effectively block viral entry (Chitsike et al., 2021). To date, there are no entry or fusion inhibitors specifically licensed for veterinary use, and such agents have not been adopted in veterinary clinical practice (Merck & Co., n.d.).

## 4. Neuraminidase (NA) Inhibitors

NA inhibitors are agents that prevent viral release from host cells. By blocking the ability of influenza virus neuraminidase to cleave sialic acid residues on the host cell surface, these drugs impede the detachment and spread of progeny virions (Heneghan et al., 2016). Oseltamivir, zanamivir and peramivir are the main representatives of this group. When administered early, NA inhibitors have been shown to shorten the duration of symptoms and provide clinical benefit in certain subpopulations (Heneghan et al., 2016; Su et al., 2022). In

veterinary medicine, neuraminidase inhibitors are not part of routine licensed treatment protocols for influenza; their use has generally been limited to the extrapolation of human preparations (Merck & Co., n.d.).

5. Uncoating/Endosomal Acidification Inhibitors

This class of agents halts early stages of viral replication by preventing genome release (uncoating) from the capsid or by inhibiting endosomal acidification. Classical examples include amantadine and rimantadine, which historically targeted seasonal influenza A virus. However, owing to widespread resistance, their clinical importance has markedly declined. Newer approaches aim at targeting endosomal/lysosomal pathways and proteases, but these remain under investigation (Jackson et al., 2022).

6. Host-Targeting Antivirals and Immunomodulators

These agents exploit the virus’s dependence on host factors such as lipid metabolism, protein kinases, endosomal trafficking, or epigenetic regulators. Targeting host pathways provides theoretical broad-spectrum activity and a greater barrier to resistance; however, the assessment of tolerability and side effects is critical (He et al., 2024; Karim et al., 2023). In SARS-CoV-2 research, host proteases and epigenetic targets have received attention—for example, cathepsin L inhibitors and the EHMT2 inhibitor UNC0638, both of which suppress viral entry (Chen et al., 2025). Broadly, reviews focusing on host dependency factors summarize emerging strategies for chronic viral infections such as HBV, HDV and HCV (Frericks et al., 2025). In veterinary medicine, recombinant feline interferon- $\omega$  (rFeIFN- $\omega$ , Virbagen Omega) is a licensed immunomodulator. According to the European Medicines Agency (EMA), it is indicated for supportive treatment in feline retroviral infections (FeLV/FIV) and for reducing mortality and clinical signs in canine parvrosis (EMA, 2018). Clinical trials in cats have reported symptomatic improvement and reduced viral shedding (Doménech et al., 2011; Gil et al., 2013).

7. Monoclonal Antibodies

Monoclonal antibodies act by binding viral surface proteins, thereby blocking entry and fusion, and are most prominently used to treat SARS-CoV-2. Their clinical effectiveness is influenced by viral variants, as escape mutations can diminish their neutralization capacity (Chitsike et al., 2021; Chen et al., 2021).

Table 1. Antiviral mechanisms of action and examples

| Mechanism                                                | Virus Examples | Antiviral Examples                |
|----------------------------------------------------------|----------------|-----------------------------------|
| Nucleoside/Nucleotide Analogs<br>(Polymerase Inhibitors) | SARS-CoV-2     | Remdesivir, Molnupiravir          |
|                                                          | HCV/HBV        | Sofosbuvir, Tenofovir, Entecavir  |
|                                                          | FHV-1          | Famciclovir                       |
| Viral Protease Inhibitors                                | HIV            | Lopinavir, Ritonavir              |
|                                                          | HCV            | Grazoprevir                       |
|                                                          | SARS-CoV-2     | Nirmatrelvir                      |
| Entry/Binding/Fusion Inhibitors                          | HIV            | Maraviroc, Enfuvirtide            |
|                                                          | SARS-CoV-2     | cathepsin L/TMPRSS2 inhibitors    |
| Neuraminidase (NA) Inhibitors                            | Influenza A/B  | Oseltamivir, Zanamivir, Peramivir |



|                                                |                                              |                                                      |
|------------------------------------------------|----------------------------------------------|------------------------------------------------------|
| Uncoating/Endosomal Acidification Inhibition   | Influenza A                                  | Amantadine, Rimantadine                              |
| Host-Targeting Antivirals and Immunomodulators | SARS-CoV-2<br>HBV/HDV/HCV<br>FeLV/FIV<br>CPV | Camostat mesylate<br><br>Bulevirtide<br><br>rFeIFN-ω |
| Monoclonal Antibodies                          | SARS-CoV-2                                   | Neutralizing antibodies                              |

Despite the availability of numerous antiviral agents that target different stages of viral replication, the emergence of resistance, high treatment costs and limited spectrum of activity highlight the need for alternative strategies. In this context, plant-derived extracts and their bioactive compounds have gained considerable attention as complementary or novel antiviral sources. Among them, phenolic acids stand out as contemporary and extensively studied molecules with promising therapeutic potential for both human and veterinary virology.

PHENOLIC ACIDS AND THEIR USE AS ANTIVIRALS

Plants are a fundamental source of biologically active molecules that are used worldwide to prevent and treat many various diseases. The isolation and characterization of plant-derived compounds have significantly contributed to the discovery of new drug candidates. Unlike synthetic compounds, natural preparations possess anti-inflammatory, antioxidant and anticancer properties through multiple mechanisms and are often considered safer for patients because of their lower incidence of adverse effects (Chojnacka, 2021). Until the 20th century, most of the therapeutic properties of medicinal plants were utilized through the use of plant extracts, which are commonly prepared as ethanol or aqueous infusions. Although the medicinal activity of these extracts likely stems from the combined action of multiple bioactive natural products within these highly complex mixtures, the presence of harmful substances cannot be excluded (Zotchev, 2024). The use of various plants in folkloric medicine has continued to this day. According to current data, natural products and their derivatives account for approximately 35% of the global pharmaceutical market, of which approximately 25% are plant-based drugs. A historical analysis of the World Health Organization (WHO) Essential Medicines List further revealed that approximately 11% of the listed drugs originate from flowering plants. These findings underscore the ongoing role of natural products—particularly those derived from plants—in the drug development process (Najmi, 2022; Veeresham, 2012).

Polyphenols are naturally occurring chemical components widely consumed in daily life through vegetables, fruits, cereals and beverages, making them an integral part of human nutrition. They constitute a complex category of more than 10,000 compounds characterized by one or more aromatic rings bound to hydroxyl (OH) groups and are generally classified as secondary metabolites (Rana et al., 2022). Secondary metabolites are derived primarily from primary metabolites (such as carbohydrates, amino acids and lipids) and function to protect plants from UV radiation; defend against viruses, bacteria and insects; and contribute to the odor, color and flavor of plant products. Polyphenols are recognized as potent natural antioxidants with diverse biological

and pharmacological activities, including anti-inflammatory, anticancer, antimicrobial, antiallergic, antiviral, antithrombotic, hepatoprotective, food additive and signaling molecule functions, among others (Saifulazmi et al., 2022). These compounds are classified into phenolic acids, flavonoids, stilbenes and lignans on the basis of the number of rings and linking elements (Chojnacka, 2021).

Phenolic acids, belonging to the polyphenol subclass, are secondary compounds originating from plants and fungi and are associated primarily with defense mechanisms against pathogens and ultraviolet damage. Recently, these compounds have gained particular attention because of their beneficial biological properties, including antiviral and anti-inflammatory activities, especially in the treatment of human viral diseases (Wu et al., 2023). Structurally, phenolic acids are a diverse group of naturally occurring aromatic acid compounds consisting of a phenolic ring and an organic carboxylic acid group. On the basis of the length of their carbon chain, they are subdivided into hydroxycinnamic acids (C3–C6) and hydroxybenzoic acids (C1–C6) (Table 2) (Oluwole et al., 2022). These compounds contain at least one aromatic ring substituted with a hydroxyl group. Phenolic acids are abundant in plants, with hydroxycinnamic acid derivatives reported as more widespread than hydroxybenzoic acid derivatives (Wu et al., 2023). They occur in various forms (free, soluble, conjugated and insoluble), and owing to their simple chemical structure, high availability, water solubility, chemical stability and biocompatibility, they can be easily absorbed in human metabolism (De Freitas Marinho et al., 2024).

**Table 2.** Classification of phenolic acids

| Phenolic Acids                           |                                         |
|------------------------------------------|-----------------------------------------|
| Hydroxycinnamic Acid Derivatives (C3–C6) | Hydroxybenzoic Acid Derivatives (C1–C6) |
| Sinapic acid                             | p-Hydroxybenzoic acid (p-HBA)           |
| p-Coumaric acid                          | Gallic acid                             |
| Ferulic acid                             | Ellagic acid                            |
| Caffeoylquinic acid (Chlorogenic acid)   | Vanillic acid                           |
| Caffeic acid                             | Genticik acid                           |
| Cinnamic acid                            | Syringic acid                           |
|                                          | Salicylic acid                          |
|                                          | Protocatechuic acid (PCA)               |

Phenolic acids have been investigated in human medicine for their antiviral potential against a broad spectrum of both enveloped and nonenveloped viruses, including HIV, SARS-CoV-2, hepatitis C virus (HCV), hepatitis B virus (HBV), herpes simplex virus (HSV), influenza A virus, RSV, dengue virus and Ebola virus, where reductions in viral titers have been reported (Table 3) (Wu et al., 2023; Langland et al., 2018). Over the past decade, the number of publications addressing the in vitro and in vivo antiviral activities of phenolic compounds has increased significantly. However, the level of evidence remains limited in terms of clinical validation and regulatory approval; in particular, studies at the single-compound level largely remain in the preclinical stage without sufficient confirmation of clinical efficacy (De Freitas Marinho et al., 2024; Wei et al., 2025).

**Table 3.** Phenolic acid compounds and the viral species investigated

| Phenolic Acid Compounds | Studied Virus Species | References |
|-------------------------|-----------------------|------------|
|-------------------------|-----------------------|------------|





|                            |                                        |                                                             |                         |
|----------------------------|----------------------------------------|-------------------------------------------------------------|-------------------------|
| Caffeic<br>Phenethyl Ester | Acid/Caffeic<br>Acid                   | Porcine reproductive and respiratory syndrome virus (PRRSV) | Cui et al., 2024        |
|                            |                                        | Canine Distemper Virus                                      | Wu et al., 2017         |
|                            |                                        | Feline Calicivirus                                          | Cui et al., 2021        |
|                            |                                        | HBV                                                         | Wang et al., 2009       |
|                            |                                        | HCV                                                         | Shirasago et al., 2019  |
|                            |                                        | Influenza A                                                 | Utsunomiya et al., 2014 |
|                            |                                        | HSV                                                         | Langland et al., 2018   |
|                            |                                        | HIV                                                         | Sonar et al., 2017      |
|                            |                                        | Ilheus Virus                                                | Saivish et al., 2023    |
|                            |                                        | Severe Fever With Thrombocytopenia Syndrome Virus           | Ogawa et al., 2021      |
| Chlorogenic Acid           | HIV                                    | Tamayose et al., 2019                                       |                         |
|                            | Influenza A                            | Ding et al., 2017                                           |                         |
|                            | HBV                                    | Lai et al., 2024                                            |                         |
|                            | Infectious Bursal Disease Virus (IBDV) | Li et al., 2021                                             |                         |
|                            | White Spot Syndrome Virus              | Chen et al., 2024                                           |                         |
|                            | Enterovirus                            | Li ve ark. 2013                                             |                         |
|                            | SARS-CoV-2                             | Gonçalves et al., 2022                                      |                         |
|                            | Rift Valley Fever Virus                | More et al., 2025                                           |                         |
| Sinapic Acid               | MERS-CoV                               | Shadid et al., 2024                                         |                         |
|                            | Dengue Virus                           | Roney et al., 2023                                          |                         |
|                            | SARS-CoV-2                             | Orfali et al., 2021                                         |                         |
| Ferulic Acid               | Porcine Parvovirus                     | Ma et al., 2020                                             |                         |
|                            | HIV                                    | Sonar et al., 2017                                          |                         |
| p-Coumaric Acid            | Dengue Virus                           | Rathnayake et al., 2021                                     |                         |
| Cinnamic Acid              | HCV                                    | Amano et al., 2017                                          |                         |
|                            | Infectious Bronchitis Virus            | Wei et al., 2025                                            |                         |
|                            | Equine Herpes Virus-1 (EHV-1)          | Gravina et al., 2011                                        |                         |
|                            | Chikungunya virus                      | Alagarasu et al., 2025                                      |                         |
|                            | Influenza A Virus                      | Pang et al., 2020                                           |                         |
|                            | HSV-1                                  | Thakka et al., 2010                                         |                         |
|                            | Dengue Virus                           | Cárdenas-Granados et al., 2025                              |                         |
| Gallic Acid                | Influenza virüs                        | Lee et al., 2016                                            |                         |
|                            | HSV-1                                  | Kratz et al., 2008                                          |                         |
|                            | HIV                                    |                                                             |                         |
|                            | HCV                                    | Govea-Salas et al., 2016                                    |                         |
|                            | Coxsackievirus                         | Choi, 2014                                                  |                         |
| Ellagic Acid               | Zika Virus                             | Acquadro et al., 2020                                       |                         |
|                            | Ebola Virus                            | Cui et al., 2018                                            |                         |
|                            | SARS-CoV-2                             | Alexova et al., 2023                                        |                         |

|                     |                                 |                                 |
|---------------------|---------------------------------|---------------------------------|
|                     | Human Papillomavirus            | Le Donne et al., 2017           |
|                     | Influenza Virus                 | Pavlova et al., 2016            |
|                     | HBV                             | Kang et al., 2006               |
|                     | HIV                             | Nutan et al., 2013              |
|                     | Porcine Epidemic Diarrhea Virus | Song et al., 2024               |
|                     | HSV-1                           | Sun et al., 2025                |
|                     | HCV                             | Lim et al., 2021                |
|                     | Rotavirus                       | Zheng et al., 2024              |
|                     | Metapneumovirus                 | Artamonova & Abdullatypov, 2022 |
| Protocatechuic Acid | HBV                             | Dai et al., 2017                |
|                     | IBDV                            | Ou et al., 2018                 |
|                     | Influenza A Virus               | Wang et al., 2022               |
|                     | SARS-CoV-2                      | Geiger et al., 2022             |

In recent years, the most extensively studied phenolic acids have included caffeic acid/CAPE, chlorogenic acid, gallic acid and ellagic acid. Meta-analyses have demonstrated that the number of publications on these compounds has increased significantly over the past five years (De Freitas Marinho et al., 2024). However, current reviews emphasize that the majority of evidence regarding the antiviral activity of phenolic acids remains limited to the in vitro and preclinical stages, with clinical validation still scarce (Denaro et al., 2020; Chojnacka et al., 2021; Wu et al., 2023). Moreover, none of the phenolic acids have been approved by the FDA as stand-alone antiviral agents (De Clercq & Li, 2016). In addition to their individual effects, combination approaches are gaining increasing importance. For example, metal chelates of caffeic acid and related compounds have demonstrated broad-spectrum activity against HSV, Ebola and vaccinia viruses (Langland et al., 2018). Nevertheless, there are currently no clinical trials in which phenolic acids are directly combined with approved antivirals. Nevertheless, the NA inhibitory effect of chlorogenic acid in influenza suggests that it may serve as an adjuvant or resistance-reducing agent when used alongside existing NA inhibitors (Ding et al., 2017; Heneghan et al., 2016; Su et al., 2022).

Although their use in veterinary medicine has not yet been translated into clinical practice, experimental evidence exists. For example, CAPE has been shown to have inhibitory effects against PRRSV, protocatechuic acid against IBDV and trans-cinnamic acid against EHV (Cui et al., 2024; Ou et al., 2018; Gravina et al., 2011). Taken together, phenolic acids stand out in antiviral research both because of their broad-spectrum mechanisms and their potential for combined applications. However, none of these compounds have yet been approved as antiviral agents by either the FDA or EMA, and most of the available evidence remains at the in vitro or experimental level (De Clercq & Li, 2016). Without clinical validation, pharmacokinetic and safety evaluations, dose–response analyses and randomized controlled clinical trials, this potential cannot be fully assessed.

Studies have demonstrated that phenolic acids suppress viral replication across different virus species through direct or indirect mechanisms (Denaro et al., 2020; Chojnacka et al., 2021). The possible mechanisms of action of these compounds can be classified as summarized below:

- 1. **Inhibition of Viral Entry and Release:** One of the frequently observed mechanisms of phenolic acids is the prevention of viral attachment and entry into host cells. Specifically, chlorogenic acid suppresses the



release of viral particles in influenza A through NA inhibition (Ding et al., 2017). Similarly, caffeic acid and its derivatives have been reported to limit cell entry in HSV and influenza (Utsunomiya et al., 2014; Langland et al., 2018).

2. **Inhibition of Viral Replication and Polymerase:** Phenolic acids can reduce replication by directly or indirectly inhibiting RNA or DNA polymerase enzymes. Ellagic acid derivatives have shown strong binding affinity for the RNA-dependent RNA polymerase of human metapneumovirus (Artamonova & Abdullatypov, 2022). Chlorogenic acid has been reported to suppress viral replication in HBV by inhibiting the JNK signaling pathway (Lai et al., 2024). Moreover, protocatechuic acid suppresses viral RNA replication in influenza A by modulating host signaling (Wang et al., 2022).
3. **Inhibition of Viral Proteins and Enzymes:** Phenolic acid derivatives may disrupt the functions of viral structural or enzymatic proteins. Cinnamic acid derivatives inhibited the replication of HCV through oxidative stress (Amano et al., 2017). Ferulic and caffeic acid esters have been reported as dual inhibitors of HIV-1 reverse transcriptase (Sonar et al., 2017). Similarly, ellagic acid and gallic acid suppress HIV protease and reverse transcriptase activity (Nutan et al., 2013).
4. **Host-Directed Modulation of Immunity and Inflammation:** Another important mechanism in the antiviral activity of phenolic acids is the regulation of host immune responses. Chlorogenic acid and baicalein exhibited antiviral activity against IBDV in a chicken embryo model while suppressing inflammation (Li et al., 2021). Salicylic acid inhibited SARS-CoV-2 replication in vitro by enhancing host defense mechanisms (Geiger et al., 2022).
5. **Multiple Mechanisms and Combination Effects:** Many phenolic acids simultaneously exert both direct antiviral and indirect (antioxidant, anti-inflammatory) effects. For example, CAPE interacts with SARS-CoV-2 proteases while also suppressing host cell inflammatory responses (Cui et al., 2021; Cui et al., 2024). Additionally, phenolic acids have been reported to exhibit synergistic effects when combined with other antiviral drugs (Chitsike & Duerksen-Hughes, 2021).

Phenolic acid compounds possess antiviral potential by acting at multiple stages, such as viral entry/exit, replication and protein inhibition (De Freitas Marinho et al., 2024). However, to date, none of these compounds have been approved by the FDA as standalone antiviral drugs (De Clercq & Li, 2016). Nevertheless, their low toxicity profile and multiple mechanisms of action make them strong candidates for advanced clinical investigations in both human and veterinary virology.

## PHENOLIC ACIDS AND THEIR ANTIVIRAL ACTIVITIES: EXAMPLES FROM INFLUENZA VIRUS, HSV, HBV, HIV AND SARS-CoV-2

### Influenza virus

Influenza A is an RNA virus responsible for annual seasonal flu outbreaks, affecting millions of people worldwide. In particular, severe complications and mortality may occur in elderly individuals, children and immunocompromised patients. The high antigenic variability of the virus (antigenic drift and shift) limits the long-term protection of current vaccines, thereby emphasizing the importance of developing new antiviral candidates. Phenolic acids have drawn attention primarily because they target viral surface glycoproteins (e.g.,

NA) and replication stages (Heneghan et al., 2016). Studies with chlorogenic acid against influenza A virus in mice and MDCK cells demonstrated that it inhibits viral release via neuraminidase inhibition and increases survival in animal models (Ding et al., 2017). Caffeic acid and its derivatives were reported to suppress influenza A viral replication in vitro (Utsunomiya et al., 2014). Gallic acid, in turn, was found to contribute to antiviral activity against influenza A and B within extract fractions, potentially via oxidative stress-related mechanisms (Lee et al., 2016). To date, research on phenolic acid compounds against influenza viruses has been limited primarily to in vitro and animal model studies, with no clinical validation available.

### HSV-1/2

HSV-1 and HSV-2 are DNA viruses responsible for latent infections in a large proportion of the global population. The clinical spectrum ranges from simple skin/mucosal lesions to severe conditions such as encephalitis and neonatal infections. Current antivirals (e.g., acyclovir) typically suppress viral replication; however, the issue of antiviral resistance has increasingly emerged (De Clercq & Li, 2016). Therefore, natural products, especially phenolic acids (caffeic, gallic, and ellagic acids), have been investigated as potential alternative or adjunctive agents against HSV (De Clercq & Li, 2016). Among these, caffeic acid, ellagic acid and gallic acid have been tested against HSV. In vitro studies with caffeic acid and gallic acid have demonstrated strong antiviral effects (Kratz et al., 2008; Langland et al., 2018). Moreover, ellagic acid has been reported to suppress HSV-1 replication and reduce brain inflammation (Sun et al., 2025).

### HBV

HBV is one of the leading causes of liver cirrhosis and hepatocellular carcinoma, with more than 300 million chronic carriers worldwide (Frericks et al., 2025). Current nucleoside/nucleotide analogs and interferon therapies suppress viral replication but fail to achieve complete eradication. Therefore, the antiviral effects of natural compounds, particularly phenolic acids (chlorogenic, caffeic and protocatechuic acids), have been intensively studied. Both in vitro and in vivo models have been reported to suppress viral replication (Wang et al., 2009; Dai et al., 2017). Chlorogenic acid and caffeic acid have been shown in both in vitro and in vivo studies to suppress HBV replication, primarily through the inhibition of JNK expression (Wang et al., 2009; Lai et al., 2024). Protocatechuic acid has been demonstrated in vitro to suppress HBV replication by activating the ERK1/2 pathways (Dai et al., 2017). While data on the use of phenolic acids against HBV are available from both cell culture and animal models, no clinical validation has yet been achieved.

### HIV

HIV remains one of the most serious global health concerns, and despite effective suppression through antiretroviral therapy (ART), a definitive cure has not yet been identified (De Clercq & Li, 2016). The nucleoside and nucleotide analogs, reverse transcriptase inhibitors and protease inhibitors included in ART regimens exhibit strong efficacy, but resistance development, toxicity and the need for lifelong drug administration remain significant challenges (Abdullah Al Awadh, 2022; Kamzeeva et al., 2023). In this context, plant-derived natural compounds, particularly phenolic acids, present complementary potential in HIV treatment. Among phenolic acids, caffeic acid and its derivatives, particularly CAPE, have shown potent inhibitory activity against HIV-1 reverse transcriptase in both in vitro and in silico studies. Similarly, ferulic acid derivatives exhibit dual inhibitory



effects on HIV-1 reverse transcriptase, highlighting the therapeutic potential of both the natural form and structural modifications of the compound (Sonar et al., 2017). Gallic acid and ellagic acid can suppress viral replication by inhibiting HIV-1 protease and reverse transcriptase activity; notably, ellagic acid has been reported as a dual-mechanism inhibitor of HIV-1 infection (Nutan et al., 2013). These findings demonstrate that phenolic acids can interfere with multiple stages of the HIV life cycle. An important advantage of phenolic acids in the context of HIV is their low toxicity profiles and antioxidant properties, which support host immune responses (Oluwole et al., 2022; Rana et al., 2022). However, most available evidence is based on in vitro or computer-assisted modeling studies, and further preclinical and clinical studies are needed to advance the clinical application of these methods. Nonetheless, the use of phenolic acids in combination with existing ART regimens appears to be a more promising approach than their use as stand-alone agents.

### SARS-CoV-2

At the end of 2019, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) triggered the coronavirus disease 2019 (COVID-19) pandemic, with profound impacts on global health, the economy and social life. Its high transmissibility, capacity for variant emergence and mortality risk have made it one of the most intensively studied pathogens in recent years (Chen et al., 2021; Jackson et al., 2022). Phenolic acids, particularly sinapic acid, chlorogenic acid derivatives and ellagic acid, have demonstrated in vitro and in silico activity against SARS-CoV-2 replication enzymes (e.g., 3CLpro and Mpro) and structural proteins. These findings underscore the potential of phenolic acids in the context of modern pandemics. Orfali et al. (2021) reported that sinapic acid inhibited SARS-CoV-2 replication in vitro with low cytotoxicity. Ellagic acid has been shown to inhibit the SARS-CoV-2 3CLpro protease, thereby blocking viral replication (Zheng et al., 2024). Gonçalves et al. (2022) demonstrated the in silico and in vitro potential of chlorogenic acid derivatives against the SARS-CoV-2 Mpro protease.

### CONCLUSION

The development of antiviral drugs is a demanding, costly and time-consuming process. Moreover, the high mutation capacity of viruses contributes to the emergence of drug resistance and facilitates the occurrence of new waves of infection. Therefore, there is a growing need for safe, effective and versatile antiviral alternatives in both human and veterinary medicine. Phenolic acids, which are naturally derived compounds with broad biological activities and wide-spectrum antiviral potential, represent a strong candidate group to meet this need. In particular, compounds such as caffeic acid and its derivatives, chlorogenic acid, gallic acid and ellagic acid, have shown promising results against clinically and economically significant viruses, including influenza, HSV, HBV, HIV and SARS-CoV-2. Their antiviral mechanisms are diverse, ranging from the inhibition of viral proteins to the modulation of immune responses. However, most of the current evidence remains limited to in vitro or preclinical stages, with clinical validation and regulatory approval processes yet to be completed. Thus, expanding research on phenolic acids, conducting comprehensive preclinical and clinical studies across different viral groups and evaluating their synergistic effects in combination therapies are of paramount importance. From this perspective, increasing the depth and scope of research is a critical step toward positioning phenolic acids not only as complementary but also as potential independent antiviral agents in the future.

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## THE EFFECT OF CURCUMIN SUPPLEMENTATION ON HEPATIC FAT ACCUMULATION AND HISTOPATHOLOGICAL FINDINGS IN LAYING HENS

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### Abstract

This study was conducted to determine the effects of different levels of dietary curcumin supplementation in laying hens on the liver fat content and histopathological findings. A total of 40 Hy-Line Brown laying hens, 68 weeks of age, were used in the experiment. The birds were randomly allocated into five groups, each consisting of 8 replicates. The first group was fed a basal diet, while the other groups received a basal diet supplemented with 100, 200, 300, or 400 mg/kg curcumin. The hens were housed individually in cages and provided with feed and water ad libitum throughout the 10-week trial period. At the end of the experiment, liver tissue samples were collected from slaughtered birds for determination of total fat content and histopathological examination. The results revealed that supplementation with 100 mg/kg curcumin significantly reduced the liver fat content ( $p<0.01$ ), and no lipid vacuoles were observed in the liver tissue of this group. The highest fat content (33.82%) was detected in the group supplemented with 400 mg/kg curcumin, where more than 66% of the liver tissue contained lipid vacuoles with macro- and microvesicles. In conclusion, supplementation of laying hen diets with 100 mg/kg curcumin had beneficial effects on the liver fat content and histopathological structure, indicating that this dose may be considered the most appropriate level.

**Keywords:** Curcumin, Fatty liver, Histopathology

### Introduction

The liver plays a vital role in a variety of functions, including the metabolism of fats, carbohydrates, proteins, vitamins and minerals; waste product removal; and detoxification. It is also the main storage site for vitamins A, D, E, K and B12, as well as glycogen and minerals such as iron and copper. <sup>1</sup> In poultry, chylomicrons pass from the portal blood system to the liver <sup>2,3</sup>, increasing the likelihood of fat accumulation. <sup>4</sup> In addition, cage systems are widely used in egg chicken farming to maximize space utilization. However, intensive feeding is required to achieve high yields, and the limited movement space in cages makes liver steatosis even more inevitable. Fatty liver disease can result in excessive fat accumulation in the liver and abdominal cavity, liver hemorrhage and sudden death. <sup>5</sup> In laying hens, fatty liver disease can reduce egg size by up to 45% within a few days. The first sign of the syndrome is a sudden drop in egg production and an increase in mortality. <sup>6</sup> Adding lipotropic agents to poultry diets is a promising strategy for reducing liver fat accumulation and improving overall productivity. One biologically active compound that has recently gained attention for this purpose is curcumin, a natural polyphenolic compound derived from *Curcuma longa*, a plant in the Zingiberaceae family. Curcumin is notable for its antioxidant, anti-inflammatory and lipid metabolism-regulating properties. The aim of this study was to determine the effects of adding different levels of curcumin to feed on the fat content and histopathology of the livers of laying hens to prevent or minimize the effects of liver steatosis.



## Material and Methods

A total of 40 Hy-Line Brown commercial laying hens were used in the 68-week study. Approval to conduct the study was obtained from the Atatürk University Animal Experiments Local Ethics Committee (2022/59). The hens were divided into five groups of eight, each of which was placed in a separate cage. The first group was fed a basal diet (C0), while the remaining groups were fed basal diets supplemented with 100 mg/kg (C100), 200 mg/kg (C200), 300 mg/kg (C300) or 400 mg/kg (C400) curcumin. The C0 diet used in the experiment was obtained from a commercial feed factory (16.52% CP and 2726 kcal/kg ME), and 99% pure curcumin was obtained from Grenera Nutrients Pvt Ltd. Feed and water were provided ad libitum throughout the ten-week experiment. At the end of the experiment, the animals were euthanized and subjected to surgical intervention. Some of the samples were analyzed via the ether extraction method<sup>7</sup> to determine the relative fat content, whereas others were fixed in a 10% formaldehyde solution for three days. The samples were then reduced in size, placed in tissue cassettes and rinsed under running tap water for one night to remove formaldehyde. The tissues were then processed through increasing concentrations of alcohol solutions, followed by xylene and hot paraffin. Finally, they were embedded in paraffin blocks via an automatic tracking device (Thermo Shandon Citadel 2000). Five-micron samples were taken from the paraffin blocks. The samples on the slides were left in a 57 °C oven for one hour to remove the paraffin wax. The samples were then stained via the hematoxylin–eosin method.

## Statistical Analyses

Values of liver fat ratio variance analysis were performed via the general linear model procedure, and importance control of important data was performed via the SPSS 17 program. Differences between groups were determined via the Duncan multiple comparison test.

## Results and Discussion

The liver plays a major role in providing lipids for use by all tissues, including itself, through a process called lipogenesis. Unlike in mammals, fat synthesis occurs at high levels in the liver of poultry and at limited levels in adipose tissue.<sup>2</sup> The lipogenesis capacity of the liver in poultry is 20 times greater than that of adipose tissue. Table 1 shows that adding 100 ppm curcumin to the diet significantly reduced the liver fat content. While no difference was observed between the control group and the curcumin 200 and 300 groups, the highest fat content was found in the curcumin 400 group. Curcumin reduces the plasma concentration of free fatty acids and mitigates their harmful effects by inhibiting excessive lipolysis and the conversion of lipids from adipose tissue, owing to its anti-inflammatory properties. Furthermore, curcumin has been reported to improve the lipid profile via its hypolipidemic and hypoglycemic properties.<sup>8</sup> A study on large yellow charlatans revealed that the addition of 0%, 0.02%, 0.04% and 0.06% curcumin to the diet reduced hepatic lipid levels and increased antioxidant activity.<sup>9</sup> The positive effects of curcumin on nonalcoholic fatty liver disease have been demonstrated in various animal species.<sup>10,11</sup>

Histopathologically, liver steatosis was examined, and scoring was performed according to fat vacuole formation (Table 2).

**Table 1.** Effect of curcumin supplementation on the liver fat ratio (%)

| Groups  | Liver Fat Ratio (%) |
|---------|---------------------|
| Control | 28.22 <sup>b</sup>  |
| C 100   | 18.37 <sup>c</sup>  |
| C 200   | 29.10 <sup>b</sup>  |
| C 300   | 29.13 <sup>b</sup>  |
| C 400   | 33.82 <sup>a</sup>  |
| SE      | 1.71                |
| P       | 0.00                |

**a-c:** The averages shown with different letters in the same column are different from each other. **Control:** basal fed group; **C100:** 100 mg of added curcumin group; **C200:** 200 mg of added curcumin group; **C300:** 300 mg of added curcumin group; **C400:** 400 mg of added curcumin group. **SE:** Standard error, \*\*: P<0.01

**Table 2.** Scoring according to fat vacuole formation

| Fat Percentage | Score |
|----------------|-------|
| <% 5           | 0     |
| %5-% 33        | 1     |
| >%33-%66       | 2     |
| >%66           | 3     |

Histopathological examination of the liver tissues in the control group revealed the presence of lipid vacuoles containing more than 33–66% macro- and microvesicles (Figure 1). With a score of 2, this group exhibited a markedly high level of hepatic fat accumulation, which was attributed to cage feeding. The hepatic fat content of this group was 28.22%, as shown in Table 1. In the group fed 100 mg/kg curcumin, hydropic degeneration was observed in the liver tissues; however, no lipid vacuoles were detected (Figure 2). This group presented the lowest hepatic fat content in the trial, with a value of 18.37% (Table 1). In the group supplemented with 200 mg/kg curcumin, lipid vacuoles containing 5–33% macro- and microvesicles were identified in the liver tissues (Figure 3). The livers of chickens receiving 300 mg/kg curcumin presented lipid vacuoles containing 33–66% macro- and microvesicles (Figure 4). In the group fed 400 mg/kg curcumin, more than 66% of the liver tissue contained lipid vacuoles with macro- and microvesicles (Figure 5). This group had the highest hepatic fat content in the study, reaching 33.82% (Table 1).

The results of the present study demonstrated that dietary supplementation with curcumin exerted a dose-dependent effect on hepatic fat accumulation in laying hens. The control group, which did not receive curcumin, exhibited marked hepatic steatosis characterized by the presence of extensive lipid vacuoles and a relatively high liver fat content. This finding is consistent with previous reports indicating that cage feeding and energy-rich diets may predispose laying hens to hepatic lipid accumulation.



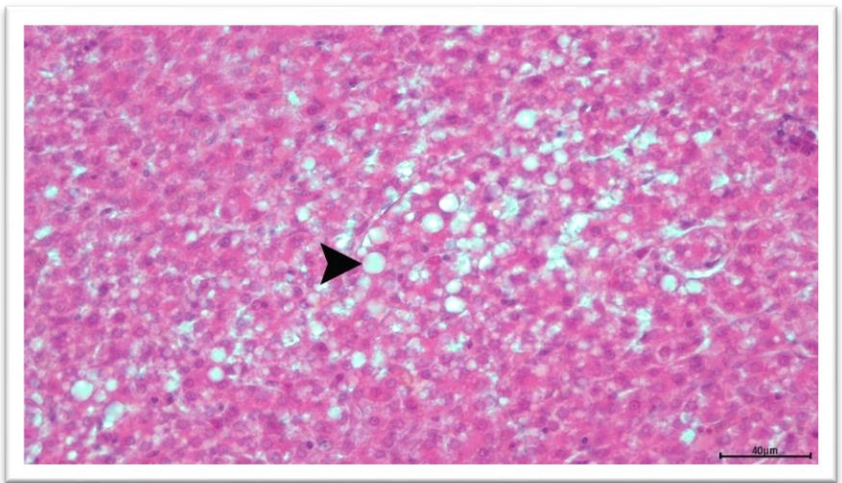


**Figure 1.** Histopathological micrograph of a liver from a control group of chickens. Macrovesicular lipid vacuole (arrowhead). H&E ×40 µm.

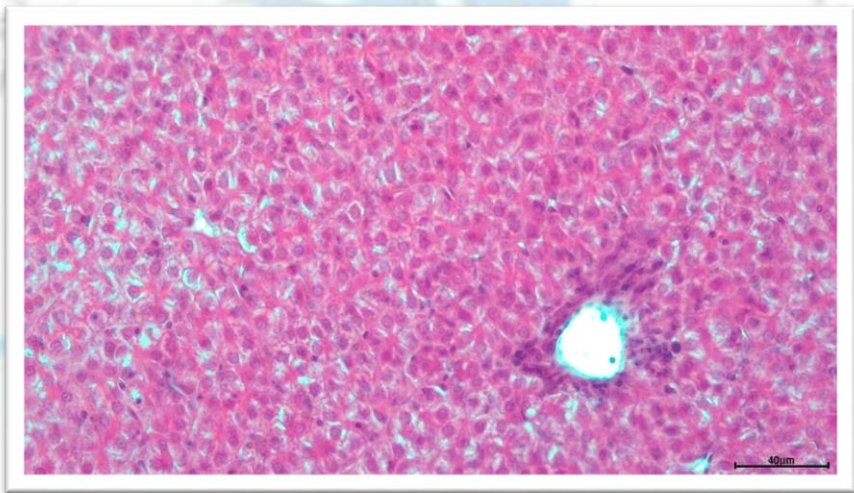


**Figure 2.** Histological micrograph of livers from chickens fed 100 mg/kg curcumin. H&E ×40 µm.

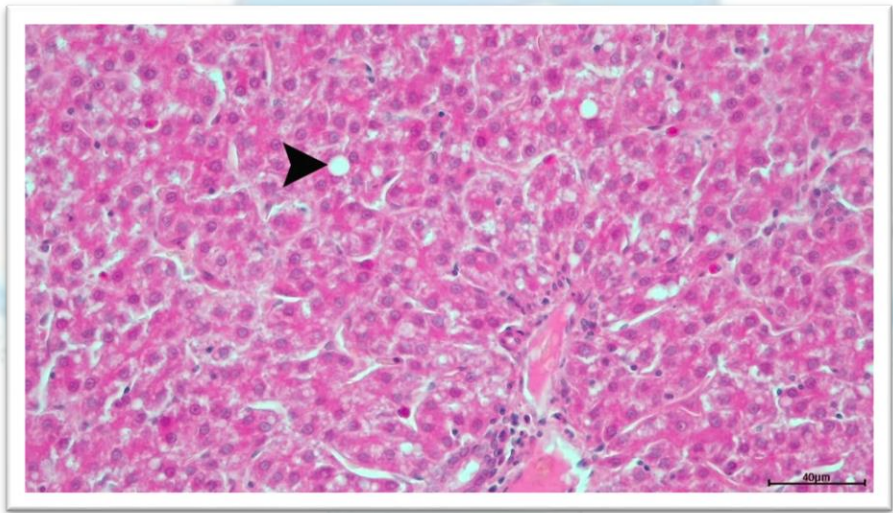


**Figure 3.** Histopathological micrograph of a liver from a chicken fed 200 mg/kg curcumin. Macrovesicular lipid vacuole (arrowhead). H&E ×40 µm.



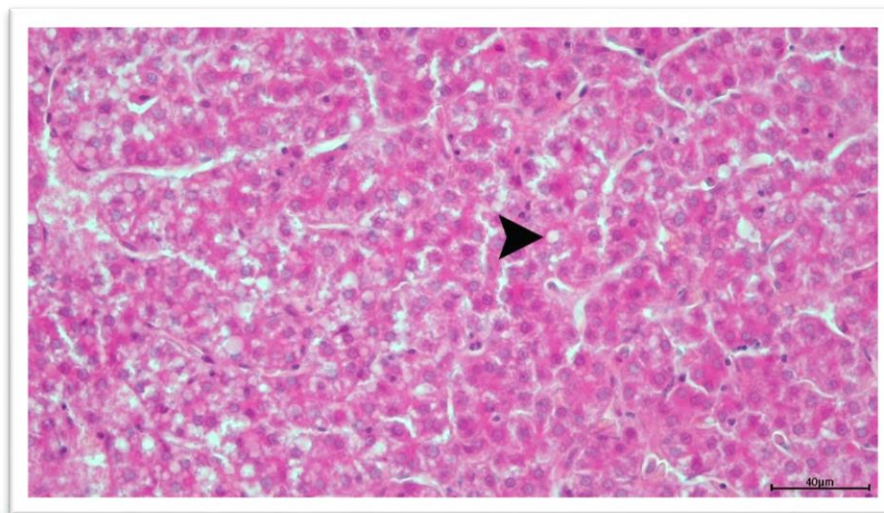


**Figure 4.** Histopathological micrograph of the liver from a chicken fed 300 mg/kg curcumin. Macrovesicular lipid vacuoles (arrowhead). H&E ×40 μm.

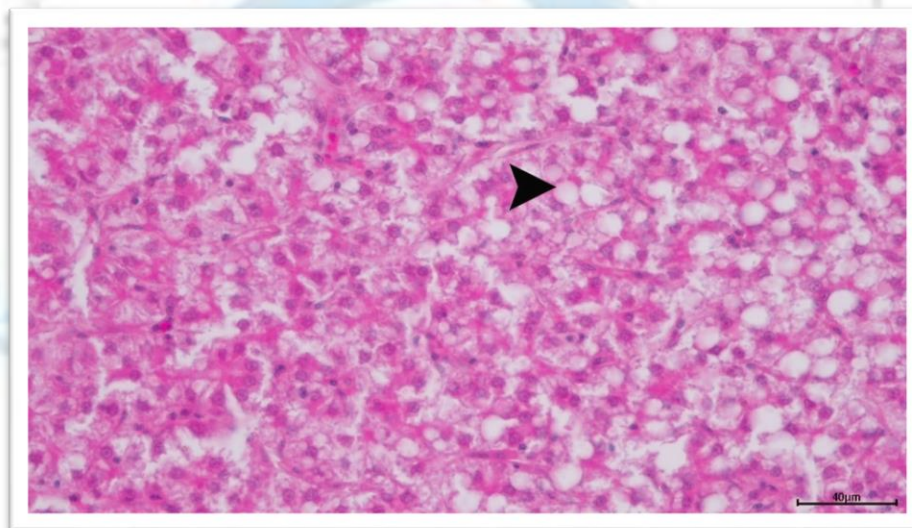


**Figure 5.** Histopathological micrograph of a liver from a chicken fed 400 mg/kg curcumin. Macrovesicular lipid vacuoles (arrowhead). H&E ×40 μm.

The lowest hepatic fat content was observed in the 100 mg/kg curcumin group, where no lipid vacuoles were detected despite the presence of hydropic degeneration. These findings suggest that curcumin at low doses may play a protective role against hepatic steatosis, possibly because of its well-documented antioxidant and anti-inflammatory properties, which can improve lipid metabolism and reduce oxidative stress in the liver. At moderate levels of curcumin supplementation (200 and 300 mg/kg), partial protection was observed, as indicated by reduced but still notable lipid vacuole accumulation. These findings may suggest that, compared with the lowest dose, curcumin at these concentrations provides only limited improvement in hepatic lipid metabolism. Interestingly, the 400 mg/kg curcumin group presented the highest level of hepatic steatosis, surpassing even the control group. These findings indicate that excessive supplementation with curcumin may have adverse effects, potentially disrupting the balance of hepatic lipid metabolism. High concentrations of curcumin may interfere with nutrient absorption, induce metabolic stress, or exert pro-oxidant effects, which could explain the increased hepatic lipid deposition observed. Similar to this study, previous research has demonstrated that curcumin reduces triglyceride synthesis by inhibiting hepatic lipogenesis enzymes while accelerating the breakdown of fatty acids



by enhancing  $\beta$ -oxidation.<sup>12</sup> Curcumin has also been reported to protect the structural integrity of hepatocytes by reducing oxidative stress and inflammation in the liver.<sup>13</sup> Overall, the findings highlight a biphasic response of curcumin supplementation: while low doses appear to alleviate hepatic fat accumulation, higher doses may counteract these benefits and even exacerbate steatosis. These results suggest that curcumin could be a promising feed additive for preventing fatty liver in laying hens, but its efficacy is highly dose dependent. Future studies should focus on elucidating the precise mechanisms underlying the hepatoprotective and hepatotoxic effects of curcumin and determining the optimal supplementation level for poultry diets.

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## POTENTIAL USE OF CBD'S ANTIOKSIDAN AND ANTI-INFLAMMATORY EFFECTS IN VETERINARY MEDICINE

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### Abstract

Cannabidiol (CBD), a nonpsychoactive cannabinoid, exhibits significant anti-inflammatory and antioxidant properties through a complex network of molecular interactions. This article reviews the mechanisms underlying these effects, with a focus on the interaction of CBD with various receptors and molecular targets. In addition to its indirect modulation of the endocannabinoid system (CB1 and CB2 receptors), CBD directly interacts with transient receptor potential (TRP) channels (TRPV1, TRPA1, and TRPM8), 5-HT<sub>1A</sub> serotonin receptors, G protein-coupled receptor 55 (GPR55), and adenosine transporters. These pleiotropic actions contribute to its broad therapeutic potential in managing inflammation, oxidative stress, pain, and neurodegenerative conditions. By elucidating these diverse receptor interactions, this review highlights the multifaceted pharmacological profile of CBD and its promise as a therapeutic agent, underscoring the need for further clinical research to establish its full application.

### Introduction

Cannabidiol (CBD), a nonpsychoactive cannabinoid derived from the *Cannabis sativa* plant, has garnered significant scientific and medical interest because of its diverse therapeutic properties. Unlike its well-known counterpart, delta-9 tetrahydrocannabinol (THC), CBD does not induce intoxicating effects, making it an attractive candidate for various clinical applications. Among its most promising attributes are its potent anti-inflammatory and antioxidant properties, which are increasingly being explored across a spectrum of medical conditions. Inflammation and oxidative stress are fundamental pathophysiological processes implicated in numerous acute and chronic diseases, including pain syndromes, neurodegenerative disorders, and cardiovascular conditions. Effective modulation of these processes is crucial for disease management and improving patient outcomes<sup>1</sup>. The ability of CBD to interact with various molecular targets, both within and outside the endocannabinoid system, makes it a versatile agent with the potential to mitigate these detrimental processes. This study aims to comprehensively review the current understanding of the anti-inflammatory and antioxidant effects of CBD, as well as their underlying molecular mechanisms, with a particular focus on its diverse receptor interactions, drawing upon contemporary literature<sup>1</sup>.

### Anti-Inflammatory Effects and Mechanisms of CBD

Inflammation is a complex biological response of the body to harmful stimuli (pathogens, damaged cells, and irritants). While acute inflammation plays a protective role, chronic inflammation can lead to tissue damage and various diseases<sup>2</sup>. CBD stands out for its ability to modulate inflammatory processes through various molecular pathways.

### Modulation of Inflammatory Pathways

The anti-inflammatory effects of CBD stem from its complex interactions with multiple cellular and molecular targets. These mechanisms include the following:



**Inhibition of the NF- $\kappa$ B Signaling Pathway:** Nuclear factor kappa B (NF- $\kappa$ B) is a central regulator of the inflammatory response, controlling the transcription of proinflammatory genes. Research indicates that CBD inhibits NF- $\kappa$ B activation, thereby reducing the production of inflammatory cytokines and chemokines<sup>2</sup>. This inhibition helps disrupt the inflammatory cycle. The interaction of CBD with the NF- $\kappa$ B pathway is a complex process that occurs at multiple levels, forming the basis of the anti-inflammatory potential of CBD<sup>3,4</sup>.

**Upstream Suppression (TLR4–MyD88 Axis and IRAK1):** The NF- $\kappa$ B pathway typically initiates with the activation of proinflammatory receptors such as Toll-like receptor 4 (TLR4). CBD has been shown to reduce TLR4 expression and MyD88 (myeloid differentiation primary response gene 88)-dependent signaling. Furthermore, it lowers IRAK1 (IL-1 receptor-associated kinase 1) levels, weakening TAK1 (TGF- $\beta$  activating kinase 1) activation and thus limiting IKK $\beta$  (I $\kappa$ B kinase  $\beta$ ) activity. This leads to inhibition in the early stages of the NF- $\kappa$ B signaling cascade<sup>23</sup>.

**IKK–I $\kappa$ B $\alpha$  and p65 (RELA) Modulation:** Phosphorylation and degradation of the I $\kappa$ B $\alpha$  protein are essential for NF- $\kappa$ B activation. CBD prevents IKK $\beta$ -mediated I $\kappa$ B $\alpha$  phosphorylation and degradation, contributing to the retention of the p65 (RELA)/p50 dimer in the cytosol. It also reduces the phosphorylation of p65 at Ser536/Ser311 and its acetylation at Lys310, thereby decreasing its transcriptional activity. The upregulation of SIRT1 (sirtuin 1) and the deacetylation of p65 appear to be critical for this effect. Consequently, nuclear NF- $\kappa$ B binding and target gene expression are suppressed.

**Nrf2–NF- $\kappa$ B Crosstalk:** CBD activates the Nrf2 (nuclear factor erythroid 2-related factor 2) system, the main regulator of the cellular antioxidant response. Nrf2 activation increases the expression of antioxidant and detoxification enzymes. Simultaneously, it creates competition between Nrf2 and NF- $\kappa$ B for the coactivator CBP/p300, limiting NF- $\kappa$ B transcription through trans-repression. Increased heme oxygenase-1 (HO-1) can also secondarily inhibit NF- $\kappa$ B activity.<sup>4</sup>

**Regulation of Redox Tone:** CBD's reactive oxygen species (ROS) scavenging and mitochondrial ROS-reducing effects weaken the ASK1 (apoptosis signal-regulating kinase 1)–IKK activation axis, thereby reducing upstream NF- $\kappa$ B triggering. A reduction in redox load plays a significant role in preventing the chronic activation of NF- $\kappa$ B (Nature Scientific Reports, 2024; Portland Press Neuronal Signaling, n.d.). Indirect Inhibition via Adenosine–A2A Receptor: CBD increases extracellular adenosine levels by inhibiting nucleoside transporters. Adenosine A2A receptor activation suppresses NF- $\kappa$ B directly or indirectly via cAMP/PKA signaling (e.g., decreased IKK activity and competition between CREB and the CBP/p300 coactivator)<sup>5</sup>

**PPAR $\gamma$ -Mediated Trans-Repression:** CBD activation of PPAR $\gamma$  (peroxisome proliferator-activated receptor gamma) reduces the transcriptional activity of NF- $\kappa$ B at target promoters through corepressor complexes. This effect suppresses the expression of cytokine and enzyme-encoding genes (COX-2, iNOS) (MDPI, 2023). Autophagy/NOTCH axis: CBD-mediated induction of autophagy via SIRT1 and suppression of NOTCH signaling indirectly reduce NF- $\kappa$ B activity, shifting the cell away from a proinflammatory phenotype<sup>3</sup>. These multiple mechanisms enable CBD to comprehensively modulate the NF- $\kappa$ B pathway, leading to potent anti-inflammatory effects<sup>67</sup>.

**Reduction in proinflammatory cytokines:** CBD can directly suppress the production of proinflammatory cytokines such as tumor necrosis factor-alpha (TNF- $\alpha$ ) and interleukin-6 (IL-6). These cytokines play key roles in the exacerbation and maintenance of the inflammatory response. A reduction in the secretion of these cytokines by CBD prevents the spread of inflammation (the mechanism by which cannabidiol (CBD) suppresses TNF- $\alpha$ )<sup>89</sup>.

**Modulation of COX and LOX enzymes:** Cyclooxygenase (COX) and lipoxygenase (LOX) enzymes are involved in the synthesis of inflammatory mediators (prostaglandins and leukotrienes). CBD has been reported to reduce the inflammatory response by modulating these enzymes. Specifically, CBD has been shown to directly suppress the formation of leukotrienes and activate a lipoxygenase enzyme, thereby shifting the inflammatory process toward inhibition<sup>101112</sup>.

**Interactions with the endocannabinoid system:** CBD indirectly interacts with components of the endocannabinoid system (ECS). Although it does not bind directly to CB1 and CB2 receptors with high affinity, it can increase the levels of endogenous cannabinoids (such as anandamide) by inhibiting fatty acid amide hydrolase (FAAH), an enzyme that degrades these compounds. The increase in endogenous cannabinoids contributes to the regulation of the inflammatory response<sup>1314</sup>. Furthermore, the antagonistic effects of CBD on CB2 receptors play a significant role in the anti-inflammatory response by suppressing mast cell degranulation and neutrophil proliferation<sup>1516</sup>.

**PPAR $\gamma$  Activation:** Peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ) is a nuclear receptor that significantly affects inflammation and metabolism. CBD has been shown to activate PPAR $\gamma$ , increasing anti-inflammatory cytokines and reducing the inflammatory response<sup>1718</sup>. This mechanism is particularly effective in reducing inflammation in cardiovascular cells. These multifaceted mechanisms make CBD a potential therapeutic agent for various inflammatory conditions<sup>192021</sup>.

### Antioxidant Effects and Mechanisms of CBD

Oxidative stress is a condition resulting from an imbalance between reactive oxygen species (ROS) and antioxidant defense systems, which can lead to cell and tissue damage. CBD, a potent antioxidant, can reduce oxidative stress and provide cellular protection through various mechanisms.

#### Reduction in oxidative stress

The antioxidant effects of CBD occur through multiple pathways:

**Ability to scavenge free radicals:** CBD can directly scavenge free radicals such as hydroxyl radicals and superoxide anions. This prevents oxidative damage that harms cell membranes, proteins, and DNA. This direct radical scavenging property allows CBD to function similarly to traditional antioxidants<sup>2223</sup>.

**Enhancement of Endogenous Antioxidant Systems:** CBD exerts indirect antioxidant effects by strengthening the body's antioxidant defense mechanisms. Specifically, it increases the gene expression of key endogenous antioxidant systems such as superoxide dismutase (SOD) and glutathione (GSH). Glutathione is a crucial antioxidant that plays a critical role in protecting cells against oxidative damage, and CBD helps maintain its effectiveness by preventing glutathione oxidation<sup>2425</sup>.

**Nrf2 pathway activation:** Nuclear factor erythroid 2-related factor 2 (Nrf2) is the main regulator of the cellular antioxidant response. When activated under oxidative stress conditions, Nrf2 increases the gene expression of antioxidant and detoxification enzymes. CBD has been shown to activate the Nrf2 pathway, thereby increasing the resistance of cells to oxidative damage (the molecular activity of cannabidiol in the regulation of the Nrf2 system). This activation significantly enhances the capacity of the cell to cope with oxidative stress<sup>26</sup>.

**Protection of Mitochondrial Function:** Mitochondria, in addition to being the main centers of cellular energy production, are also a significant source of ROS production. Oxidative stress can lead to mitochondrial dysfunction, causing further ROS production and cellular damage. CBD has been reported to protect cells by preserving mitochondrial function and reducing mitochondrial oxidative stress<sup>27</sup>. These antioxidant mechanisms support the therapeutic potential of CBD in various pathologies in which oxidative stress plays a role, such as neurodegenerative diseases, ischemic injury, and inflammatory conditions<sup>28</sup>.



## CBD's Receptor Interactions and Other Molecular Targets

The therapeutic effects of CBD are not limited to its interactions with endocannabinoid system (ECS) receptors (CB1 and CB2); it also interacts with numerous other receptors and ion channels. This multitargeting enables CBD to exhibit a broad pharmacological profile.

### Endocannabinoid System Receptors (CB1 and CB2)

CBD does not bind directly to CB1 and CB2 receptors with high affinity. However, it can act as an allosteric modulator on these receptors, meaning that it can affect the receptor's activity from another binding site. Specifically, the antagonistic effects of CBD on CB2 receptors play a role in modulating inflammatory processes<sup>27</sup>.

### Transient Receptor Potential (TRP) Channels

CBD exerts significant physiological effects by modulating various TRP channels. These channels play a role in processes such as pain, temperature sensation, and inflammation:

**Transient Receptor Potential Vanilloid 1 (TRPV1):** TRPV1, also known as the capsaicin receptor, is an ion channel that plays a critical role in the perception of pain and inflammation. CBD acts as an agonist of the TRPV1 channel, meaning that it activates it. However, at low concentrations, it can inhibit or desensitize TRPV1 signaling (Anand et al., 2020; Louis-Gray et al., 2022). This desensitization contributes to analgesic effects by reducing the transmission of pain signals. The dual effect of CBD on TRPV1 highlights its complex role in pain and inflammation management<sup>22</sup>.

**TRPA1 (transient receptor potential ankyrin 1):** TRPA1 is another ion channel activated by cold, irritating chemicals, and inflammatory mediators. It plays an important role in pain and inflammation processes. CBD has been shown to modulate the TRPA1 channel, and this interaction contributes to its anti-inflammatory and analgesic properties<sup>29</sup>.

**TRPM8 (transient receptor potential melastatin 8):** TRPM8 is a channel activated by cold sensation and compounds such as menthol. CBD has also been reported to affect the TRPM8 channel, but the full physiological significance of these interactions is still under investigation<sup>30</sup>.

**Serotonin receptors (5-HT1A):** CBD acts as an agonist of the serotonin 5-HT1A receptor. This receptor plays a role in various physiological processes, such as anxiety, depression, pain perception, and nausea. The activation of the 5-HT1A receptor by CBD contributes to its anxiolytic, antidepressant, and antiemetic effects<sup>31</sup>.

**GPR55 (G protein-coupled receptor 55):** GPR55 is a recently discovered G protein-coupled receptor thought to have cannabinoid-like properties. GPR55 plays a role in processes such as pain, inflammation, and bone metabolism. CBD can act as an antagonist of the GPR55 receptor, meaning that it can block its activity. This antagonistic effect may contribute to the anti-inflammatory and analgesic properties of CBD<sup>32 33</sup>

**Adenosine transporters:** CBD can exert indirect effects by increasing extracellular adenosine levels. Adenosine is an important neuromodulator with potent anti-inflammatory and immunosuppressive effects. CBD inhibits the uptake of adenosine into cells by inhibiting nucleoside transporters, thereby increasing the extracellular adenosine concentration. This increase, particularly through A1 and A2A adenosine receptors, leads to potent anti-inflammatory effects and constitutes one of CBD's critical roles in reducing inflammation<sup>34</sup>.

**Other Potential Targets:** Other potential molecular targets with which this broad range of targets explains the multifaceted effects of CBD on various physiological and pathological processes. The interactions of CBD with these receptors and ion channels contribute to its anti-inflammatory, antioxidant, analgesic, anxiolytic, and neuroprotective properties. This complex network of receptor interactions enables CBD to be a pleiotropic agent that acts through multiple pathways rather than a single target<sup>35</sup>.

## Clinical Applications

The anti-inflammatory and antioxidant properties of CBD offer significant therapeutic potential in various clinical conditions. This section discusses the relevant clinical applications of CBD.

### Pain Management

CBD has demonstrated analgesic effects in both neuropathic and inflammatory pain models. Its ability to reduce inflammation and modulate oxidative stress makes it a promising agent for the treatment of chronic pain syndromes. The use of CBD as part of multimodal analgesia approaches is being investigated. It has the potential to minimize side effects by reducing dependence on traditional analgesics<sup>36</sup>.

### Neuroprotective effects

The antioxidant and anti-inflammatory properties of CBD make it a potential neuroprotective agent in conditions such as neurodegenerative diseases and acute brain injury. Oxidative stress and neuroinflammation play central roles in the pathogenesis of many neurological disorders. The ability of CBD to modulate these processes has the potential to reduce neuronal damage in conditions such as ischemic stroke, traumatic brain injury, and Alzheimer's/Parkinson's disease<sup>37</sup>.

### Cardiovascular Protection

Inflammation and oxidative stress are important factors in the development and progression of cardiovascular diseases. The anti-inflammatory and antioxidant effects of CBD may have protective effects on cardiovascular conditions such as hypertension, ischemia–reperfusion injury, and atherosclerosis<sup>37</sup>.

The effects of CBD on the gastrointestinal system's anti-inflammatory properties have also been investigated in gastrointestinal disorders such as inflammatory bowel disease (IBD). It can improve gut barrier function by reducing inflammation and oxidative stress<sup>38</sup>.

## Conclusion

The anti-inflammatory and antioxidant potential of CBD has increased its potential as a promising agent for the treatment of various diseases. Its pleiotropic effects, demonstrated through multiple receptor interactions and molecular targets, explain its broad therapeutic spectrum. Future research will further clarify the clinical applications of CBD and enable the development of new treatment strategies.

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## PROANTHOCYANIDINS IN VETERINARY MEDICINE: BIOAVAILABILITY, APPLICATIONS, AND EFFECTS ON ANIMAL HEALTH

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### Abstract

Proanthocyanidins are polyphenolic compounds found in fruit seeds, peels, nuts, cereals, legumes, wine, and chocolate. These compounds influence the nutritional value and sensory properties of foods while exhibiting protective effects against cardiovascular diseases, cancers, urinary tract infections, diabetes, and age-related metabolic disorders. Although their bioavailability is limited depending on the degree of polymerization, they are metabolized by the gut microbiota into phenolic acids and their derivatives, which mediate their biological activities. In veterinary medicine, proanthocyanidins are applied to support the urinary tract, oral health, and infectious disease management in cats and dogs and to increase rumen fermentation, protein digestibility, and control of parasitic infections in ruminants. The current literature confirms their antioxidant, anti-inflammatory, and metabolic regulatory properties, highlighting their potential use in veterinary nutraceuticals and feed additives. This review summarizes the bioavailability, veterinary applications, and effects of proanthocyanidins on animal health on the basis of recent studies and discusses directions for future research.

**Keywords:** Proanthocyanidins, Veterinary medicine, Urinary tract health, Rumen fermentation, Antioxidant

### Introduction

Interest in the use of natural plant compounds for animal health has increased in recent years. Phenolic compounds, in particular, have gained prominence as supportive therapies and feed additives in both small animal medicine and farm animals due to their antioxidant and antimicrobial properties. Proanthocyanidins (PACs) are polyphenolic compounds belonging to the flavonoid group; they have oligomeric or polymeric structures and are composed of the monomers catechin and epicatechin.<sup>1</sup> The strong antioxidant capacity of PACs is associated with their ability to scavenge free radicals, inhibit lipid peroxidation, and regulate the inflammatory response.<sup>2</sup> Owing to its antimicrobial effects, it can also be used as a supplement, especially in the context of urinary system infections and gastrointestinal disorders.<sup>3</sup> Studies in veterinary medicine have shown that PACs offer significant potential in maintaining intestinal health in dogs<sup>4</sup>, improving growth performance and meat quality in pigs<sup>1,2</sup>, and supporting immunity and improving product quality in aquaculture.<sup>5</sup>

### Chemistry and Natural Sources

Polyphenols are widespread secondary metabolites in plants that exhibit considerable diversity in size, structure, and function. Although their chemical configurations and biological roles are not fully understood, they commonly interact with proteins and metal ions via multiple hydroxyl groups.<sup>6</sup> Plant-derived phenolics include a variety of secondary metabolites, including phenolic acids, flavonoids, coumarins, stilbenes, hydrolysable and condensed tannins, lignans, and lignins. These compounds display antimicrobial, antioxidant, and anti-inflammatory properties, and their biological activities are largely determined by their molecular structure.<sup>7</sup>



Proanthocyanidins are naturally occurring polyphenolic antioxidants widely distributed in fruits, vegetables, nuts, seeds, flowers, and bark.<sup>8,9</sup> Their monomeric units consist of (epi)catechin or (epi)gallocatechin, which are linked covalently through C4–C8 or C4–C6 bonds.<sup>10</sup> These flavan-3-ol units typically polymerize to form oligomeric and polymeric structures, with degrees of polymerization ranging from 2–11, and are classified as condensed tannins according to Bate-Smith and Swain (1962).<sup>11</sup> The broad occurrence and structural diversity of proanthocyanidins contribute to their potent biological activities, including antioxidant, anti-inflammatory, and antimicrobial effects, highlighting their relevance in nutritional and veterinary research.

PACs are oligomeric and polymeric forms of flavan-3-ols, such as catechin and epicatechin, that exist as B-type (C–C linked) and A-type (C–O–C and C–C linked) structures. Cranberries are particularly rich in A-type PACs, which are responsible for antiadhesion effects against *E. coli*.<sup>12</sup> Berries and fruits represent some of the richest natural sources of proanthocyanidins, with edible varieties such as lingonberry, cranberry, black elderberry, black chokeberry, black currant, and blueberry containing particularly high levels.<sup>13</sup> Raw persimmon, banana, carob bean, and Chinese quince exhibit strong astringency because of their abundant proanthocyanidin content. Previous studies have reported the highest concentrations per fresh weight in chokeberries, rose hips, and cocoa products.<sup>14,15</sup> Furthermore, proanthocyanidins are widely present in a variety of other fruits, including medlar, mulberry, plum, apricot, walnut, silverberry, and pomegranate, highlighting their ubiquitous distribution across the plant kingdom.<sup>16</sup>

### Bioavailability and Gut Microbiota Metabolism

PACs exhibit poor direct absorption, especially at high degrees of polymerization.<sup>17</sup> Monomers and dimers can be absorbed in the small intestine, but most PACs reach the colon, where microbiota metabolize them into phenolic acids and valerolactones with systemic bioactivity.<sup>18</sup> Thus, PAC–microbiota interactions play a central role in mediating biological functions.<sup>19</sup> Plant polyphenols, as secondary metabolites with phenolic structures, can dynamically interact with the intestinal microbiota and play a crucial role in maintaining gut health in animals.<sup>20</sup> These compounds are able to modulate the composition and diversity of intestinal microorganisms, promoting the growth of beneficial bacteria and thereby enhancing microbial homeostasis within the gut.<sup>21</sup> Notably, grape seed proanthocyanidin (GSP), a naturally occurring polyphenol, has been reported to alter intestinal microbial communities and activate the farnesoid X receptor (FXR) signaling pathway, contributing to the prevention of intestinal inflammation in rodent models.<sup>22</sup> The bioavailability of polyphenols is strongly influenced by their metabolism by the gut microbiota, as microbial enzymes can convert these compounds into bioactive metabolites that are more readily absorbed and biologically active. Consequently, the interplay between polyphenols and intestinal microbes not only determines their systemic effects but also mediates their protective roles in gastrointestinal health.<sup>22</sup>

### Molecular Mechanisms of Action

Proanthocyanidins (PACs) are the second most abundant natural phenolic compounds after lignin<sup>23</sup> and exhibit diverse biological activities, including anti-inflammatory, antioxidant, lipid-modulating, gut-protective, and anticancer effects.<sup>4,24–26</sup> PACs have been shown to possess stronger antioxidant capacities than other well-known antioxidants, such as vitamin E, vitamin C, and  $\beta$ -carotene, and can be rapidly absorbed in the body.<sup>27,28</sup> The potential of dietary PACs as health-promoting agents has attracted considerable attention in nutritional research.<sup>29,30</sup> Epidemiological studies indicate that the regular intake of proanthocyanidin-rich foods is associated

with a reduced risk of inflammatory disorders and multifactorial diseases, including metabolic syndrome, atherosclerosis, and cancer.<sup>31-33</sup> Recent experimental evidence further suggests that dietary supplementation with PACs can enhance growth performance, reduce inflammation and oxidative stress, modulate the gut microbiota, and improve the quality of animal-derived products in pigs, poultry, and other livestock species.<sup>34-38</sup>

### **Preclinical evidence and species-specific veterinary applications**

Recent studies have indicated that dietary supplementation with proanthocyanidins (PACs) can enhance growth performance, attenuate inflammation and oxidative stress, modulate the composition of the gut microbiota, and improve the quality of animal-derived products in pigs, poultry, and other livestock species.<sup>34-38</sup>

**Dogs and Cats:** Zhang et al. (2023)<sup>4</sup> studied how grape seed proanthocyanidin (GSP) supplementation affects Labrador Retrievers with moderate inflammatory bowel disease (IBD). They reported that GSP reduced intestinal inflammation by increasing Ruminococcaceae, Faecalibacterium, the Ruminococcus torques group, and the Lachnospiraceae NK4A136 group and improving beneficial bile acid profiles. This study used fecal microbiota transplantation to establish that the therapeutic efficacy of GSPs was dependent on gut microbiota modulation, highlighting the importance of a polyphenol-based diet for canine intestinal health.<sup>4</sup>

**Ruminants:** In dairy cattle, supplementation with grape seed proanthocyanidin extract (GSPE) has been shown to increase milk protein yield compared with that in control groups. Consistent with this, Moate et al. (2014)<sup>39</sup> reported that the inclusion of 5.0 kg/day of dried grape marc in the diet of late-lactation cows resulted in a modest increase in milk protein levels. Similarly, Gessner et al. (2015)<sup>40</sup> demonstrated that supplementation with grape seed and grape marc meal extract (GSGME) led to improved milk protein production in cows during early lactation.

**Poultry:** Evidence from previous studies indicates that dietary supplementation with grape seed proanthocyanidin extract (GSPE) markedly reduces mortality rates and enhances growth performance in chickens following *Eimeria tenella* infection. The protective efficacy of GSPE in broilers was shown to be dose dependent. While the antiprotozoal and anthelmintic activities of GSPE have been documented previously<sup>41-44</sup>, its influence on broiler performance in response to *Eimeria* infection has been specifically highlighted in these experimental findings.

**Horses and rabbits:** In horses, Davies et al. (2009)<sup>45</sup> reported no health hazards from grape seed extract. Grape seed extract had no detrimental effects on horse feed intake, respiration, pulse, or body temperature.<sup>45</sup> Some findings indicate that appropriate dietary supplementation with GSPE supports liver development in rabbits.<sup>46</sup>

### **Delivery Strategies and Formulation**

The bioavailability of PACs is influenced by their molecular size, as monomers and dimers are absorbed and detected in the bloodstream at relatively low concentrations, whereas compounds with higher degrees of polymerization are not directly absorbed.<sup>47,48</sup> Larger PAC oligomers, once metabolized by the colonic microflora, are broken down into smaller, more readily absorbable compounds that yield metabolites with beneficial health effects.<sup>49,50</sup> Accordingly, it has been suggested that the depolymerization of polymeric PACs into oligomeric forms could markedly increase both their bioaccessibility and bioavailability.<sup>51,52</sup> In summary, the current body of research remains insufficient to definitively determine the bioavailability of proanthocyanidins. Existing evidence suggests that only proanthocyanidins with low degrees of polymerization, or their metabolites generated



in the colon, are effectively absorbed. Furthermore, metabolic transformation and conjugation can substantially modify their biological activities. Therefore, additional in vivo investigations are critically needed to validate these initial findings.

### Safety and potential drug interactions

At nutritional doses, PACs are safe for pets. Dogs treated with 4, 20, or 40 mg/kg proanthocyanidin for 24 weeks were safe and effective.<sup>53</sup> Given these concerns, PACs are safe and may benefit veterinary diets and supplements when managed. The optimization of treatment results requires careful evaluation of species-specific metabolism and interactions.

### Antibacterial, antifungal, antiviral, and antiprotozoal activities

The antimicrobial properties of PAs against bacteria and fungi have long been recognized. Scalbert (1991)<sup>54</sup> comprehensively reviewed the antibacterial and antifungal activities of tannins, including proanthocyanidins, and proposed that tannins exert their inhibitory effects through three principal mechanisms. First, the characteristic astringency of tannins can inhibit extracellular microbial enzymes. Second, tannins may directly interfere with microbial metabolic processes, as evidenced by the inhibition of oxidative phosphorylation by tannic acid. Third, tannins have the ability to chelate metal ions that are essential for microbial growth, thereby restricting microbial proliferation.<sup>54</sup> Recent investigations have assessed the antimicrobial efficacy of various proanthocyanidins against several gram-positive and gram-negative bacteria, as well as the yeasts *Candida albicans* and *Cryptococcus neoformans*.<sup>55,56</sup> Both studies reported that the tested proanthocyanidins exhibited only moderate to weak antibacterial activity. Notably, B-type proanthocyanidins demonstrated greater effectiveness than A-type proanthocyanidins against *C. albicans* and *C. neoformans*, with minimum inhibitory concentrations (MICs).<sup>55,56</sup> Değirmençay (2023)<sup>57</sup> reported that the use of proanthocyanidin in dogs with CDV had an antiviral effect by reducing viral shedding. Few studies have examined the antiprotozoal effects of PAs. In vitro, proanthocyanidins were tested against *Leishmania donovani* amastigotes and promastigotes. All the chemicals significantly inhibited amastigote intracellular survival. Positive controls included sodium stibogluconate (Pentostam) and amphotericin B, which have predicted intracellular antileishmanial activity.<sup>58</sup>

### Conclusions

Proanthocyanidins (PACs) are versatile polyphenolic compounds with significant potential in veterinary medicine and animal nutrition. Their diverse biological activities, including antioxidant, anti-inflammatory, antimicrobial, and metabolic regulatory effects, have been well documented across multiple species, including dogs, cats, ruminants, poultry, horses, and rabbits. The health-promoting effects of PACs are closely linked to their molecular structure, degree of polymerization, and interactions with the gut microbiota, which mediate their bioavailability and generate biologically active metabolites. Dietary supplementation with PACs has demonstrated benefits such as improved intestinal health, enhanced growth performance, better product quality, and protection against oxidative stress and infectious agents. Although preclinical and experimental studies provide promising evidence, the variability in PAC composition, bioavailability, and species-specific responses underscores the need for further controlled in vivo studies to optimize dosage, delivery strategies, and long-term safety. Overall, PACs represent a promising class of nutraceuticals and functional feed additives with potential applications for improving animal health, productivity, and welfare.

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## PHARMACOLOGICAL POTENTIAL OF RESVERATROL IN VETERINARY INTERNAL MEDICINE

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### Abstract

Resveratrol is a naturally occurring stilbene polyphenol present in grapes, berries, peanuts, and red wine. In recent years, it has gained increasing attention in veterinary medicine because of its strong antioxidant, anti-inflammatory, cardioprotective, and anticancer properties. The pharmacological activities of resveratrol are mediated mainly through the activation of SIRT1, the inhibition of NF- $\kappa$ B, and the modulation of MAPK signaling pathways, which together contribute to the reduction in oxidative stress, the suppression of inflammatory responses, and the delay of cellular aging. Studies in veterinary medicine have reported a variety of potential applications. In dogs, experimental and clinical findings indicate that resveratrol exerts anti-inflammatory and chondroprotective effects on the management of osteoarthritis while also supporting metabolic functions in age-related disorders. In cats, its antioxidant properties help counteract oxidative damage and maintain metabolic balance. In farm animals, dietary resveratrol supplementation has been associated with improved immune responses, reduced oxidative stress markers, and, in some cases, enhanced reproductive performance. Additional experimental data suggest potential roles in the management of metabolic syndrome, obesity, and infectious diseases. Despite these promising results, one of the main limitations of resveratrol is its low oral bioavailability and rapid metabolism, which significantly restrict its clinical efficacy. To overcome this challenge, new formulations, such as nanoencapsulation and structural derivatives, are being investigated to improve their absorption and stability. In conclusion, resveratrol appears to be a promising nutraceutical and pharmacological agent for use in veterinary internal medicine, particularly under inflammatory, metabolic, and degenerative conditions. However, further well-designed controlled trials are necessary to establish optimal dosages, long-term safety, and clinical effectiveness across different animal species.

**Keywords:** Resveratrol, Pharmacology, Veterinary medicine, Anti-inflammatory, Antioxidant

### Introduction

Resveratrol (3,5,4'-trihydroxy-trans-stilbene) is a naturally occurring stilbene polyphenol synthesized in response to stress in various plant species, including grapes, berries, peanuts, and the roots of *Polygonum cuspidatum*.<sup>1,2</sup> In veterinary medicine, interest in its potential application for managing hepatobiliary, metabolic, and age-related disorders in companion and production animals is increasing owing to its pleiotropic bioactivities, which include antioxidant, anti-inflammatory, metabolic, and immunomodulatory effects.<sup>3,4</sup> The ability of resveratrol to modulate key signaling pathways, such as the SIRT1, NF- $\kappa$ B, and MAPK cascades, and increase endogenous antioxidant defenses support its therapeutic potential across species.<sup>5,6,7</sup> However, translating these properties into clinical utility in animals is contingent upon overcoming pharmacokinetic limitations, particularly poor bioavailability due to rapid metabolism and low systemic exposure—challenges broadly shared with other



polyphenols.<sup>8,9</sup> This review aims to provide a veterinary-focused synthesis of resveratrol's chemistry and sources, species-specific pharmacokinetics, and implications for therapeutic applications in various animal species.

### Chemical and Natural Sources of Resveratrol

Chemically, resveratrol is a stilbene derivative composed of two phenolic rings connected by an ethene double bond (C<sub>6</sub>–C<sub>2</sub>–C<sub>6</sub> backbone), with a molecular weight of 228 Da.<sup>10</sup> It exists as cis and trans isomers, with the trans form recognized as the more biologically active configuration.<sup>11</sup> In plants, resveratrol functions as a phytoalexin that is synthesized in response to pathogen attack, UV radiation, or mechanical injury.<sup>12,13</sup> Significant dietary and nutraceutical sources include the skin of grapes (*Vitis vinifera*), various berries (Vaccinium, raspberries, mulberries), peanuts, and common traditional medicinal plants such as Japanese knotweed (*Polygonum cuspidatum*).<sup>14</sup> The resveratrol concentration varies with plant species, cultivar, tissue (skin vs. pulp), environmental factors, and processing methods, such as fermentation in winemaking, which involves extracting it from grape skins into red wine.<sup>15</sup>

### Pharmacokinetics and Bioavailability (Species Differences)

Resveratrol displays low oral bioavailability across species, primarily due to rapid absorption coupled with extensive phase II metabolism, including glucuronidation and sulfonation in the intestine and liver, resulting in the predominant presence of conjugated metabolites in circulation.<sup>16</sup> In a canine-specific study using LC–MS/MS methods, resveratrol, resveratrol glucuronide, and sulfate metabolites were quantified after 13 weeks of oral administration; these findings confirmed rapid biotransformation and highlighted the predominance of conjugated metabolites.<sup>17</sup> Additionally, pilot pharmacokinetic data in dogs following the ingestion of a resveratrol-containing joint supplement revealed that neither the aglycone nor its glucuronide metabolites reached quantifiable plasma concentrations, underscoring the challenges posed by low systemic exposure and detection sensitivity.<sup>18</sup> Species differences are notable: monogastric species such as dogs absorb resveratrol more readily, albeit with rapid clearance, whereas in ruminant species, ruminal microbial metabolism significantly reduces bioavailability; thus, systemic exposure tends to be negligible unless protected delivery systems are utilized.<sup>19,20</sup> Consequently, effective dosing regimens and delivery strategies (e.g., encapsulation, phytosome formulations, and nanoparticle carriers) are critical considerations in veterinary applications across species.

### Molecular Mechanisms of Action

Resveratrol exerts diverse biological effects by interacting with multiple molecular pathways, many of which are relevant to veterinary internal medicine. One of the best-characterized mechanisms is the activation of the NAD<sup>+</sup>-dependent deacetylase sirtuin 1 (SIRT1), which modulates mitochondrial biogenesis, lipid metabolism, and glucose homeostasis. Through SIRT1 activation, resveratrol enhances peroxisome proliferator-activated receptor gamma coactivator-1 $\alpha$  (PGC-1 $\alpha$ ), thereby improving oxidative phosphorylation and energy utilization in hepatocytes, myocytes, and immune cells.<sup>21</sup>

Another critical pathway involves inhibition of the nuclear factor-kappa B (NF- $\kappa$ B) transcription factor. NF- $\kappa$ B is central to the expression of proinflammatory cytokines, adhesion molecules, and acute-phase proteins. Resveratrol suppresses NF- $\kappa$ B nuclear translocation and reduces tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), interleukin-6 (IL-6), and cyclooxygenase-2 (COX-2) expression, which contributes to its hepatoprotective, cardioprotective, and anti-infective effects.<sup>22</sup>

Resveratrol also modulates the mitogen-activated protein kinase (MAPK) signaling cascade, influencing cellular proliferation, apoptosis, and the stress response. In hepatocytes and immune cells, resveratrol has been shown to attenuate extracellular signal-regulated kinase (ERK1/2) activation while enhancing c-Jun N-terminal kinase (JNK) and p38 MAPK phosphorylation under oxidative stress conditions, favoring adaptive cytoprotection over apoptosis.<sup>23</sup>

A prominent component of its pharmacology is the reinforcement of endogenous antioxidant defenses. Resveratrol directly scavenges reactive oxygen species (ROS) and upregulates the expression of antioxidant enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase via nuclear factor erythroid 2-related factor 2 (Nrf2) pathway activation.<sup>24,25</sup> In addition, resveratrol modifies the gut microbiome by promoting beneficial taxa such as *Lactobacillus* and *Bifidobacterium* while inhibiting pathogenic bacteria that contribute to systemic inflammation and hepatic stress.<sup>26</sup> Collectively, these molecular actions underpin the rationale for the potential use of resveratrol in veterinary hepatology, cardiometabolic medicine, and immunology.

### **Preclinical evidence (in vitro and laboratory animal models)**

Extensive preclinical evidence supports the hepatoprotective and systemic benefits of resveratrol. In vitro studies using hepatocyte and endothelial cell lines demonstrated reduced lipid peroxidation, decreased ROS generation, and increased mitochondrial membrane potential following resveratrol supplementation.<sup>27,28</sup> In murine macrophage models, resveratrol suppresses lipopolysaccharide-induced TNF- $\alpha$  and IL-1 $\beta$  production, confirming its potent anti-inflammatory properties.<sup>29</sup> Rodent models have been pivotal in defining its pharmacological spectrum. In mice exposed to carbon tetrachloride (CCl<sub>4</sub>), resveratrol significantly decreased the serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels, preserved hepatic histoarchitecture, and upregulated Nrf2-regulated antioxidant enzymes.<sup>30</sup> Similarly, in rats subjected to high-fat diets, resveratrol ameliorated hepatic steatosis, improved insulin sensitivity, and reduced oxidative stress biomarkers, establishing its utility in metabolic syndrome.<sup>31</sup> In addition to its effects on liver disease, resveratrol has been shown to have cardioprotective effects on rabbit and guinea pig models of ischemia–reperfusion injury, which is mediated by SIRT1 and endothelial nitric oxide synthase (eNOS) activation.<sup>32</sup> Neuroprotective properties have also been identified in rodent Parkinson’s disease and Alzheimer’s disease models, with improved neuronal survival and decreased neuroinflammation.<sup>33</sup> Collectively, these studies highlight the broad spectrum of cellular and systemic benefits that may be translatable to veterinary patients.

### **Species-specific Evidence & Clinical Applications**

#### **Dogs**

In dogs, resveratrol has been investigated primarily for its antioxidant and anti-inflammatory effects on degenerative joint disease and hepatic stress. Pilot supplementation trials demonstrated improved oxidative stress markers and mobility scores in osteoarthritic dogs, although the clinical efficacy of these methods remains variable.<sup>18</sup> Davis et al. (2021) examined the hemodynamic response and acute kidney damage (AKI) after seven days of oral resveratrol in a canine hemorrhagic shock model. Twelve grayhounds received resveratrol or a placebo before hemorrhaging. The resveratrol group needed more blood volume loss to reach hypotension, but AKI biomarkers and renal damage scores were not significantly different. Resveratrol enhances blood pressure tolerance to severe hemorrhage but does not protect the kidneys.<sup>34</sup>



### Cats

Although evidence in cats is limited, *in vitro* studies suggest that their hepatoprotective and antioxidant effects are similar to those in dogs and rodents. In a 4-week supplementation study, resveratrol did not affect body weight, body condition score (BCS), BUN, or insulin in obese cats but did reduce triglyceride, free fatty acid, SAA, and LDH activity while increasing adiponectin. These findings indicate potential benefits of resveratrol in managing lipid metabolism disorders, including obesity, in cats.<sup>35</sup>

### Ruminants

In cattle, resveratrol supplementation mitigates mycotoxin-induced hepatotoxicity and oxidative stress, enhancing growth, antioxidant capacity, and immune responses in aflatoxin-exposed calves.<sup>36</sup> In dairy cows, resveratrol has been studied as a feed additive for improving energy metabolism during the transition period, with preliminary data indicating reduced markers of oxidative stress and improved reproductive outcomes.<sup>37</sup> Salzano et al.<sup>38</sup> reported that resveratrol in bovine culture medium improved fertility, cell proliferation, blastocyst development, and embryo cryotolerance. Similarly, pretreatment of cow mammary epithelial cells with resveratrol reduced H<sub>2</sub>O<sub>2</sub>-induced ROS formation and preserved cell viability, suggesting its therapeutic potential against oxidative stress in nursing animals.<sup>39</sup>

### Poultry

Resveratrol supplementation in poultry diets has been widely studied. The benefits of aflatoxin B1 include reduced mortality under heat stress, increased feed efficiency, and mitigation of aflatoxin B1-induced hepatic injury through improved antioxidant enzyme activity and decreased lipid peroxidation.<sup>40,41</sup> Moreover, immunomodulatory effects, including increased antibody titers following vaccination, have been reported.<sup>42</sup>

### Horses

Equine data remain sparse. Experimental studies suggest that it protects against oxidative damage and fibrosis. Aging and lameness increase oxidative stress in horses, and resveratrol supplementation can increase antioxidant capacity, providing protective effects.<sup>43</sup> Anecdotal evidence suggests its application as an antioxidant supplement in performance horses to mitigate exercise-induced oxidative damage. A four-month administration of a resveratrol-containing supplement (Equithrive Joint®) in horses exhibiting lameness and suboptimal performance in their hind limbs led to a decrease in lameness.<sup>44</sup>

### Formulations, Delivery Systems and Strategies to Improve Bioavailability

A major limitation of resveratrol in veterinary medicine is its low oral bioavailability due to first-pass metabolism and rapid clearance.<sup>16</sup> To address this, various formulations have been explored. Nanoencapsulation with polymeric nanoparticles, liposomes, or solid lipid carriers improves stability, solubility, and circulation.<sup>45</sup> Compared with unmodified resveratrol, phytosome complexes with phosphatidylcholine show superior absorption in dogs and other monogastric species.<sup>46</sup>

### Safety, adverse effects, and drug–herb interactions

Resveratrol is generally well tolerated across species at nutraceutical doses. In dogs, subchronic oral administration did not result in significant hematological, biochemical, or histopathological abnormalities.<sup>47</sup> Rodent studies confirm a high margin of safety, with adverse effects reported only at supra-nutritional doses exceeding 1 g/kg/day.<sup>48</sup> Drug–herb interactions in veterinary medicine are underexplored. Resveratrol can inhibit

or induce cytochrome P450 enzymes, including CYP3A4, affecting the metabolism of drugs such as NSAIDs, antifungals, and anticonvulsants.<sup>49</sup> It may also enhance anticoagulant effects by inhibiting platelet aggregation.<sup>50</sup> Therefore, clinical use requires careful monitoring, particularly in polypharmacy cases.

## Conclusions

Resveratrol is a multifunctional nutraceutical with considerable potential in veterinary medicine. It has antioxidant, anti-inflammatory, antifibrotic, and metabolic effects. Preclinical evidence supports hepatoprotective, cardioprotective, and immunomodulatory roles, whereas emerging veterinary studies indicate applications in companion and production animals. Thus, resveratrol may serve not only as a supportive nutraceutical but also as a pharmacological candidate in veterinary hepatology, metabolic medicine, and food safety. However, its clinical application is limited by poorly defined species-specific pharmacokinetics, especially in cats, horses, and ruminants, as well as sparse long-term safety data in companion animals. Most evidence is derived from rodent or in vitro studies, with few controlled veterinary trials. Prior research areas include chronic liver disease, metabolic syndrome, osteoarthritis, infectious diseases, mycotoxin mitigation, reproduction, and food safety. Innovative delivery systems—such as nanoparticles, phytosomes, and prodrugs—along with multicenter trials and formulation advances, are essential to unlock the therapeutic potential of resveratrol.

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## CURCUMIN IN VETERINARY MEDICINE: THERAPEUTIC POTENTIALS

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### Abstract

Curcumin, a polyphenolic compound derived from *Curcuma longa*, has been widely studied for its therapeutic potential in veterinary medicine. The evidence consistently highlights its antioxidant and anti-inflammatory properties, along with additional antimicrobial, antiviral, and anticancer activities. In cats, curcumin has shown promise in feline infectious peritonitis virus models, obesity-related inflammation, osteoarthritis, and mammary tumor cells. In dogs, it has been evaluated in osteoarthritis, periodontal disease, wound healing, and cancer, with supportive effects reported in both clinical and experimental settings. In cattle and calves, studies suggest protective effects against mastitis, modulation of immune and oxidative responses postweaning, and supportive effects under heat stress, with additional benefits observed in respiratory and enteric diseases. Equine studies indicate adjunctive potential in osteoarthritis, supported by pharmacokinetic evidence of systemic exposure, whereas sheep studies report improvements in subclinical mastitis, growth, metabolism, meat quality, and resilience under coccidiosis or heat stress. In poultry, curcumin supplementation has been associated with protection against aflatoxin and pesticide toxicity, improved performance under heat stress, modulation of oxidative and immune markers, supportive roles against *Eimeria*-associated coccidiosis and necrotic enteritis, and *in vitro* antiviral activity against Newcastle disease virus. Overall, while curcumin has broad therapeutic potential across species, challenges related to bioavailability, formulation differences, and reliance on multicomponent products remain. Future research should focus on standardized preparations, pharmacokinetic/pharmacodynamic validation, and well-designed randomized controlled trials to ensure reliable clinical translation.

**Keywords:** Anti-inflammatory, Antioxidant, Curcumin, Nanoparticles, Veterinary medicine

### Therapeutic Effects of Curcumin

Curcumin, the principal bioactive compound of *Curcuma longa*, has attracted considerable attention in both human and veterinary medicine because of its broad pharmacological properties.<sup>1</sup> At the core of these effects lies its potent antioxidant activity.<sup>2</sup> Curcumin can directly scavenge reactive oxygen species, increase the levels of enzymatic antioxidants such as superoxide dismutase, catalase, and glutathione peroxidase, and activate the nuclear factor erythroid 2-related factor 2 pathway.<sup>3</sup> Through these mechanisms, it protects against oxidative damage by increasing the activity of phase II detoxification enzymes and reducing lipid peroxidation.<sup>4</sup>

In addition to its antioxidant capacity, curcumin also exerts strong anti-inflammatory effects. It suppresses nuclear factor kappa B activation, downregulates cyclooxygenase-2 and inducible nitric oxide synthase, and limits the production of proinflammatory cytokines such as tumor necrosis factor-alpha (TNF- $\alpha$ ), interleukin-1 $\beta$  (IL-1 $\beta$ ), and interleukin-6 (IL-6).<sup>5</sup> In veterinary medicine, curcumin has demonstrated potential for alleviating



pain and inflammation in osteoarthritis, as well as mitigating inflammatory processes associated with hepatopathies and infectious diseases.<sup>6</sup>

Another important property of curcumin is its broad-spectrum antimicrobial and antiviral activity. It is active against gram-positive and gram-negative bacteria, including *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Salmonella typhimurium*, with nanoparticle-based formulations enhancing its efficacy against multidrug-resistant strains.<sup>7</sup> Antiviral studies have reported inhibitory effects on HIV, hepatitis C virus, herpes simplex virus, influenza virus, and feline infectious peritonitis virus, primarily through interference with viral entry, replication, and nuclear factor kappa B (NF- $\kappa$ B) signaling.<sup>8</sup>

The anticancer potential of curcumin is also noteworthy. By modulating signaling pathways such as the phosphoinositide 3-kinase/AKT, STAT3, and MAPK pathways, curcumin induces apoptosis, inhibits angiogenesis, and suppresses metastasis.<sup>9</sup> *In vitro* and *in vivo* studies have demonstrated significant tumor-suppressive effects in models of hepatocellular carcinoma, breast carcinoma, osteosarcoma, and melanoma. Furthermore, curcumin enhances chemosensitivity and reduces treatment-associated inflammation, supporting its role as a potential adjunctive therapy.<sup>10</sup>

The aim of this review is to systematically summarize current scientific evidence on the use of curcumin in different animal species, elucidate its underlying mechanisms of action, and identify the existing knowledge gaps that need to be addressed for clinical translation.

### **Efficacy of Curcumin in Cats**

The low oral bioavailability of curcumin limits its clinical application in cats. To overcome this, chitosan-based nanoparticles have been developed, which reduce IL-1 $\beta$ , IL-6, and TNF- $\alpha$  expression in feline infectious peritonitis virus-infected Crandell-Rees feline kidney cells, achieve greater selectivity than free curcumin, and reach significant maximum plasma concentrations after oral administration.<sup>11</sup>

In obese cats, dietary intervention with *Curcuma longa* extract decreased acute-phase proteins, including  $\alpha$ 1-acid glycoprotein and haptoglobin, whereas changes in peripheral blood mononuclear cell cytokine mRNA expression remain limited.<sup>12</sup> In naturally occurring osteoarthritis, a therapeutic diet containing omega-3 fatty acids, hydrolyzed collagen, and turmeric extract improved objective measures such as peak vertical force, the Montreal Instrument for Cat Osteoarthritis-Veterinary version, and activity monitoring over 13 weeks, with good tolerability and no significant adverse effects.<sup>13</sup> In oncology, curcumin exhibited dose-dependent antiproliferative and proapoptotic effects on feline mammary tumor cells, as confirmed by Annexin-V/PI flow cytometry and G0/G1 phase arrest.<sup>14</sup>

### **Efficacy of Curcumin in Dogs**

In dogs, low oral bioavailability also restricts clinical use, leading studies to test standardized turmeric extracts or technologies that enhance absorption (e.g., comiconization, liposomal intravenous formulations). In a randomized, double-blind, placebo-controlled trial, the turmeric-derived extract P54FP showed no significant differences in force plate analysis, but overall clinical assessments favored treatment.<sup>15</sup> Among multicomponent formulations, diets containing curcuminoids, hydrolyzed collagen, and green tea extract improved pain and mobility outcomes in osteoarthritic dogs.<sup>16</sup> Similarly, palmitoyl-glucosamine comiconized with curcumin promoted analgesic effects while reducing the meloxicam dosage.<sup>17</sup>

In periodontal disease, the chemically modified curcumin derivative CMC2.24, which is used as an adjunct to scaling and root planning, reduces the gingival index, pocket depth, and number of pockets  $\geq 4$  mm and decreases matrix metalloproteinases and IL-1 $\beta$  in gingival crevicular fluid and tissues.<sup>18</sup> Follow-up studies also reported further decreases in early biomarkers such as MMP-9. In dermatology and wound healing, a topical gel combining curcumin, chitosan, and *aloe vera* accelerated epithelialization and granulation in aseptic surgical wounds, shortening the healing time compared with mupirocin.<sup>19</sup>

In oncology, curcumin has been shown to have dose- and time-dependent antiproliferative and proapoptotic effects on D-17 canine osteosarcoma cells.<sup>20</sup> Clinically, a small pilot study using liposomal IV curcumin in dogs with cancer reported disease stabilization in some cases, although no objective tumor regression was observed. Treatment was well tolerated, but larger controlled trials are needed to confirm its efficacy.<sup>21</sup>

### **Efficacy of Curcumin in Cattle and Calves**

Bovine models and studies conducted on buffalo mammary epithelial cells have demonstrated that *Curcuma longa*-derived extracts suppress TLR4/NF- $\kappa$ B signaling pathways and downregulate proinflammatory cytokines such as TNF- $\alpha$  and IL-6, suggesting a potential anti-inflammatory role of curcumin in mastitis management.<sup>22</sup>

In calves, postweaning studies have reported that dietary curcumin improved rumen fermentation, increased total protein and globulin levels, decreased IL-1 $\beta$  and IL-6, elevated IL-10, and increased oxidative stress markers. Nevertheless, improvements in growth performance and feed efficiency have not been consistent across studies.<sup>23</sup> Under environmental stress conditions, nanomicelle curcumin supplementation supported feed intake and skeletal development without adverse effects on digestive parameters.<sup>24</sup>

In systemic inflammation models, nanocurcumin suppressed proinflammatory responses in LPS-challenged calves, with effects comparable to those of glucocorticoids.<sup>25</sup> Field observations are also promising for respiratory and enteric diseases: curcumin supplementation reduces retreatment requirements for bovine respiratory disease (BRD) in newly arrived calves,<sup>26</sup> whereas in dairy calves, it decreases oocyst shedding and oxidative stress in coccidiosis, supporting body weight gain.<sup>27</sup>

### **Efficacy of Curcumin in Horses**

In horses, curcumin has been most frequently studied for the management of osteoarthritis and associated lameness. In a double-blind, placebo-controlled trial with the Longvida® formulation, improvements in functional measures such as peak vertical force were reported, whereas equine gastric ulcer syndrome scores did not worsen.<sup>28</sup> These results provide preliminary support for the use of curcumin as an adjunctive option in osteoarthritis. From a pharmacokinetic perspective, plasma measurements via LC-ESI-MS/MS confirmed systemic exposure to free curcuminoids and their conjugated metabolites following oral supplementation, highlighting formulation-dependent differences in bioavailability and the need for plasma-level confirmation in clinical trials.<sup>29</sup>

Mechanistically, *in vitro* experiments in equine neutrophils revealed that curcuminoids dose-dependently suppressed reactive oxygen species production and myeloperoxidase activity, indicating potential utility in conditions driven by NF- $\kappa$ B-mediated inflammation and oxidative stress.<sup>30</sup> Additionally, liposomal curcumin demonstrated *in vitro* antagonistic activity against opportunistic hindgut bacteria such as *Clostridium* spp. and the *Streptococcus bovis/equinus* complex, although *in vivo* evidence for this effect is still lacking.<sup>31</sup>



### Efficacy of Curcumin in Sheep

In sheep, curcumin has been most frequently studied in relation to subclinical mastitis. Dietary supplementation with curcumin in dairy ewes improved California mastitis test scores, increased milk- and health-related biochemical parameters, and improved performance outcomes.<sup>32,33</sup> Conversely, an evaluation of intramammary curcumin treatment in subclinical mastitis patients failed to achieve a cure; in some groups, the somatic cell count even increased, and milk quality was negatively affected, indicating that intramammary administration is not supported by current evidence.<sup>34</sup>

With respect to growth and metabolic health, curcumin supplementation in lambs improved rumen fermentation, microbial protein synthesis, and antioxidant capacity, whereas in nursing lambs, it increased body weight gain, energy metabolism, and immune performance.<sup>35,36</sup> Curcumin also favorably modulates the fatty acid profile of lamb meat, reducing saturated fatty acids and increasing polyunsaturated fatty acids, thereby improving oxidative stability and nutritional value.<sup>37</sup>

In the context of disease and environmental stress, dietary curcumin reduces oocyst shedding and oxidative stress in natural coccidiosis infections, supporting overall health.<sup>38</sup> In summary, supplementation of Hu sheep increased their antioxidant capacity, enhanced their immune response, and was associated with the upregulation of testicular gene expression related to male reproduction.<sup>39</sup>

### Efficacy of Curcumin in Poultry

In poultry, the most consistent evidence for curcumin involves attenuation of oxidative damage and enhancement of immune responses. Under aflatoxin B1 exposure, broiler kidneys presented increased activities of superoxide dismutase, catalase, and glutathione peroxidase, with reductions in malondialdehyde and improvements in total antioxidant capacity.<sup>40</sup> Similarly, under imidacloprid toxicity, curcumin improved growth performance and the feed conversion ratio while enhancing antioxidant defense and modulating immune-related gene expression.<sup>41</sup>

Curcumin has also shown promise in the treatment of protozoal infections. In *Eimeria tenella*-infected broilers, dietary supplementation increased the anticoccidial index and survival rates, preserved cecal integrity, and ameliorated oxidative stress markers, effects attributed to the suppression of proinflammatory cytokines and reinforcement of intestinal barrier function.<sup>42</sup> With respect to bacterial enteropathies, evidence for necrotic enteritis is encouraging but inconsistent. Some field and semifield trials with turmeric/curcumin-based products revealed a reduced *Clostridium perfringens* load and improved gut morphology and microbiota profiles. However, studies using multicomponent formulations (e.g., *Lonicerae flos* combined with turmeric) complicate the attribution of effects specifically to curcumin.<sup>43</sup>

Under heat stress, curcumin nanoparticles improved growth performance, intestinal histomorphometry, and humoral immunity.<sup>44</sup> These findings are consistent with recent meta-analyses, which reported overall benefits of curcumin on performance, antioxidant status, and intestinal health under high environmental temperatures.<sup>45</sup> In the antiviral domain, evidence remains mainly *in vitro*. Curcumin and chitosan-curcumin nanoparticles reduce the replication and hemagglutination titers of Newcastle disease virus.<sup>46</sup> However, field evidence linking these effects to clinical protection and performance in poultry flocks remains limited.

## Conclusion

Curcumin has broad therapeutic potential in veterinary medicine, with evidence indicating that it has antioxidant, anti-inflammatory, antimicrobial, antiviral, and anticancer effects across species. Despite these promising findings, most data arise from small-scale or multicomponent studies. Future work should prioritize standardized formulations, species-specific pharmacokinetic validation, and well-designed clinical trials to ensure reliable translation into practice.

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## EFFECTS OF OREGANO OIL ON DIARRHEA, IMMUNITY AND PERFORMANCE IN YOUNG RUMINANTS

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### Abstract

In recent years, there has been a significant increase in the use of aromatic plants and their essential oils as feed additives in animal nutrition. One of the main reasons for this trend is the ban in the European Union on the use of antibiotic growth promoters as feed additives, citing their potential to contribute to the development of resistance in pathogens. The potential negative effects of chemical additives on both animal and human health have increased interest in natural plant-based additives. Studies have revealed the therapeutic properties of oregano essential oil, including antimicrobial, antifungal, and antioxidant properties. These properties further strengthen the potential use of oregano oil in the livestock sector. A review of the literature indicates that oregano essential oil does not have a negative effect on performance, immune function, or diarrhea in young ruminants, whereas some studies report limited positive effects. However, inconsistencies in the findings highlight the need for more comprehensive and controlled studies on the subject. This review examines current research on the use of oregano essential oil in young ruminants.

**Keywords:** Diarrhea, Immunity, Oregano oil, Performance, Young ruminants

### Introduction

In recent years, trends and searches for natural feed additives have increased in the livestock sector. The restriction of antibiotic use in animal husbandry in some countries has further increased interest in alternative natural solutions. In this context, plant secondary compounds and essential oils are among the potential additives attracting attention in animal nutrition.<sup>1,2</sup> Plant essential oils are volatile liquids obtained through distillation from various parts of aromatic plants and contain numerous biologically active compounds. These oils are widely recognized for their broad spectrum of biological activities, including antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory effects.<sup>3</sup> The acceptance of these products as safe for human and animal consumption has increasingly led to their widespread use as feed additives globally.<sup>4</sup>

Ruminants, especially calves and lambs, are highly vulnerable to disease during the early stages of life because of their immature postnatal immune system.<sup>5</sup> This period carries a high risk of diarrhea and infections, which negatively impact growth performance and increase mortality rates.<sup>6</sup> Essential oils reduce the risk of diarrhea and support digestive health by limiting the proliferation of pathogenic microorganisms in the gut.

Oregano (*Origanum*, *Thymus*, *Satureja*, *Thymbra*) is a plant that grows naturally in the meadows and pastures of China and provides significant benefits in terms of animal nutrition and health. Oregano, a member of the *Lamiaceae* (*Labiatae*) family, is known as both a valuable source of essential oil and a widely used spice. While many species are classified under the name "oregano," those that contain carvacrol and/or thymol as the primary components in their essential oils are considered true oregano. Among these species, plants belonging to



the *Origanum* genus are particularly prominent, and those of greatest commercial importance in our country are Izmir Oregano (*O. onites*), Istanbul Oregano (*O. vulgare ssp. hirtum*), Sütçüler Oregano (*O. multiflorum*), Alanya Oregano (*O. majorana syn. O. dubium*) and Syrian Oregano (*O. syriacum var. bevanii*) are reported to be widespread in the Aegean, Mediterranean and Southeast Anatolia regions.<sup>7</sup> Some images of different oregano species are presented in Figures 1 and 2.



**Figure 1.** *Origanum dubium*<sup>7</sup> **Figure 2.** *Origanum onites*<sup>7</sup>

Oregano oil is rich in phenolic monoterpenes, primarily thymol and carvacrol. These compounds exhibit antimicrobial, antioxidant, antiseptic, and flavor-modifying properties.<sup>4,8</sup> The use of oregano essential oil in ruminant nutrition has significant potential for regulating ruminal fermentation, enhancing growth performance, increasing feed utilization efficiency, and improving animal health. Additionally, the high levels of carvacrol and thymol, along with their potent antimicrobial and antioxidant properties, make oregano oil a natural alternative to synthetic antioxidants.<sup>9</sup> These properties suggest that oregano essential oil could be a valuable natural alternative for improving growth performance and supporting health in young ruminants. Its antimicrobial and immunomodulatory properties may reduce the risk of infection and reduce antibiotic dependence. Studies investigating the effects of oregano oil on calves are quite limited. This study aimed to evaluate the effects of oregano oil on performance, the immune system, and diarrhea in young ruminants.

#### **Components and Different Uses of Oregano Essential Oil**

The essential oil obtained from oregano is rich in components with high biological activity and is therefore widely used in both scientific research and industrial applications.<sup>10</sup> The most distinctive feature of oregano essential oil is its high content of phenolic monoterpenes. Carvacrol is generally the major component and is largely responsible for the strong antimicrobial and antioxidant activity of oil.<sup>11</sup> In addition, compounds such as thymol, p-cymene,  $\gamma$ -terpinene, linalool, myrcene, and  $\alpha$ -terpinene are present in lower amounts.<sup>12</sup> The percentage of these compounds may vary depending on many parameters, such as plant species (e.g., *Origanum vulgare*, *Origanum onites*), growing conditions, ecological factors, harvest period, plant part (leaf, flower), and extraction method.

**Table 1.** Major components found in oregano essential oil<sup>12</sup>

| Compound    | %    |
|-------------|------|
| Carvacrol   | 70.6 |
| Linalool    | 9.7  |
| p-Cymene    | 7    |
| γ-Terpinene | 2.1  |
| Thymol      | 1.8  |
| Myrcene     | 1    |
| α-Terpinene | 1    |

Phenolic compounds, particularly carvacrol and thymol, damage bacterial cell membranes because of their lipophilic structure, increasing permeability and disrupting cellular metabolism.<sup>13</sup> Oregano essential oil is valuable in numerous areas because of its rich chemical composition and biological activity. In the food industry, it is used to prolong shelf-life, delay spoilage, and act as a natural preservative because of its natural antimicrobial and antioxidant properties.<sup>14</sup> In animal nutrition, it is reported to improve intestinal health, balance rumen fermentation, support the immune system, and enhance animal performance when used as a feed additive.<sup>12</sup> As a natural alternative to antibiotics, it is gaining importance, especially in countries where antibiotic growth promoters are banned.<sup>15</sup> In medical and pharmacological uses, it has been widely evaluated in traditional medicine and modern pharmacology because of its antimicrobial, antifungal, antiviral, and anti-inflammatory properties.<sup>16</sup> It plays a supportive role in respiratory infections, digestive system disorders, skin diseases, and inflammatory processes. In cosmetics and personal care products, oregano essential oil is used as a natural fragrance and antimicrobial additive in products such as perfumes, soaps, creams, and shampoos. Furthermore, its use in the cosmetics industry is increasing because of its positive effects on skin health.<sup>17</sup>



**Figure 3.** Therapeutic Properties of Oregano EOs



### Effects of Oregano Oil on the Performance of Young Ruminants

Plant-derived essential oils can be useful tools for improving nutrient utilization efficiency in ruminants and reducing the environmental impact of their production. Oregano oil, an important essential oil, is widely used in many sectors, including pharmaceuticals, cosmetics, and food, and is also incorporated into animal nutrition practices.

In a study conducted on lambs, Simitzis et al.<sup>18</sup> reported that the addition of oregano essential oil to their diets at a level of 1 ml/kg did not significantly affect fattening performance or feed intake. Similarly, Gümüş et al.<sup>19</sup> reported that the addition of oregano essential oil to lamb diets at different doses (200 mg/kg and 400 mg/kg) did not significantly affect lamb performance. On the other hand, Canbolat and Karabulut<sup>20</sup> reported that adding oregano oil at a rate of 5 g/kg DM to diets formulated with different energy and protein levels did not significantly affect the fattening performance of lambs. In a similar study, Ünal<sup>21</sup> investigated the effects of the addition of 250 ppm and 500 ppm oregano oil to the rations of male lambs on performance parameters, and the results revealed that the applied dose did not lead to a statistically significant change in fattening performance. Aydın et al.<sup>22</sup> investigated the effects of oregano oil on the performance of Tuj lambs (1 mL/lamb and 2 mL/lamb daily). The results revealed that the addition of oregano oil did not result in statistically significant differences in live weight gain, average daily live weight gain, feed consumption or the feed conversion ratio. However, Tapkı et al.<sup>23</sup> reported that adding 100 and 150 mg/L oregano oil to milk had a positive effect on live weight gain in suckling calves but had no effect on feed efficiency. Additionally, Nora et al.<sup>24</sup> reported a significant increase in live weight gain in Holstein calves supplemented with a 5 g/kg/MR blend of essential oils (cinnamon, oregano, and eucalyptus).

### Effects of Oregano Oil on Immunity in Young Ruminants

In young ruminants, immunity is initially provided by colostrum, whereas active immunity develops over time as the immune system matures. Different Oregano species have been reported to have immunomodulatory effects.<sup>25</sup> During this critical transition period, the bioactive compounds contained in oregano can support immune function and increase resistance to infection. In a study conducted on calves supplemented with 1000 g/l attapulgit + oregano in starter feed, Liu et al.<sup>26</sup> reported that there was no difference in serum Ig levels. In contrast, Nora et al.<sup>24</sup> reported that supplementing calves with milk replacer supplemented with a mixture of essential oils obtained from cinnamon and eucalyptus plants, including oregano, significantly increased serum IgA levels on days 45 and 60, whereas total protein and globulin levels remained unchanged. In another study,<sup>27</sup> calves were given 4 g, 6 g, or 8 g of oregano essential oil daily, and the supplementation significantly increased the serum IgA and IgM concentrations.

### Effects of Oregano Oil on Diarrhea in Young Ruminants

Diarrhea, an important economic problem in young ruminants, leads to significant financial losses in livestock farms due to high mortality rates.<sup>28</sup> Recent studies indicate that the therapeutic use of medicinal plants in the treatment of diarrhea is increasing. There is evidence that these plants increase the recovery rate, shorten the recovery time, reduce mortality, and improve growth performance in animals. Therefore, medicinal plants offer potentially effective alternative treatment options for diarrhea treatment.<sup>29</sup> The use of oregano (*O. vulgaris*) and thyme (*Thymus vulgaris*) plant extracts has positive effects on animal health, performance, and metabolic status by suppressing intestinal pathogens that cause diarrhea in ruminants. Furthermore, probiotics, which

include live microorganisms that can benefit the host when they are consumed in adequate amounts, are considered important supplements in animal nutrition.

A study conducted by Heisler et al.<sup>30</sup> revealed that 60 mg of oregano oil did not significantly affect the fecal scores of Jersey calves. Similarly, Selvi and Tapki<sup>31</sup> reported that the addition of 893 mg of oregano oil to milk replacer did not have a notable effect on the fecal scores of dairy calves. Similar results have also been reported by Bampidis et al.<sup>32</sup>, who reported no significant difference in the fecal scores of calves supplemented with oregano essential oil. Similarly, Ünlü and Erkek<sup>33</sup> reported that the addition of 250 mg of oregano or garlic oil to milk did not have a statistically significant effect on the fecal score or the number of days with diarrhea. In contrast, Tapki et al.<sup>23</sup> reported that the addition of 100–150 mg/l oregano oil to milk reduced the fecal score in calves. Furthermore, calves receiving oregano oil via oral syringe supplementation at a dose of 12.5 mg/kg BW/day exhibited significant improvements in gastrointestinal health, as evidenced by reductions in both the incidence of daily diarrhea and fecal consistency scores.<sup>34,35</sup>

### Conclusion

The studies conducted on the effects of oregano oil supplementation on young ruminant diets suggest that, given its therapeutic properties, this herbal supplement may offer potential benefits for performance, immunity, and digestive health. However, findings in the literature are often inconsistent. Some studies report improvements in performance criteria, whereas others find no statistically significant differences. Similarly, while studies on the immune system have reported positive changes in immunoglobulin levels, some trials have reported no significant effects. Research on diarrhea has generally revealed no consistent effect on fecal scores; however, favorable results can occur under certain dosing regimens and application methods. Overall, the effectiveness of oregano essential oil appears to vary significantly depending on the dose, animal species, and environmental factors. Therefore, future studies are crucial for determining the optimal dose, investigating long-term effects in different species and age groups, and clarifying its potential use as an antibiotic substitute.

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## EFFECT OF RHODIOLA ROOT ON SEIZURE ACTIVITY IN RATS WITH PENTYLENETETRAZOLE - INDUCED EPILEPSY

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### Abstract

Epilepsy is a neurological condition involving the brain that makes people more susceptible to having recurrent seizures. It is one of the most common disorders of the nervous system and affects people of all ages, races, and ethnic backgrounds<sup>1</sup>. Approximately 700,000 million Turkish citizens live with epilepsy. Anything that interrupts the normal connections between nerve cells in the brain can cause a seizure. This includes a high fever, low blood sugar, high blood sugar, alcohol or drug withdrawal, or a brain concussion. Under these circumstances, anyone can have one or more seizures. However, when a person has two or more seizures, he or she is considered to have epilepsy<sup>1</sup>. There are many possible causes of epilepsy, including an imbalance of nerve-signaling chemicals called neurotransmitters, tumors, strokes, and brain damage from illness or injury, or some combination of these. In the majority of cases, there may be no cause that can be discovered for epilepsy. In this study, the effects of an extract obtained from Rhodiola plant roots on pentatetrazole-induced epileptic seizures in experimental animals were investigated. In herbal medicine, the part of the plant used is its root. Although several types of Rhodiola exist, only *Rhodiola rosea*, a plant of the Crassulaceae family, contains compounds of interest, such as rosavins, salidroside, tyrosol and phenolic acids<sup>2</sup>. The animals used in the experimental study were divided into two groups: the first group, the control group (n= 8), and the second group, the experimental group (n= 8). All the studies were performed under anesthesia. Urethane was used as the anesthesia agent. One gram of urethane was weighed and dissolved in 4 cc distilled water, and then a 25% solution was prepared at a dose of 1.25 g/kg before the surgical procedure and administered intraperitoneally (i.p.). After the animals were anesthetized one by one, surgical procedures were performed. The skull bone was cut from the parietal region, and the brain tissue was removed. The control group animals were subsequently fixed to the sterile device, and epilepsy seizures were induced by injecting 2.5 microlitres (µL) of pentylenetetrazole directly into the brain tissue. Epilepsy seizures were recorded for 120 min with a Biopac MP 100 device. After all the animals in the control group were treated in this way, the experimental group of animals were started. The experimental group of animals were injected with 2.5 microlitres (µL) of the plant extract obtained from the root of the *R. rosea* plant directly into the brain tissue 10 min after the onset of epilepsy seizures induced by pentylenetetrazole, as in the control group, and epilepsy seizures were recorded for 120 minutes with the Biopac MP 100 device. Then, statistical analyses of the recordings were performed, and the strength (amplitude) and frequency of epileptic seizures in the control group and the experimental group were compared. We observed that *R. rosea* plant extract significantly suppressed epileptic seizures. As shown in the table above, there was a 44% reduction in the frequency of epilepsy attacks as well as epilepsy. There was a 66% decrease in the power of epileptic seizures. The total number of frequencies decreased from 578 to 284 in 2 h, a 44% decrease. The amplitude decreased from 364 mV to 241 mV in 120 min. The decrease is 66%.

**Keywords:** Rhodiola, Epilepsy, EEG, Neurological disorder



## Introduction

Epilepsy is a nervous system disorder that occurs as a result of sudden, excessive and irregular discharge of neurons in the brain. Unpredictable irregularities can be observed in consciousness, behavior and motor activity. Epilepsy, as well as human medicine, is important in veterinary medicine. Epilepsy is a disease that reduces the quality of life of animals. At most, epilepsy is observed in cats and dogs; more sparsely, it is observed in horses and even in cattle.<sup>1,2</sup> In this study, we investigated the effects of *Rhodiola* on epilepsy. *Rhodiola rosea*, also known as *Rhodiola rosea* "golden root" or "rose root", belongs to the plant family Crassulaceae. *R. rosea* is a phytomedicinal herb with adaptogenic effects that can increase the body's nonspecific resistance to physical and mental stresses and normalize its functions.<sup>2</sup> In traditional treatment, the chemicals found in the roots of *R. rosea* are used to treat phenols, rosavin, resin, rosarin, organic acids, terpenoids, phenolic acids and their derivatives, flavonoids, anthraquinones, alkaloids, tyrosol and salidroside, fatigue, depression, and nervous system disorders. *R. rosea* also has numerous beneficial pharmacological activities, including anti-inflammatory, antioxidant, antidepressive, antifibrotic, antiallergic, anticancer and antiapoptotic activities.<sup>4</sup>

## Materials and Methods

### Rhodiola Root Extract

The *Rhodiola* (golden root) used in our study was collected from the countryside of the Yusufeli district of ARTVIN. The obtained *R. rhodiola* roots were divided into stems and leaves. *Rhodiola* was transported to the laboratory environment with aluminum foil, shock freezing was performed via liquid nitrogen, the mixture was stored at -30 °C, and extraction buffer was used to prepare the crude extract. Two grams of *rhodiola* roots were placed in a mortar and crushed into flour by adding liquid nitrogen. Five milliliters of extraction buffer was added to 1 gram of root nodules.

**The extraction buffers used were** 0.01 M phosphate, 0.01 M ascorbic acid, and 0.5% polyvinylpyrrolidone (PVP) at pH = 5.20. The mixture was subsequently centrifuged in a cooled centrifuge at 20,000 rpm and 5 °C for 30 min.<sup>5</sup>

**Preparation of the epilepsy model in rats:** Experimental animals (n=16) were obtained from the Erzurum Provincial Directorate of Animal Protection of the Ministry of Agriculture of the Republic of Türkiye with the approval of the ethics committee.<sup>6</sup>

### Anesthetic and surgical procedures

All experimental animals were anesthetized, the skull bone tissue of each animal was cut, and the brain tissue was removed under anesthesia. Urethane was given for anesthesia.

**Preparation of the anesthetic agent:** Later, under anesthesia before the surgical procedure, 1 g of urethane was weighed, dissolved in 4 cc distilled water and given intraperitoneally (i.p.) at 1.25 g/kg.<sup>7</sup>

### Grouping of animals

**First Group** (Control Group) (n = 8): The anesthetic substance urethane was administered from the prepared 25% solution at a dose of 1.25 g/kg, and 1.500 µL was administered intraperitoneally (i.p.), and electrocorticography (ECoG) recordings were taken for 120 min.<sup>8</sup>

**Second Group** (*Rhodiola rosea*) (n=8): *Rhodiola rosea* 500 IU (2.5 µL) was given as i.c. (Intracortical), and ECoG recordings were taken for 120 min.

### Electrophysiological recordings

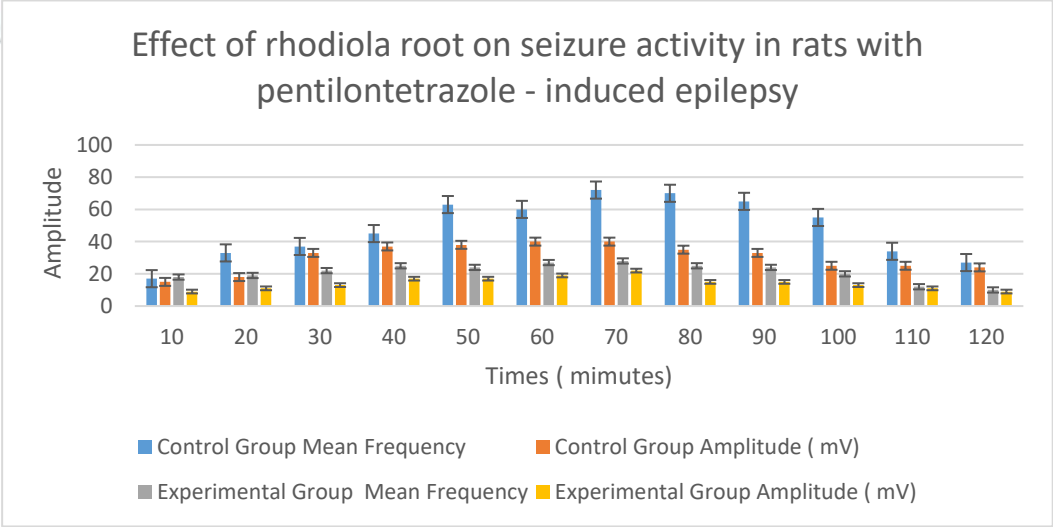
Electrodes connected to the EEG device were placed on the brain tissues of the animals fixed to the stereotaxis device, and the brain activities were recorded with the Biopac MP100 device.<sup>9</sup>

Occurrence of epileptic seizures

Pentylentetrazole was used for the occurrence of epileptic seizures. Pentylentetrazole 50–60 mg/kg was injected intracranially into the animals fixed to the sterile device, and epileptic seizures were induced. EEG recordings were recorded on the basis of the frequency and amplitude of epileptic seizures. The first seizure was observed approximately 7–10 min later, and 20–25 min were allowed for the seizures to stabilize. Then, 2.5 µl (microliter) of the previously prepared supernatant was injected intracranially only into the experimental group, and 120-min recordings were taken.<sup>10</sup> Records can be seen in Figure 1, Graf1

**Table 1:** Mean spike frequency (spike/min) and amplitude (mV) (millivolts) according to groups with 10 min intervals

| Time (minut) | Control Grp. Mean Frequency | Control Grp. Amplitude (mV) | Experimental Grp. Mean Frequency | Experimental Grp Amplitude(mV) | P    |
|--------------|-----------------------------|-----------------------------|----------------------------------|--------------------------------|------|
| 10           | 17                          | 15                          | 18                               | 9                              | ,010 |
| 20           | 33                          | 18                          | 19                               | 11                             | ,084 |
| 30           | 37                          | 33                          | 22                               | 13                             | ,023 |
| 40           | 45                          | 37                          | 25                               | 17                             | ,013 |
| 50           | 63                          | 38                          | 24                               | 17                             | ,028 |
| 60           | 60                          | 40                          | 27                               | 19                             | ,014 |
| 70           | 72                          | 40                          | 28                               | 22                             | ,030 |
| 80           | 70                          | 35                          | 25                               | 15                             | ,045 |
| 90           | 65                          | 33                          | 24                               | 15                             | ,003 |
| 100          | 55                          | 25                          | 20                               | 13                             | ,001 |
| 110          | 34                          | 25                          | 12                               | 11                             | ,001 |
| 120          | 27                          | 24                          | 10                               | 9                              | ,001 |



**Graph 1:** Graphical representation of mean amplitude values according to groups with 10 min intervals



## Results

As shown in the table above, there was a 44% reduction in the frequency of epilepsy attacks as well as epilepsy. There was a 66% decrease in the power of epileptic seizures. The total number of frequencies decreased from 578 to 284 in 2 h, a 44% decrease. The amplitude decreased from 364 mV to 241 mV in 120 min. The decrease is 66%.

## Discussions

In this study, we investigated the effect of *Rhodiola* on the nervous system. In previous scientific studies of this plant, extracts obtained from the roots were shown to have serious positive effects on diseases such as cardiovascular disease and depression. Its effect on epileptic seizures has yielded better results than expected. In this study, as shown in the graphs above, it reduces the power of epileptic seizures by almost half and reduces their frequency by 60%. Although we believe that this activity is likely due to its effect on ion channels, it may also affect GABA (gamma-aminobutyric acid). Such studies should be further developed, and even combined plant extracts should be prepared such that their effects can be expected. Another mechanism of action may involve glycine, since glycine is found in intermediate neurons in the gray matter, especially in the medulla spinalis. The glycine A receptor opens chloride channels such as the GABA-A receptor and may suppress epileptic seizures by exerting an inhibitory effect.

## Conclusion

This study revealed that this plant strongly suppresses epileptic seizures in terms of both frequency and power. In this study, many chemicals were present in the roots of the *Rhodiola* plant, and it is necessary to investigate which substances are most effective in this suppression. We believe that such studies need to be developed; they should be tested on other animals, and it would be healthier to reach a conclusion accordingly.

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## EFFECT OF DIETARY ZINC BORATE SUPPLEMENTATION ON CHEMICAL AND MICROBIOLOGICAL QUALITIES OF MEAT IN LAYING HEN

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### Abstract

This study investigated the effects of different concentrations of zinc borate (ZB) in laying hen feed on the chemical and microbial properties of breast meat. The study used feed with ZB concentrations of 100 mg/kg (ZB100), 200 mg/kg (ZB200), and 400 mg/kg (ZB400), as well as a control group. The ZB100 group was characterized by a lower pH value, lower TBARS values, and a lower total number of psychrophilic aerobic bacteria (TPAB). In contrast, higher levels of ZB (200–400 mg/kg) led to an increase in *Enterobacteriaceae* and an impairment of oxidative stability. ZB supplementation affects the chemical properties of the meat, such as pH, water activity, color and oxidative stability, as well as the microbial load. The results suggest that ZB has dose-dependent effects on meat quality and that a low dosage (100 mg/kg) may be the optimal supplementation level.

**Keywords:** Zinc borate, Chicken meat, Chemical quality, Microbial quality, Oxidation

### Introduction

Zinc is a vital trace mineral in poultry nutrition and is involved in numerous physiological processes, such as enzyme activation, the immune response, antioxidant defense, and skeletal development [1,2]. Adequate dietary zinc has been shown to increase growth performance, carcass yield, and breast muscle quality in poultry [3,4]. Boron, although less widely applied in animal feed, affects mineral metabolism, bone strength, hormonal regulation, and the gut microbial balance [5]. Recent evidence also highlights its role in supporting intestinal barrier integrity and antioxidant capacity [6]. Zinc borates, which are primarily known for their industrial applications, have recently attracted attention because of their antibacterial, antioxidant, and wound-healing properties [7,9]. Despite these promising biological effects, information on the nutritional use of zinc borates in poultry remains limited. Considering the essential functions of zinc and boron and the potential synergistic effects of their combination, this study was designed to evaluate the influence of dietary zinc borate supplementation on breast meat quality in laying hens.

### Materials and Methods

A total of 144 Lohman Brown laying hens, 60 weeks of age, were used in this study. The experiment was conducted with four groups—one control and three treatment groups—each consisting of six replicates. While the control group received the basal diet, the treatment groups were supplemented with 100, 200, or 400 mg/kg zinc borate. Feed and water were provided ad libitum, and the trial lasted for 12 weeks. At the end of the experiment, breast meat samples were collected from one bird per replicate, totaling 24 birds, and subjected to quality and microbiological analyses. This study was approved by Atatürk University, Unit Ethics Committee of the Faculty of Veterinary Medicine (Approval Date: 27.12.2023; Approval Number: 2023/37).



For the microbial analyses, 25 g of the breast meat sample was weighed and homogenized with 225 ml of sterile Ringer's solution. Serial dilutions were then prepared, and 1 ml of the corresponding dilutions was inoculated onto plates with previously prepared sterile agar via the pour plate method, after which the hepatocytes were spread [10]. PCA medium was used to determine the TMAB count, and the samples were incubated at 37 °C for 24 h. PCA medium was used to determine the TPAB count, and the samples were incubated at 10 °C for 7 days. VRBDA medium was used for the enumeration of *Enterobacteriaceae*, which were subsequently incubated at 37 °C for 24 h. MSA medium was used for the enumeration of Micrococcus-Staphylococcus, which were incubated at 37 °C for 48 h. Lactobacillus spp. MRS agar was cultured and incubated anaerobically at 37 °C for 72 h to determine the number of *Lactococcus* spp. To determine the number of *Lactococcus* spp., M17 agar was cultivated and incubated for 24 h at 37 °C. The results of the microbiological count were expressed as log cfu/g.

The sample size varies depending on the chemical analysis to be performed. To determine the water activity, a sample was placed in the AQUALAB 4TE device and measured. To determine the pH value, 10 g of the sample was weighed and homogenized with 100 ml of distilled water in an Ultra-Turraxta and measured with a pH meter. For color analysis, measurements were taken on different sections of the breast meat samples via a colorimeter. The values of L, \*a\* and b\* were determined for color measurement. For the analysis of the reactive substance thiobarbituric acid (TBARS), 2 g of the meat sample was weighed, and 12 mL of TCA solution was added and homogenized. Then, 3 mL of the filtrate was filtered through a Whatman 1 paper filter, and 3 mL of TBA solution (0.02 M) was added. The test tubes were heated to 100 °C for 40 min and then cooled. Centrifugation was performed at 4 °C for 5 min. After centrifugation, the absorbance values were measured at 530 nm with a spectrophotometer, and the TBARS values were calculated as  $\mu\text{mol malonaldehyde/kg}$  [11].

### Statistical analysis

All the statistical analyses were performed via the SPSS 20.00 software package. One-way analysis of variance (ANOVA) was used to determine differences in chemical and microbiological parameters between groups. Duncan's post hoc test was used. The data are expressed as the means  $\pm$  standard errors (SEMs). Differences were considered significant at  $P < 0.05$ .

### Findings

No difference was found between the groups in terms of TMAB counts in the breast meat samples after varying amounts of Zinc-Borate were supplemented in the basal rations ( $p > 0.05$ ). The use of high amounts of ZB increased the number of *Enterobacteriaceae*, and this increase was statistically significant ( $p < 0.05$ ). The application significantly reduced the number of Micrococcus-Staphylococcus bacteria ( $p < 0.05$ ). *Lactobacillus* and *Lactococcus* counts were significantly greater in the ZB-supplemented groups than in the control group ( $p < 0.05$ ). ZB supplementation reduced the number of TPAB, and this difference was significant compared with that in the control group ( $p < 0.05$ ) (Table 1).

The addition of ZB to the basal rations at different rates had a statistically significant effect on the aw and pH values ( $p < 0.05$ ). ZB was found to have no effect on the TBARS value, an oxidation parameter ( $p > 0.05$ ). While ZB used as a feed additive did not affect the L\* and a\* values ( $p > 0.05$ ), it was found to have a statistically significant effect on the b value ( $p < 0.05$ ) (Figure 2).

**Table 1.** Microbial properties of laying hen breast meat samples supplemented with different concentrations of zinc-borate in their diets (log cfu/g) (mean±S.E.)

| Group    | TMAB      | <i>Enterobacteriaceae</i> | <i>Micrococcus-Staphylococcus</i> | <i>Lactobacillus</i> spp. | <i>Lactococcus</i> spp. | TPAB       |
|----------|-----------|---------------------------|-----------------------------------|---------------------------|-------------------------|------------|
| Control  | 1.95±0.02 | 1.24±0.06b                | 1.91±0.02a                        | 1.30±0.00c                | 1.58±0.04c              | 2.00±0.00a |
| ZB100    | 1.90±0.03 | 1.42±0.08b                | 1.89±0.08a                        | 1.44±0.04b                | 1.70±0.05c              | 1.94±0.01a |
| ZB200    | 2.15±0.15 | 1.98±0.02a                | 1.89±0.03a                        | 1.32±0.02bc               | 2.01±0.01b              | 1.84±0.02b |
| ZB400    | 2.30±0.00 | 1.89±0.11a                | 1.60±0.06b                        | 1.65±0.05a                | 2.32±0.02a              | 1.78±0.03b |
| p değeri | 0.061     | 0.006                     | 0.030                             | 0.005                     | 0.000                   | 0.006      |

ZB100: 100 mg/kg zinc borate; ZB200:200 mg/kg zinc borate; ZB400: 400 mg/kg zinc borate; a-c: Differences between groups

**Table 2.** Chemical properties of laying hen breast meat samples supplemented with different concentrations of zinc-borate in their diets (mean±S.E.)

| Group    | Aw            | pH         | TBARS     | L*         | a*        | b*         |
|----------|---------------|------------|-----------|------------|-----------|------------|
| Control  | 0.994±0.001a  | 5.83±0.01a | 0.45±0.02 | 54.75±1.13 | 3.44±0.07 | 1.71±0.44b |
| ZB100    | 0.989±0.002b  | 5.66±0.01c | 0.53±0.13 | 56.18±1.59 | 2.91±0.56 | 3.94±0.79a |
| ZB200    | 0.992±0.000ab | 5.76±0.01b | 0.74±0.07 | 54.29±0.80 | 2.63±0.22 | 2.36±0.19b |
| ZB400    | 0.994±0.000a  | 5.82±0.03a | 0.64±0.04 | 54.70±0.95 | 2.55±0.13 | 2.10±0.28b |
| p değeri | 0.029         | 0.000      | 0.083     | 0.980      | 0.193     | 0.019      |

ZB100: 100 mg/kg zinc borate; ZB200:200 mg/kg zinc borate; ZB400: 400 mg/kg zinc borate; a-c: Differences between groups

Discussion and Conclusion

Owing to its antibacterial and antioxidant properties, the zinc borate used in this study was added to the feed of laying hens to investigate its effects on the microbial and chemical quality of the breast meat. While the TMAB content in the breast meat samples of ZB was similar to that in the control group, the number of *Enterobacteriaceae* was greater in the groups supplemented with ZB than in the control group. These findings suggest that high ZB supplementation may have negative effects on the gut microbiota and meat contamination. Conversely, the levels of *Lactobacillus* spp. and *Lactococcus* spp. were low in the control group and high in the other groups, with the highest levels in the high-ZB dose groups (400 mg/kg). This increase in lactic acid bacteria suggests that ZB may have a stimulatory effect at low doses. Zinc derivatives have been reported in the literature to affect microbial balance and promote the growth of lactic acid bacteria [14]. The lowest TPAB levels were detected in the ZB100 group, suggesting that low-dose ZB may be beneficial for cold storage stability. The microbiological data obtained from the breast meat samples are consistent with studies demonstrating the antimicrobial effects of ZB compounds [7,9,12].

ZB added to the feed of laying hens influenced the aw values. While the water activity values in the low-dose ZB groups were similar to those in the control group, the values in the group receiving 400 mg/kg ZB were greater. The lowest aw value was found in the ZB100 group, suggesting that the use of low-dose ZB may reduce the WHC of the meat. While the pH values of the control and ZB400 groups were similar, the pH values of the ZB100 and ZB200 groups were lower. Lowering the pH of meat can slow microbial growth and extend the shelf-life of meat [13]. No statistically significant difference was found in the TBARS values, an indicator of lipid



oxidation, but oxidation was relatively greater in the ZB200 group. These findings suggest that moderate ZB applications have a pro-oxidant rather than an antioxidant effect. Similarly, the application of high doses of some inorganic compounds can also disturb the oxidative balance [15].

When the color parameters were examined, the L\* and a\* values were similar between the control and treatment groups. However, a\* difference was observed in the b\* value between the groups, and an increase in the yellow color of the meat was observed in the ZB100 group. A change in meat color is an important quality criterion for consumer acceptance, and the effects of additives on color are often highlighted [16].

Supplementation with zinc borate resulted in limited changes in the quality characteristics of laying hen breast meat. In particular, a ZB dose of 100 mg/kg lowered the pH, controlled lipid oxidation and limited the development of TPAB. It also had a positive effect on the growth of lactic acid bacteria. However, applications at a concentration of 200–400 mg/kg caused a limited increase in *Enterobacteriaceae* counts and impaired oxidative stability. In conclusion, ZB can be an effective additive to improve meat quality, with the optimal effect being achieved at lower concentrations.

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## SOME MEDICINAL PLANTS IN THE TREATMENT OF DIABETES MELLITUS AND ITS COMPLICATIONS

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### Abstract

Diabetes mellitus (DM) is one of the most common and serious metabolic disorders, affecting millions of people worldwide and representing a growing public health problem. The prevalence of DM continues to rise due to factors such as obesity, sedentary lifestyles, and genetic predispositions. Although numerous pharmaceutical agents are currently available to manage DM, conventional therapies are often associated with limitations, including high cost, adverse side effects, and reduced patient adherence. For this reason, novel therapeutic approaches that are both effective and safe are increasingly needed. Complementary and alternative medicine (CAM) has gained global attention as a supportive strategy for the management of chronic diseases, particularly diabetes mellitus. Among CAM modalities, phytotherapy stands out because of its reliance on medicinal plants and natural compounds. Many plant-derived preparations have traditionally been used in different cultures, and scientific studies have confirmed that several of these plants exhibit antidiabetic properties. In addition to their hypoglycemic effects, phytotherapeutic agents are reported to provide antihypertensive, nephroprotective, cardioprotective, hepatoprotective, and retinoprotective benefits, helping to reduce the burden of diabetes-related complications. These mechanisms include the enhancement of antioxidant activity, the inhibition of  $\alpha$ -glucosidase and  $\alpha$ -amylase enzymes, the suppression of advanced glycation end product (AGE) formation, and the regulation of inflammatory pathways. Furthermore, phytochemicals can increase the expression of glucose transporter type 4 (GLUT-4) and peroxisome proliferator-activated receptor (PPAR), thereby improving glucose uptake and lipid metabolism. The objective of this study was to systematically review medicinal plants that are effective in the management of diabetes mellitus and its complications. By emphasizing the therapeutic potential of phytotherapy, this work seeks to highlight alternative and complementary approaches that may be integrated with conventional treatment strategies to achieve better outcomes in the long-term management of DM.

**Keywords:** Diabetes mellitus, Phytotherapy, Plants

### Introduction

Diabetes mellitus (DM) is widely recognized as one of the most prevalent and chronic metabolic disorders, affecting a substantial proportion of the global population across both developed and developing countries.<sup>1</sup> The incidence and prevalence of diabetes are steadily increasing due to factors such as a sedentary lifestyle, poor dietary habits, genetic predispositions, and overall increases in obesity rates.<sup>2</sup> Despite the availability of numerous pharmaceutical agents and advanced therapeutic strategies designed for the treatment and management of this condition, diabetes mellitus continues to pose a significant global health challenge.<sup>3</sup> The currently used pharmacological interventions, while effective to a certain degree, are often associated with considerable limitations, including reduced patient compliance, high cost of long-term treatment, and the occurrence of adverse side effects that may compromise overall quality of life.<sup>4</sup> Consequently, the need for novel, safer, and more



effective therapeutic interventions remains paramount to address these prevailing challenges and improve long-term disease outcomes.

In this context, complementary and alternative medicine (CAM) has emerged as a treatment method that has gained increasing popularity worldwide for the management of numerous chronic and metabolic diseases, particularly diabetes mellitus.<sup>5</sup> Among the wide spectrum of CAM approaches, phytotherapy—utilizing natural plant-derived compounds—has attracted considerable attention from researchers and healthcare practitioners.<sup>6</sup> Herbal medicines, which have been traditionally employed in various cultures for centuries, are now being systematically investigated for their potential role in modern medicine.<sup>7</sup> Certain antidiabetic plants employed in phytotherapy not only demonstrate hypoglycemic effects but also exhibit a wide range of additional beneficial properties.<sup>8</sup> These include antihypertensive, antioxidant, nephroprotective, hepatoprotective, cardioprotective, and retinoprotective activities, which provide significant advantages in combating the most common and debilitating complications associated with diabetes mellitus.<sup>9</sup>

The mechanism of action of phytotherapy on diabetes mellitus (DM) is multifactorial and involves several interrelated processes.<sup>1</sup> These include the enhancement of antioxidant defense systems, the inhibition of carbohydrate-hydrolyzing enzymes such as  $\alpha$ -glucosidase and  $\alpha$ -amylase, the suppression of advanced glycation end product (AGE) formation, the modulation of inflammatory pathways, and the regulation of insulin secretion and sensitivity.<sup>10</sup> Additionally, phytotherapeutic agents have been shown to increase the expression of glucose transporter type 4 (GLUT-4) and peroxisome proliferator-activated receptor (PPAR), both of which play crucial roles in improving glucose uptake and lipid metabolism.<sup>11</sup> Through these mechanisms, medicinal plants contribute not only to the regulation of blood glucose levels but also to the prevention of long-term complications that result from chronic hyperglycemia.<sup>12</sup>

Given the multifaceted nature of diabetes mellitus and its associated complications, there is a growing scientific interest in identifying and systematically reviewing medicinal plants that can serve as potential therapeutic agents.<sup>6</sup> The objective of this study is therefore to provide a comprehensive and systematic review of medicinal plants that are useful for the treatment and management of diabetes mellitus (DM) and its related complications. By shedding light on the mechanisms of action, clinical evidence, and therapeutic potential of these plants, this work aims to highlight the role of alternative and complementary methods in diabetes treatment. Ultimately, the integration of phytotherapy with conventional therapeutic approaches may pave the way for the development of safer, more sustainable, and patient-friendly strategies for the long-term management of diabetes mellitus.

### **Prevalence of Diabetes in Animals and Risk Factors**

**Diabetes in Cats:** In a study involving 193,563 cats, the incidence of diabetes in cats was found to be 0.58%. It was also determined that certain breeds (Tonkinese, Norwegian Forest, Burmese) carry a relatively high risk.<sup>13</sup> Body weight increases the risk of developing diabetes (e.g., OR = 32.4 in cats weighing  $\geq 8$  kg). As age increases (e.g., between 12 and 14.9 years), the risk increases exponentially (OR  $\approx 27.6$ ). It has been determined that male cats are at greater risk than females are (OR  $\approx 1.6$ ).

**Diabetes in Dogs:** Type 1 diabetes, which is generally insulin dependent, develops in dogs. The immune system's attack of beta cells in the pancreas is a significant contributing factor. In some cases, type 2 diabetes can also develop due to a decrease in insulin production. Temporary gestational diabetes may also occur due to

pregnancy or oestrus. Breeds genetically predisposed to this condition include Keeshond, Samoyed, Australian Terrier, miniature poodle, Schnauzer, and husky. A study conducted on an island dog population reported an annual prevalence of 0.56% and an incidence of approximately 0.37%.<sup>14</sup>

**Diabetes in Zoo Primates:** According to a survey of members of the Association of Zoos and Aquariums (AZA), approximately 30% of participating institutions reported having at least one primate with diabetes, the majority of which were Old World monkeys.<sup>15</sup>

### Medicinal Plants Used for the Management of Diabetes

#### *Allium sativum* L. (Garlic)

Garlic (*Allium sativum* L.) is a widely used medicinal plant that is traditionally valued for its culinary and therapeutic properties. Its bioactive sulfur compounds, particularly allicin and S-allyl cysteine, are responsible for diverse pharmacological effects, including antioxidant, anti-inflammatory, and cardioprotective activities.<sup>16,17</sup> Recent evidence suggests that garlic also plays a promising role in metabolic disorders such as diabetes mellitus, improving glucose regulation and insulin sensitivity while offering protection against diabetic complications.<sup>18</sup> In recent studies, the administration of garlic or its derivatives (e.g., allicin, aged garlic extract, garlic oil) has been shown to result in substantial reductions in blood glucose and HbA1c levels, accompanied by improvements in liver and kidney function and increased insulin levels.<sup>19,20</sup> According to meta-analyses, garlic supplementation has been found to have positive effects on fasting blood sugar; lower lipid parameters such as triglycerides, total cholesterol, and LDL; and regulate blood pressure.<sup>21</sup>

#### *Allium cepa* (Onion)

*Allium cepa*, or onion, has shown some promise in fighting diabetes in experimental and preliminary clinical studies. In rats with diabetes caused by a chemical, streptozotocin, eating onions lowered fasting blood glucose levels, improved cholesterol and triglyceride levels, and increased antioxidant enzyme activities. These findings suggest that onions have both blood sugar-lowering and antioxidant effects.<sup>22</sup> Similarly, when diabetic rats were supplemented with onion, a decrease in blood sugar and serum lipid levels and normalization of oxidative stress markers such as glutathione peroxidase, reductase and S-transferase activities were observed.<sup>23</sup> The hypoglycemic effect of *Allium cepa* is mediated through the modulation of important hepatic enzymes involved in glucose and lipid metabolism, including hexokinase, glucose-6-phosphatase, and HMG-CoA reductase.<sup>24</sup> Some clinical evidence suggests that red onion extract reduces fasting blood glucose and advanced glycation end products (AGEs), increases serum insulin levels, suppresses inflammatory gene expression, and may support pancreatic  $\beta$ -cell function and enzyme pathways related to glycolysis and gluconeogenesis.<sup>25</sup>

#### *Zingiber officinale* Roscoe (Ginger)

Ginger (*Zingiber officinale*) is a widely used medicinal plant and spice rich in bioactive compounds such as gingerols, shogaols, paradols, and zingerone, which contribute to its pharmacological properties. Traditionally, ginger has been used for its anti-inflammatory, antioxidant, and digestive benefits. In recent years, numerous experimental and clinical studies have explored its potential role in the management of metabolic disorders, particularly diabetes mellitus.<sup>26,27</sup> In diabetic animal models, ginger supplementation has been shown to reduce fasting blood glucose, improve insulin sensitivity, and enhance pancreatic  $\beta$ -cell function. Its bioactive compounds can modulate glucose metabolism by increasing hepatic glucokinase activity, enhancing glycogen storage, and inhibiting key enzymes such as  $\alpha$ -glucosidase and  $\alpha$ -amylase, thereby reducing postprandial glucose



levels.<sup>27</sup> Additionally, ginger exhibits antioxidant activity by decreasing reactive oxygen species (ROS) and increasing the activity of antioxidant enzymes such as superoxide dismutase (SOD) and glutathione peroxidase (GPx), which help protect against diabetes-related oxidative stress.<sup>28</sup> Clinical trials also support the antidiabetic potential of ginger. Several studies have reported significant reductions in fasting blood glucose, HbA<sub>1c</sub>, and insulin resistance in type 2 diabetic patients after ginger supplementation, along with improvements in lipid profiles and inflammatory markers. Overall, these findings indicate that ginger may serve as a complementary therapeutic agent for glycemic control and metabolic health in diabetic patients.<sup>29</sup>

### ***Cinnamomum verum* J. Presl (Cinnamon tree)**

*Cinnamomum verum*, more commonly referred to as Ceylon cinnamon, is a medicinal plant that has been utilized for therapeutic purposes for many years. The presence of bioactive compounds, including cinnamaldehyde, cinnamic acid, and polyphenols, has been identified as the primary factor contributing to its pharmacological properties (Ranasinghe et al., 2013). Cinnamon has a long history in the management of metabolic disorders, including diabetes mellitus, due to its reported effects, including antidiabetic, antioxidant, and anti-inflammatory properties.<sup>30</sup> Experimental studies have shown that taking cinnamon supplements can significantly reduce fasting blood glucose and HbA<sub>1c</sub> levels while increasing insulin sensitivity. Cinnamon enhances the translocation of glucose transporter type 4 (GLUT-4) to the cell membrane and activates insulin receptor kinase, thereby increasing intracellular glucose uptake. It also improves glycemic control by modulating key enzymes involved in glucose metabolism, such as glycogen synthase and phosphoenolpyruvate carboxykinase. Furthermore, cinnamon protects pancreatic  $\beta$ -cells from free radicals by reducing oxidative stress and increasing the activity of endogenous antioxidant enzymes.<sup>31</sup> Clinical studies conducted on patients with type 2 diabetes have reported that cinnamon supplementation significantly reduces fasting blood sugar, HbA<sub>1c</sub> and total cholesterol levels. These effects enable cinnamon to be used as an alternative agent in addition to existing treatment methods used to combat diabetes.<sup>30,32</sup>

### ***Ocimum basilicum* (Sweet basil)**

Sweet basil (*Ocimum basilicum*) is a widely known plant that is frequently used in kitchens. A subspecies of basil with a milder or liquorice flavor.<sup>33</sup> Recent studies suggest that it may have beneficial effects on the management of diabetes mellitus.<sup>34</sup> Sweet basil has demonstrated antidiabetic potential in various preclinical studies. Extracts obtained from its leaves exhibit inhibitory effects on important carbohydrate digestion enzymes, such as  $\alpha$ -amylase and  $\alpha$ -glucosidase, contributing to the reduction in postprandial blood sugar levels.<sup>35</sup> Sweet basil is also rich in antioxidant compounds that can reduce the oxidative stress associated with chronic hyperglycemia and protect pancreatic and hepatic tissues.<sup>36</sup> In streptozotocin-induced diabetic rats, the administration of *O. basilicum* extract led to significant decreases in fasting blood glucose and improvements in oral glucose tolerance and lipid profiles.<sup>37</sup> Additionally, it has antioxidant and anti-inflammatory effects by increasing the levels of endogenous antioxidant enzymes (such as SOD and CAT) and reducing the levels of oxidative stress markers.<sup>38</sup>

### **Doses in animal models**

**Garlic:** In animal diabetes models, garlic extract has been administered at 250–350 mg/kg orally in rabbits<sup>39</sup>, 50–125 mg/kg in streptozotocin-induced diabetic rats (with 125 mg/kg being the most effective)<sup>40</sup>, 500

mg/kg/day intraperitoneally in fructose-induced insulin-resistant rats<sup>41</sup>, and 100–600 mg/kg/day intraperitoneally as aged garlic extract (AGE).<sup>42</sup>

**Onion:** In animal models, onion extract has been administered as onion peel extract (OPE) added to the diet at 0.5–1% for 8 weeks<sup>43</sup>, at 25–100 mg/kg intramuscularly in alloxan-induced hyperglycemic mice (100 mg/kg being the most effective) as red onion extract<sup>44</sup>, and at 500 mg/kg orally as Welsh onion root extract in streptozotocin-induced diabetic rats.<sup>45</sup>

**Ginger:** In animal diabetes models, ginger extract has been administered at 500 mg/kg intraperitoneally for 7 weeks in STZ-induced diabetic rats (aqueous raw ginger extract lowering serum glucose, cholesterol, triglycerides, and proteinuria)<sup>46</sup>, at a single oral dose of 500 mg/kg in diabetic rats (promoting pancreatic islet regeneration)<sup>47</sup>, and at 200 mg/kg/day orally for 30 days in diabetic rats (modulating CD4/CD8 expression).<sup>48</sup>

**Cinnamon:** In animal diabetes models, cinnamon extract has been administered at 50–200 mg/kg/day orally for 6 weeks (resulting in significant antihyperglycemic effects)<sup>49</sup> and at 40 mg/kg/day orally for 8 weeks in STZ-induced diabetic rats, normalizing hyperglycemia and oxidative stress markers.<sup>50</sup>

**Sweet basil:** In animal diabetes models, aqueous basil extract has been administered orally at 100–400 mg/kg body weight (showing dose-dependent antihyperglycemic effects in STZ-induced diabetic rats)<sup>51</sup>, at 200 mg/kg orally in microvesicle-formulated basil extract (with or without MKC acid), yielding significant antihyperglycemic effects in alloxan-induced hyperglycemic rats<sup>52</sup>, and at much higher doses of 100, 300, and 1000 mg/kg BW orally in diabetic rats to assess blood glucose and advanced glycation end products)<sup>53</sup>.

## Results

Diabetes mellitus is a multifactorial metabolic disorder that continues to represent a major health challenge not only in humans but also in animals, where it negatively affects welfare, longevity, and productivity. Conventional antidiabetic drugs, while clinically effective, are often associated with adverse effects, high cost, and limitations in long-term compliance, thus necessitating the exploration of complementary and alternative therapeutic approaches. In this context, medicinal plants such as garlic, onion, ginger, cinnamon, and sweet basil have gained increasing attention because of their hypoglycemic, antioxidant, and multiorgan protective effects, which have been demonstrated in various animal models.

Evidence from preclinical studies indicates that these plants act through multiple mechanisms, including inhibition of carbohydrate-digesting enzymes ( $\alpha$ -amylase and  $\alpha$ -glucosidase), improvement of insulin secretion and sensitivity, modulation of hepatic glucose and lipid metabolism, suppression of oxidative stress and inflammatory pathways, and protection of pancreatic  $\beta$ -cells. In animal models of diabetes, effective doses vary widely depending on the plant species and extract type: garlic (50–600 mg/kg), onion (0.5–1% diet or 25–500 mg/kg), ginger (200–500 mg/kg), cinnamon (40–200 mg/kg), and sweet basil (100–1000 mg/kg). These dose ranges consistently produced antihyperglycemic and antioxidative effects, highlighting their therapeutic potential.

Despite these promising findings, significant gaps remain before routine application in veterinary or human medicine can be recommended. Most available studies are preclinical, and differences in extract preparation, dose standardization, and experimental design limit the generalizability of the results. Moreover, safety profiles at higher doses, potential herb–drug interactions, and long-term effects require further investigation.



In conclusion, garlic, onion, ginger, cinnamon, and sweet basil show strong potential as supportive therapeutic agents in diabetes management. Their integration into future therapeutic regimens, guided by evidence-based research, may offer safer, cost-effective, and patient-friendly alternatives to conventional therapy in both animals and humans.

**Conflicts of interest:** The authors have no conflicts of interest.

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## TWO MODEL AGENTS AND A PARADOXICAL COMPOUND IN HEPATOTOXICITY: CCL<sub>4</sub>, SUNITINIB, AND LONG-TERM OLEANOLIC ACID EFFECTS

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### Abstract

Hepatotoxicity is one of the most frequently encountered toxic reactions in drug development processes and is highly relevant for both clinical practice and experimental research. Owing to the central role of the liver in drug metabolism, understanding the mechanisms leading to hepatocellular injury is critical for drug safety and therapeutic management. In this review, carbon tetrachloride (CCl<sub>4</sub>), sunitinib, and oleanolic acid are discussed as different models used to investigate hepatotoxicity. CCl<sub>4</sub>, a classical experimental hepatotoxicity model, induces oxidative stress, lipid peroxidation, mitochondrial dysfunction, and inflammatory responses through cytochrome P450-mediated reactive radical formation. Sunitinib, a multitarget tyrosine kinase inhibitor used in clinical practice, can lead to hepatotoxicity via mitochondrial damage and disturbances in energy metabolism despite its anticancer efficacy. Oleanolic acid, a paradoxical compound, exerts hepatoprotective effects at low doses but causes hepatotoxicity in long-term or high-dose applications through cholestasis and oxidative stress. The fact that all three agents converge on common pathways, such as oxidative stress, mitochondrial dysfunction, and inflammatory processes, highlights the shared mechanisms underlying hepatocellular injury. These findings demonstrate that mechanistic insights obtained from experimental models overlap with clinical manifestations of hepatotoxicity, underscore the need to reassess the safety profile of herbal products and provide a translational framework for the early diagnosis, prevention, and treatment of drug-induced liver injury. Notably, oleanolic acid shows hepatoprotective effects at low doses but hepatotoxic effects with long-term or high-dose exposure, a dual behavior that has also been documented in experimental studies, emphasizing the importance of careful dose selection.

**Keywords:** Carbon tetrachloride, Hepatotoxicity, Oleanolic acid, Sunitinib

### Introduction

Hepatotoxicity is a major concern in both clinical practice and experimental research. The liver is a vital organ that regulates many fundamental functions in the human body, including drug detoxification <sup>1</sup>, lipid metabolism <sup>2</sup>, and glucose metabolism. <sup>3,4</sup> Owing to its central role in drug metabolism, the liver is of particular importance in pharmacological research. Once administered, drugs enter systemic circulation and are metabolized, detoxified, and eliminated by the liver. Evaluating whether drugs accumulate in the body, impair detoxification capacity, or induce hepatotoxicity represents a critical step in drug development and safety validation. Moreover, the liver is frequently affected by toxic reactions during drug development. <sup>5</sup> Indeed, hepatotoxicity accounts for nearly one-third of drug development project discontinuations. <sup>6</sup>

Drug-induced liver injury (DILI) poses a significant concern for consumers, clinicians, the pharmaceutical industry, and regulatory authorities alike. For consumers, it represents a marginal but ever-present risk; for clinicians, it presents a diagnostic and therapeutic challenge; and for the pharmaceutical industry and regulators, it constitutes a major obstacle to successfully bringing and maintaining a product on the market. The difficulties associated with DILI partly arise from the incomplete understanding of its underlying mechanisms, making experimental models crucial for investigation. DILI animal models are technically straightforward; typically, high doses of a drug in animal models induce hepatotoxicity. However, their correct application requires a fundamental understanding of the specific mechanisms involved in each model.<sup>7</sup>

Carbon tetrachloride (CCl<sub>4</sub>) is not a drug; however, at high doses ( $\geq 1$  mL/kg), it reproducibly induces acute liver injury resembling DILI. It represents one of the most widely used models of xenobiotic-induced acute liver injury.<sup>8</sup> Owing to their biological similarity to humans, rats are considered the most suitable experimental animals for studying CCl<sub>4</sub>-induced hepatotoxicity.<sup>7</sup> Studies have demonstrated that the histopathological features of CCl<sub>4</sub> toxicity in rats resemble those observed in humans.<sup>9</sup>

Sunitinib is an orally administered, multitargeted tyrosine kinase inhibitor (TKI). Clinical studies have shown that sunitinib prolongs survival in patients with gastrointestinal stromal tumors, pancreatic neuroendocrine tumors, and metastatic renal cell carcinoma.<sup>10</sup> However, sunitinib therapy has also been associated with hepatotoxicity—sometimes fatal—as well as nephrotoxicity, both of which pose significant threats to patient safety. Several clinical case reports have documented sunitinib-induced liver and kidney injury.<sup>11</sup> This review aims to examine the hepatotoxicity pathways of CCl<sub>4</sub> and sunitinib, addressing their mechanisms, histopathological findings, and translational implications.

## Materials and Methods

### Hepatotoxicity Induced by Carbon Tetrachloride (CCl<sub>4</sub>)

#### Trichloromethyl Radical ( $\bullet\text{CCl}_3$ ) and Peroxyl Radicals: The Initiating Events of CCl<sub>4</sub> Hepatotoxicity

CCl<sub>4</sub> undergoes hepatic metabolism primarily through CYP2E1 and other cytochrome P450 enzymes, producing the trichloromethyl radical ( $\bullet\text{CCl}_3$ ). In the presence of oxygen, this radical rapidly transforms into a highly reactive peroxyl radical ( $\bullet\text{OOCCL}_3$ ). These reactive intermediates covalently bind to nucleic acids, proteins, and lipids, leading to DNA damage, protein dysfunction, and disturbances in lipid metabolism. Consequently, fatty degeneration (steatosis) and hepatocellular injury occur.<sup>12-17</sup>

#### Lipid Peroxidation, Oxidative Stress, and Mitochondrial Dysfunction in CCl<sub>4</sub> Hepatotoxicity

Free radicals generated during CCl<sub>4</sub> metabolism initiate lipid peroxidation, thereby compromising cell membrane integrity. Additionally, increased production of reactive oxygen species (ROS) contributes to oxidative stress. Free radicals also trigger the opening of mitochondrial permeability transition (mPT) pores, increasing intracellular calcium levels. This results in decreased mitochondrial membrane potential, impaired oxidative phosphorylation, and activation of apoptosis/necrosis pathways. The release of cytochrome c and other low-molecular-weight solutes into the cytoplasm further accelerates cell death.<sup>16,18-21</sup>

#### Hepatocellular Necrosis, Fibrosis, and the Inflammatory Response Associated with CCl<sub>4</sub>

The most characteristic histopathological findings following CCl<sub>4</sub> exposure include hepatocellular necrosis and fat accumulation. Severe cases may progress to fibrosis and inflammation. Furthermore, CCl<sub>4</sub> activates Kupffer cells, promoting neutrophil infiltration, ROS release, and inflammatory signaling. These



processes exacerbate hepatocyte damage, and when the regenerative capacity is exceeded, acute liver failure may ensue. At high exposure levels, systemic toxicity—such as central nervous system depression and respiratory failure—may develop, leading to death. <sup>12-15,18,19</sup>

### **Translational Research with CCl<sub>4</sub>: From Animal Models to Clinical Implications**

CCl<sub>4</sub> is one of the best-characterized xenobiotics known to induce free radical-mediated hepatotoxicity. For this reason, it has become one of the most commonly employed experimental models for studying both acute and chronic liver injury. In particular, rats serve as the most suitable model owing to their biological similarity to humans and ease of accessibility. Consequently, CCl<sub>4</sub> is widely used as a standard model to study drug toxicity, evaluate hepatoprotective agents, and investigate liver regeneration. <sup>7,12-14,19</sup>

### **Results**

#### **Hepatotoxicity Induced by Sunitinib**

##### **Clinical Use**

Sunitinib is an orally available multitarget tyrosine kinase inhibitor (TKI) with significant therapeutic efficacy. It is approved for the treatment of metastatic renal cell carcinoma, imatinib-resistant gastrointestinal stromal tumors, and pancreatic neuroendocrine tumors. <sup>12,13</sup>

##### **Clinical Findings**

Sunitinib can cause unexpected toxic effects, including potentially fatal hepatotoxicity. A meta-analysis of 5,658 patients reported elevated liver enzyme levels in 40% of cases. <sup>15</sup> Another meta-analysis revealed hepatic dysfunction in 33% of patients with primary hepatocellular carcinoma and 23% of patients with gastrointestinal stromal tumors. <sup>22,23</sup> Furthermore, 3–4% of patients receiving sunitinib developed liver injury, and 0.3% experienced liver failure. A population-based cohort study indicated that alanine transaminase (ALT) levels doubled in 7.4–9.3% of patients, whereas bilirubin levels increased in 8.6–18.1% of patients. <sup>23,24</sup> Clinical cases of liver failure following sunitinib administration have been documented. <sup>23,25</sup> In addition, the U.S. Food and Drug Administration (FDA) issued a black box warning concerning rare but life-threatening hepatotoxicity associated with sunitinib therapy. <sup>10</sup>

##### **Hepatotoxic Mechanisms**

Studies suggest that mitochondrial damage, inhibition of glycolysis, and metabolic activation play key roles in sunitinib-induced hepatotoxicity. <sup>24</sup> Sunitinib exerts its therapeutic effects primarily by inhibiting multiple receptor tyrosine kinases involved in cell growth and angiogenesis. These targets include VEGF receptors (VEGFR-1, VEGFR-2, VEGFR-3), PDGF receptors (PDGFRA, PDGFRB), c-KIT, FLT3, CSF-1R, and RET. <sup>26</sup> In skeletal muscle, glucose uptake occurs via glucose transporters and can be triggered through both insulin-dependent and contraction-mediated mechanisms. During muscle contraction, nitric oxide synthase (NOS) becomes activated, increasing the production of nitric oxide (NO), a crucial mediator of exercise-induced glucose uptake. Inhibition of NOS impairs this process. By blocking VEGFR, sunitinib reduces NOS activity and NO production, thereby decreasing glucose uptake in muscle cells. Moreover, VEGFR signaling is also mediated through protein kinase C (PKC). Sunitinib indirectly suppresses this pathway, further impairing calcium-dependent glucose uptake. Thus, both insulin-dependent and insulin-independent glucose uptake mechanisms are disrupted. <sup>26-28</sup> The precise mechanisms underlying sunitinib-associated hepatotoxicity remain incompletely understood. Nonetheless, studies have demonstrated that sunitinib disrupts oxygen production, decreases the

mitochondrial membrane potential, and increases mitochondrial ROS generation. These findings, observed at clinically relevant concentrations, suggest that mitochondrial dysfunction is a central contributor to sunitinib-induced hepatotoxicity.<sup>29</sup>

### **Paradoxical Compounds: Hepatotoxicity Associated with Long-Term Use of Oleanolic Acid**

Clinically, liver injury related to traditional medicine accounts for 25.7% of all drug-induced liver injuries.<sup>30</sup> Paradoxically, traditional medicines and their extracts, which are generally considered hepatoprotective, may also possess hepatotoxic potential. Among various herbal constituents, triterpenoids have been reported to exhibit both hepatoprotective and hepatotoxic properties.<sup>31</sup> Oleanolic acid, a triterpenoid present in many medicinal plants, has been shown to exert hepatoprotective effects at low doses, whereas reports indicate that high doses may lead to cholestasis.<sup>32</sup> Furthermore, oleanolic acid has the capacity to reprogram the liver to protect against various hepatotoxic agents; however, at high doses, it becomes hepatotoxic.<sup>33</sup>

### **Discussion**

#### **Common Pathways in Experimental Hepatotoxicity Models: Carbon Tetrachloride, Sunitinib, and the Paradoxical Effects of Oleanolic Acid**

All three agents can trigger oxidative stress, mitochondrial dysfunction, and inflammatory responses, thereby converging on common pathways in the development of hepatotoxicity. CCl<sub>4</sub> induces hepatocellular damage through cytochrome P450-mediated free radical production; sunitinib exerts its hepatotoxic effect primarily via inhibition of the mitochondrial respiratory chain, whereas oleanolic acid, at high doses, overwhelms antioxidant defenses, leading to excessive reactive oxygen species (ROS) generation and cholestasis. Despite their distinct origins (a chemical toxin, an anticancer drug, and an herbal compound), these agents converge on overlapping pathways, leading to hepatocellular injury.

#### **Translational Implications and Clinical Relevance**

Findings from experimental hepatotoxicity models provide direct translational insights into clinical practice. The CCl<sub>4</sub> model demonstrates that oxidative stress, lipid peroxidation, and mitochondrial dysfunction lie at the core of hepatocellular injury, mechanisms that also overlap with clinical cases of sunitinib-associated hepatotoxicity. Similarly, the example of oleanolic acid reveals that herbal compounds traditionally regarded as hepatoprotective may paradoxically cause hepatotoxicity upon high-dose or long-term use, highlighting the need to reassess the safety profile of herbal products. Thus, the convergence of these three agents on common mechanistic pathways underscores the predictive value of experimental findings for clinical hepatotoxicity, not only in terms of drug safety but also in terms of the use of herbal products, while offering a translational framework for the early diagnosis, prevention, and management of liver injury.

### **Limitations**

Limitations of this work include reliance on animal models and literature-derived data, which do not always predict human hepatotoxic responses owing to interspecies differences in metabolism and toxicity concordance.<sup>34</sup> In particular, idiosyncratic or immune-mediated forms of drug-induced liver injury are difficult to reproduce in standard animal models, limiting the ability to model some clinically relevant hepatotoxic mechanisms.<sup>35</sup> To improve translational relevance, future studies should experimentally validate our findings via human-relevant systems (e.g., primary human hepatocytes, 3D liver organoids, or humanized mouse models) and



incorporate mechanistic biomarkers and clinically grounded dose regimens.<sup>36</sup> Such experimental validation will be critical before extrapolating these results to humans and informing safe dose selection in clinical contexts.<sup>37</sup>

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## SILYMARIN AND HEPATOPROTECTION IN VETERINARY MEDICINE

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### Abstract

Silymarin, a flavonolignan complex extracted from *Silybum marianum* (milk thistle), has been widely studied for its hepatoprotective properties in both human and veterinary medicine. Its bioactive compounds, including silibinin, isosilybin, silychristin, and silydianin, exhibit potent antioxidant, anti-inflammatory, antifibrotic, and membrane-stabilizing activities. These effects are primarily mediated through the scavenging of free radicals, the inhibition of lipid peroxidation, and the modulation of intracellular signaling pathways related to apoptosis and inflammation. In veterinary practice, hepatic disorders represent a significant clinical challenge, particularly in dogs and cats with chronic hepatitis, toxic hepatopathies, and cholestatic diseases. Several experimental and clinical studies have demonstrated that silymarin supplementation improves hepatic enzyme profiles (ALT, AST, ALP, GGT), enhances glutathione concentrations, and promotes hepatocellular regeneration. Moreover, in ruminants, silymarin has been reported to mitigate aflatoxin-induced hepatotoxicity, reduce oxidative stress markers, and improve overall productivity parameters such as milk yield and feed efficiency. Despite its promising effects, the bioavailability of silymarin is limited due to poor water solubility and extensive enterohepatic circulation. Recent advancements, such as complexation with phosphatidylcholine (phytosome technology) and nanoparticle-based formulations, have significantly improved absorption and clinical efficacy in veterinary patients. Overall, silymarin represents a valuable phytopharmaceutical agent in the management of hepatopathies in companion and farm animals. However, further controlled clinical trials are needed to establish optimal dosing regimens, long-term safety, and potential drug–herb interactions, particularly in patients receiving concurrent hepatotoxic or immunosuppressive therapies.

**Keywords:** Silymarin, Hepatoprotection, Veterinary medicine, Oxidative stress, Liver disease

### INTRODUCTION

Liver diseases represent a significant concern in veterinary medicine because of the central role of organs in metabolism, detoxification, and immune regulation. The liver is a primary target for toxic agents, infectious pathogens, and metabolic disturbances in both companion animals and livestock. Hepatic disorders can lead to decreased productivity in production animals, chronic illness in companion animals, and, in severe cases, death. Among the various therapeutic strategies explored to protect and restore hepatic function, phytochemicals have gained increasing interest because of their wide safety margins and multifaceted mechanisms of action. Silymarin, a flavonolignan complex extracted from the seeds of *Silybum marianum* (milk thistle), is one of the most extensively studied plant-derived hepatoprotective agents.

Hepatotoxic insults in veterinary species arise from diverse causes, including mycotoxins (aflatoxin B1), drugs (acetaminophen, phenobarbital, carprofen), plants (pyrrolizidine alkaloids), infectious diseases (leptospirosis, viral hepatitis in dogs), and metabolic disorders such as hepatic lipidosis in cats or ketosis in cattle.<sup>1–3</sup> In food-producing animals, aflatoxicosis remains one of the most devastating liver diseases, resulting

in economic losses and food safety concerns.<sup>4</sup> Likewise, in small animals, chronic hepatopathies are increasingly diagnosed via advanced imaging and biomarker technologies.<sup>5</sup> Current treatment options often rely on supportive therapy, nutritional management, and withdrawal of the offending agent. However, few pharmacological therapies directly protect hepatocytes or enhance regeneration.

Silymarin has drawn attention because of its pleiotropic effects, including antioxidant, anti-inflammatory, antifibrotic, immunomodulatory, and regenerative properties.<sup>6</sup> These properties make it an attractive adjunct in the management of liver disorders across species. Furthermore, its favorable safety profile has led to its inclusion in veterinary nutraceutical formulations marketed for both companion and farm animals.<sup>7</sup>

### Phytochemistry of Silymarin

Silymarin is a standardized extract obtained from the seeds (achenes) of *Silybum marianum*, a member of the *Asteraceae* family. The primary bioactive constituents are flavonolignans, which are unique secondary metabolites formed by the oxidative coupling of a flavonoid and a phenylpropanoid lignan. The total silymarin complex typically contains 65–80% flavonolignans and approximately 20–35% fatty acids and other polyphenolic compounds.<sup>8</sup> The major flavonolignans in silymarin include silybin (silibinin, ~50–60%), which is the most pharmacologically active component. Isosilybin (A and B, ~5%), Silychristin (~20%), Silydianin (~10%), and Taxifolin (a flavanone, ~2–3%).<sup>9</sup>

Silybin is considered the primary determinant of hepatoprotective activity because of its potent antioxidant and membrane-stabilizing effects.<sup>8</sup> Structural analyses revealed that the flavonolignan skeleton allows free radical scavenging and metal chelation while also interacting with cellular membranes to prevent lipid peroxidation. The phytochemical content of silymarin extracts can vary depending on the plant genotype, cultivation conditions, extraction methods, and standardization protocols.<sup>10</sup> For veterinary applications, this variability raises concerns about the consistency and reproducibility of therapeutic effects. Commercial veterinary formulations often contain mixtures of silymarin with other hepatoprotective agents, such as phosphatidylcholine, vitamin E, or N-acetylcysteine, to enhance bioavailability and synergistic activity.<sup>11</sup> Recent advances in analytical chemistry, such as HPLC–MS/MS, have enabled precise quantification of individual flavonolignans and have facilitated pharmacokinetic studies in animals.<sup>12</sup> Moreover, nanoparticle-based delivery systems, liposomal encapsulation, and complexation with phosphatidylcholine (silipide) have been developed to overcome the challenges of poor solubility and bioavailability.<sup>13</sup>

### Pharmacokinetics and Bioavailability of Silymarin in Animals

The pharmacokinetics of silymarin in veterinary species have been less extensively studied than those in humans, but the available data provide valuable insights. Silymarin has poor water solubility, resulting in low oral bioavailability.<sup>14</sup> In monogastric animals such as dogs and cats, absorption is limited, with peak plasma concentrations occurring within 2–4 h post-administration.<sup>15</sup> Moreover, silymarin undergoes extensive enterohepatic recirculation, prolonging its systemic half-life but also complicating dose optimization.<sup>16</sup> To address bioavailability issues, several formulations have been developed, including silymarin-phosphatidylcholine complexes (phytosomes), nanoparticle-based systems, and self-emulsifying drug delivery systems, which have shown increased absorption in experimental models.<sup>13</sup> In ruminants, the ruminal degradation of polyphenolic compounds poses an additional challenge. However, encapsulation technologies and controlled-release formulations are being investigated to overcome these barriers and ensure effective delivery to



the target organ, the liver.<sup>17</sup> Comparative studies indicate species-specific variations; for example, silymarin is rapidly metabolized and excreted in poultry<sup>18</sup>, whereas in dogs, hepatic metabolism through glucuronidation plays a dominant role.<sup>19</sup> Understanding these pharmacokinetic differences is essential for tailoring therapeutic regimens in veterinary practice.

### Hepatoprotective Mechanisms of Action

The hepatoprotective effects of silymarin are mediated through multiple molecular pathways that act synergistically to preserve liver function.

**Antioxidant Activity:** Silymarin scavenges free radicals and increases the expression of endogenous antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, and catalase.<sup>20</sup> This antioxidant defense is crucial in preventing the oxidative damage associated with toxins, drugs, and infectious agents.

**Anti-inflammatory effects:** Silymarin modulates the NF- $\kappa$ B signaling pathway, reducing the production of proinflammatory cytokines such as TNF- $\alpha$ , IL-1 $\beta$ , and IL-6.<sup>21</sup> This effect is particularly relevant in chronic hepatitis, where persistent inflammation drives fibrosis.

**Antifibrotic Properties:** Silymarin inhibits hepatic stellate cell activation, a key step in the progression of hepatic fibrosis. The downregulation of transforming growth factor-beta (TGF- $\beta$ 1) and collagen synthesis has been documented in both experimental and clinical studies.<sup>22</sup>

**Membrane Stabilization:** By interacting with hepatocyte plasma membranes, silymarin prevents entry of toxins, including amanitin from *Amanita phalloides* and various xenobiotics.<sup>23</sup>

**Regenerative Effects:** Silymarin stimulates ribosomal RNA polymerase I activity, enhancing hepatocyte protein synthesis and promoting tissue regeneration.<sup>24</sup>

These multifaceted mechanisms highlight the potential of silymarin as a versatile hepatoprotective agent applicable to a wide spectrum of veterinary diseases.

### Applications in Small Animal Practice

In dogs and cats, liver disease, ranging from toxic hepatopathies to chronic hepatitis and lipidosis, is a common clinical problem.

**Canine hepatopathies:** Studies in dogs with chronic hepatitis have demonstrated improved liver enzyme profiles (ALT, ALP, AST) following silymarin supplementation, suggesting hepatocellular protection and improved metabolic function.<sup>25</sup> Randomized clinical evidence supports the use of a combined silybin + S-adenosylmethionine product (Denamarin®) to reduce lomustine (CCNU)-associated hepatopathy in tumor-bearing dogs: dogs receiving concurrent Denamarin had smaller increases in ALT, AST, ALP and bilirubin and were less likely to require treatment delay or discontinuation.<sup>26,27</sup>

**Feline hepatic lipidosis:** Cats with hepatic lipidosis, a condition often associated with obesity and anorexia, benefit from silymarin through both antioxidant support and stimulation of hepatocellular regeneration. While clinical trials remain limited, case reports suggest enhanced recovery when silymarin is combined with supportive therapy.<sup>28</sup>

**Drug-induced hepatotoxicity:** Silymarin has been used to counteract hepatotoxicity caused by drugs such as ketoconazole, phenobarbital, and NSAIDs in small animals. Its protective effects are attributed to both antioxidant and detoxifying pathways.<sup>29</sup> Experimental and toxicological studies in cats have revealed that silymarin has hepatoprotective effects on acetaminophen (APAP)-induced hepatotoxicity models: silymarin and

N-acetylcysteine have been shown to have protective effects on biochemical markers and oxidative stress endpoints in controlled feline experiments. These data support silymarin as a potential adjunct in selected feline hepatotoxicoses, although clinical trials in spontaneously affected cats are scarce.<sup>11</sup>

### **Applications in Farm Animals**

Ruminant species such as cattle, sheep, and goats are highly vulnerable to hepatotoxic insults, particularly from dietary mycotoxins, plant alkaloids, and parasitic infections. Silymarin has been evaluated both experimentally and clinically as a supportive hepatoprotective therapy in these species.

### **Aflatoxicosis**

Aflatoxin B1 contamination of feed is a major cause of chronic hepatopathy in dairy and beef cattle. Studies have demonstrated that dietary supplementation with silymarin reduces serum transaminases, improves antioxidant enzyme activity, and significantly lowers aflatoxin residues in milk.<sup>30,31</sup> This dual role — protecting hepatocytes and reducing toxin carry-over into animal products — is of particular importance for food safety.

### **Fascioliasis and Parasitic Hepatopathies**

Liver fluke (*Fasciola hepatica*) infestation induces chronic cholangiohepatitis and fibrosis in cattle and sheep. The antifibrotic and antioxidative effects of silymarin attenuate collagen deposition and preserve hepatic architecture.<sup>32</sup> Supplementation has also been linked to improved weight gain and feed conversion efficiency in infected animals.<sup>33</sup>

### **Metabolic Stress in Dairy Cows**

Periparturient dairy cows undergo substantial metabolic stress, predisposing them to hepatic lipidosis and ketosis. Experimental supplementation with silymarin extract during transition periods has been shown to reduce  $\beta$ -hydroxybutyrate levels, improve liver function biomarkers, and enhance reproductive performance.<sup>31</sup> These findings suggest a role for silymarin in preventive and therapeutic contexts.

### **CONCLUSION**

Silymarin, which has multiple pharmacological properties owing to its unique flavonolignan composition, represents one of the most promising phytotherapeutic agents in veterinary internal medicine. In companion and food-producing animals, it consistently exerts hepatoprotective effects through antioxidant, anti-inflammatory, antifibrotic, and regenerative mechanisms while maintaining an excellent safety profile. Evidence from experimental models and clinical applications supports its role in mitigating toxin-induced liver injury, enhancing recovery in metabolic and infectious hepatopathies, and improving overall animal health and productivity. Importantly, its capacity to reduce aflatoxin carry-over in milk and meat highlights not only its therapeutic relevance but also its importance in safeguarding food safety and public health.

Despite these encouraging findings, several gaps remain. The bioavailability of conventional formulations is limited, leading to variable clinical outcomes. Advances in delivery systems, such as phytosome complexes and nanotechnology-based formulations, offer promising solutions to enhance absorption and therapeutic efficacy in different species. However, standardized dosing regimens, species-specific pharmacokinetics, and long-term safety profiles remain insufficiently defined. Controlled multicenter clinical trials in naturally affected animals are urgently needed to validate experimental findings and establish evidence-based guidelines for clinical practice.



In conclusion, silymarin should be regarded as more than a supportive nutraceutical; it is an evolving pharmacological tool with the potential to become an integral component of veterinary hepatology. Its integration into preventive health programs, therapeutic protocols, and even food animal production systems could redefine approaches to managing liver disease in veterinary medicine. Future research must aim not only to clarify its clinical utility but also to unlock its translational potential as a bridge between nutraceutical innovation and evidence-based veterinary care.

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## BREAKING THE FIBROSIS–INFLAMMATION AXIS: THE ROLE OF RESVERATROL AND TYPE 3 IMMUNITY IN LIVER DISEASES

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### Abstract

The liver is one of the most complex organs in the body because of its metabolic, immunological, and detoxification functions. During the progression of chronic liver diseases, fibrosis and inflammation form a self-perpetuating cycle. While fibrosis leads to hepatic dysfunction, the inflammatory response accelerates this process, contributing to the development of cirrhosis and hepatocellular carcinoma. Therefore, disrupting the fibrosis–inflammation axis has emerged as a critical therapeutic target. Resveratrol, a natural polyphenol, has gained considerable attention as a potential therapeutic agent in liver diseases because of its antioxidant and anti-inflammatory properties. Recent studies have demonstrated that resveratrol modulates type 3 immune responses by suppressing Th17 cell differentiation and IL-17 production, thereby attenuating inflammation. Moreover, it may promote hepatocyte regeneration and tissue repair through IL-22–mediated mechanisms. Both *in vitro* and *in vivo* studies have shown that resveratrol inhibits hepatic stellate cell activation, reduces oxidative stress, and protects hepatocytes. Collectively, these findings highlight resveratrol as a promising candidate for regulating immune responses and controlling fibroinflammatory processes. Considering its regulatory effects on type 3 immunity, resveratrol has potential as a novel therapeutic strategy for chronic liver diseases.

**Keywords:** Liver fibrosis, Type 3 immunity, Resveratrol, Inflammation, Hepatoprotection

### Introduction

The liver is one of the most complex organs in the human body because of its essential roles in metabolism, immunological regulation, and detoxification. In chronic liver diseases, fibrosis and inflammation create a continuous self-amplifying cycle. Progressive fibrosis leads to hepatic dysfunction, while the inflammatory response accelerates this process, ultimately resulting in severe conditions such as cirrhosis and hepatocellular carcinoma. Consequently, disrupting the fibrosis–inflammation axis has become a critical target for the development of novel therapeutic strategies. The roles of natural compounds such as resveratrol and specific immune response subsets in this axis have attracted significant research attention in recent years. 1,2

Chronic liver diseases, including metabolic, alcoholic, viral, or autoimmune disorders, are characterized by persistent inflammation that creates a pathogenic link between parenchymal cell death and the progression of fibrosis to end-stage cirrhosis. In response to repeated liver injury, interactions between different populations of myeloid and lymphoid cells stimulate the activation of hepatic stellate cells (HSCs), which are the primary fibrogenic cells of the liver.3-5 Consequently, HSCs acquire a myofibroblastic phenotype, accumulate at sites of injury, produce extracellular matrix components of altered composition, and secrete cytokines and chemokines that sustain the inflammatory process.4-6 Although recent studies have focused primarily on innate immune responses and the roles of macrophage subsets in fibrogenesis, both experimental models and human studies have demonstrated that specific T-cell subsets—particularly Th17 cells—are critical sources of proinflammatory and fibrogenic mediators.3,5,7 Th17 cells produce a wide range of cytokines, including interleukin (IL)-17A,

interferon-gamma (IFN- $\gamma$ ), and granulocyte–macrophage colony–stimulating factor (GM-CSF), which contribute to fibroinflammatory signaling.<sup>5,8,9</sup> The hepatic fibrogenic process is tightly regulated by coordinated interactions between fibrogenic and microenvironmental cells, including parenchymal and immune cells. In addition to immune cells, hepatocytes and HSCs play central roles in the immune regulation of liver fibrosis because of their ability to secrete cytokines and chemokines that perpetuate immune cell recruitment and sustain inflammatory responses.<sup>3,5</sup> One of the key drivers of liver fibrosis is the development of type 3 inflammation, characterized by the production of IL-17A and GM-CSF, which target both hepatic myofibroblasts and hepatocytes while promoting the polarization of macrophages toward a profibrogenic phenotype.<sup>3,5,10</sup>

### **Biological Properties of Resveratrol**

Resveratrol (RES; 3,4',5-trihydroxy-trans-stilbene) is a natural polyphenolic compound abundantly found in grapes, peanuts, and various other plants.<sup>11-15</sup> Numerous studies have reported that RES has antioxidant and anti-inflammatory effects by reducing lipid peroxidation and DNA damage.<sup>15,16</sup> After absorption in the human body, RES undergoes phase II metabolism in the liver, where it is converted into trans-resveratrol-3-O-glucuronide and trans-resveratrol-3-sulfate via conjugation enzymes.<sup>15,17</sup> Approximately 75% of orally ingested RES is absorbed by the small intestine and transported to the liver through the bloodstream, where it undergoes biotransformation, which is primarily mediated by cytochrome P450 enzymes, into its active glucuronidated form, trans-resveratrol.<sup>15,18</sup> Through its strong antioxidant and anti-inflammatory properties, RES has demonstrated potential in reducing reactive oxygen species (ROS) accumulation, inhibiting inflammatory cytokine release, and protecting hepatocytes against injury. Furthermore, RES modulates key molecular signaling pathways, including the NF- $\kappa$ B, SIRT1, and Nrf2 pathways, which are associated with its hepatoprotective effects at the molecular level.<sup>19,20</sup>

### **Type 3 Immunity and Liver Diseases**

Type 3 immunity is mediated primarily by Th17 cells and their associated cytokines, including IL-17 and IL-22. While this immune response plays a protective role against pathogens, particularly at mucosal surfaces, its dysregulated activation can drive chronic inflammation and promote the progression of fibrosis. In liver diseases, IL-17 has been shown to increase inflammatory cell infiltration, thereby accelerating fibrogenesis, whereas IL-22 plays dual roles by promoting tissue repair and hepatocyte regeneration while simultaneously contributing to inflammation in certain contexts. Thus, type 3 immunity serves as a central regulator in both the progression of chronic liver diseases and as a promising therapeutic target for future treatments.<sup>21,22</sup>

### **Interaction Between Resveratrol and Type 3 Immunity**

In recent years, increasing attention has been given to the immunomodulatory effects of resveratrol on type 3 immune responses. Studies have demonstrated that RES inhibits Th17 cell differentiation and IL-17 production, thereby suppressing proinflammatory signaling cascades and reducing tissue damage. Additionally, RES may enhance IL-22–mediated hepatocyte regeneration, contributing to tissue repair and recovery.<sup>23,24</sup> This dual regulatory role positions RES as a promising candidate for disrupting the fibrosis–inflammation axis and improving therapeutic outcomes in chronic liver diseases.

### ***In Vivo* and *In Vitro* Studies**

Multiple *in vitro* studies have demonstrated the hepatoprotective effects of RES across various cell lines. In human hepatic myofibroblasts, transresveratrol has been shown to dose-dependently inhibit cell proliferation. Additionally, RES induces apoptosis in human hepatic LX-2 stellate cells.<sup>14,25</sup>



Another study reported that RES effectively protects hepatocytes from oxidative stress by increasing the activities of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), NADPH quinone oxidoreductase, and glutathione-S-transferase (GST).<sup>14,26</sup> Moreover, RES has been shown to protect cells from hydroquinone-induced toxicity, potentially via its antioxidant activity and ability to suppress cytochrome P450 2E1 expression.<sup>14,27</sup> Studies involving hepatic stellate cells and Kupffer cells revealed that RES treatment regulates their activity, highlighting its potential as a therapeutic intervention against liver injury.<sup>14</sup> High concentrations of RES were also found to prevent apoptosis in primary mouse hepatocytes and to increase cell viability in a dose-dependent manner.<sup>27</sup> In hepatocytes isolated from rats, RES was shown to inhibit short-chain fatty acid synthesis—particularly palmitic acid formation—and suppress triglyceride synthesis.<sup>28</sup> Additionally, comparative studies between RES and silymarin pretreatment demonstrated that RES effectively reduced tert-butyl hydroperoxide (t-BHP)-induced apoptotic and necrotic markers, suggesting that RES confers significant protection against oxidative stress-induced hepatotoxicity.<sup>29</sup> The protective and therapeutic effects of resveratrol on fibrosis and inflammation have been demonstrated in several rat models. For example, in dimethylnitrosamine (DMN)-induced liver fibrosis, oral administration of resveratrol at 20 mg/kg/day significantly decreased the serum ALT, AST, ALP, and bilirubin levels while reducing type I collagen mRNA expression, hepatic hydroxyproline content, and  $\alpha$ -SMA—positive hepatic stellate cell (HSC) activation and preserving hepatic glutathione and antioxidant defenses. <sup>30</sup> Similarly, in carbon tetrachloride (CCl<sub>4</sub>)-induced liver injury, resveratrol suppressed the production of proinflammatory cytokines such as TGF- $\beta$ , IL-6, and TNF- $\alpha$ ; modulated the NF- $\kappa$ B, PI3K/Akt, and PTEN signaling pathways; enhanced autophagy; protected hepatocytes; and reduced fibrotic tissue accumulation. <sup>31,32</sup> Moreover, in a thioacetamide (TAA)-induced fibrosis model, the administration of 30 mg/kg resveratrol inhibited NF- $\kappa$ B activation and oxidative stress marker expression, improved liver function, and significantly decreased the histological fibrosis score. <sup>33</sup> Collectively, these findings indicate that resveratrol exerts potent *in vivo* antifibrotic and anti-inflammatory effects by targeting HSC activation, inflammatory cytokine production, oxidative stress, and hepatocyte apoptosis, thereby effectively disrupting the fibrosis–inflammation axis.

## Conclusion

Breaking the fibrosis–inflammation axis represents a strategic therapeutic goal for preventing the progression of chronic liver diseases. Resveratrol, through its potent antioxidant, anti-inflammatory, and immunomodulatory properties, has emerged as a promising candidate in this context. Preclinical findings strongly support the hepatoprotective effects of RES, particularly through the regulation of type 3 immune responses. An evaluation of the modulation of Th17-driven cytokine pathways revealed that resveratrol has significant potential in the development of novel therapeutic strategies for chronic liver diseases.

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# EVALUATION OF BINDING PROPENSITIES OF PLANT-DERIVED COMPOUNDS TO L-TYPE AND T-TYPE VOLTAGE-DEPENDENT CALCIUM CHANNELS THROUGH MOLECULAR DOCKING: A COMPARISON WITH AMLODIPINE

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## Abstract

This study aimed to investigate *in silico* the binding propensities and potential binding modes of plant-derived compounds (carvacrol, thymol, eugenol, oleuropein) to the CaV1.2 and CaV3.2 subtypes compared with the antihypertensive calcium channel blocker amlodipine. Receptor structures were obtained and prepared from RCSB PDB (CaV1.2: 8WE8, CaV3.2: 9AYG). Potential binding pockets were scanned via CB-Dock2; the pockets yielding the best scores for amlodipine were selected, and grids were defined accordingly (CaV1.2: C4, CaV3.2: C5; with an additional +5 Å safety margin for dimensions). Ligands were retrieved from PubChem in 3D-SDF format, protonated at pH 7.4 via Open Babel, assigned Gasteiger charges, and converted to PDBQT. Blind docking simulations were performed with AutoDock Vina v1.2.7 using uniform parameters (exhaustiveness = 8, num\_modes = 10, seed = 12345). The best modes were converted into 2D interaction maps via the BIOVIA Discovery Studio Visualizer. According to Vina, the binding free energies ( $\Delta G$ , kcal·mol<sup>-1</sup>) for CaV1.2/C4 were as follows: oleuropein -8.010, amlodipine -6.339, eugenol -5.599, carvacrol -5.592, thymol -5.177; and for CaV3.2/C5, oleuropein -6.817, amlodipine -6.078, carvacrol -5.792, thymol -5.476, eugenol -4.939. Oleuropein consistently yielded better/similar scores than amlodipine did for both targets, demonstrating notable superiority, particularly for CaV1.2 ( $\Delta\Delta G$  s  $\approx -1.2$ ). 2D maps revealed that oleuropein is stabilized by multiple H-bonds (Ser/Asp/Asn/Gln) and  $\pi$ -interactions, whereas the binding of phenolic monoterpenes (carvacrol/thymol/eugenol) involves primarily hydrophobic/ $\pi$ -alkyl characteristics. The larger volume of the C4 pocket ( $\sim 2.9 \times 10^3$  Å<sup>3</sup>) explains the more advantageous binding of bulky ligands (oleuropein/amlodipine) in C4, whereas the more compact C5 is relatively superior for smaller phenolics. Oleuropein emerged as a prominent compound in calcium channel subtypes—especially in CaV1.2—exhibiting binding energies surpassing those of the reference amlodipine and a robust network of H-bonds and  $\pi$ -stacking. These findings provide pharmacophore insights (multiple H-bond donor/acceptor motifs + aromatic surfaces) for rational derivative design. Validation of selected poses through molecular dynamics simulations and electrophysiological tests will contribute to elucidating subtype selectivity.

**Keywords:** Amlodipine, Antihypertensive, Calcium channel blockers, Molecular docking, Oleuropein

## Introduction

As the global burden of hypertension continues to increase for veterinary and human health, calcium channel blockers (CCBs) remain the cornerstone of first-line treatment interventions. Hypertension constitutes a significant public health concern, affecting approximately 1.28 billion adults aged 30--79 worldwide, with a relatively high prevalence observed in low- and middle-income countries <sup>1</sup>. Calcium channel blockers represent one of the long-standing and widely prescribed drug classes in the management of hypertension <sup>2,3</sup>. Dihydropyridine-class calcium channel blockers, in particular, are favored as first-line treatment options because of their potent blood pressure-lowering effects and favorable tolerability profiles <sup>4,5</sup>. Amlodipine, a representative of the dihydropyridine class, is extensively utilized in clinical practice owing to its prolonged duration of action,

robust peripheral vasodilation, and proven efficacy reflected in cardiovascular outcomes<sup>6,7</sup>. The influx of Ca<sup>2+</sup> ions through L-type voltage-gated Ca<sup>2+</sup> channels (CaV1.2; CACNA1C) in vascular smooth muscle is indispensable for intracellular Ca<sup>2+</sup> loading and the generation of vascular tone; pharmacological or genetic suppression of this channel is intimately associated with a reduction in arterial blood pressure<sup>8,9</sup>. Structural investigations have elucidated the specific binding pocket for dihydropyridines in CaV1.2 and the conformations of its activation-inactivation gates, thereby supporting rational design and repositioning approaches<sup>10</sup>. Conversely, T-type channels (particularly CaV3.2) play pivotal roles in diverse physiological processes, including neuronal excitability and thalamocortical rhythmicity; these channels have been intensively investigated as pharmacological targets because of their unique contributions to cardiovascular autonomic control and analgesia<sup>11,12</sup>. For plant-derived secondary metabolites such as carvacrol (*Origanum spp.*), thymol (*Thymus/Trachyspermum ammi*), eugenol (*Syzygium aromaticum*), and oleuropein (*Olea europaea*), reports indicate vascular smooth muscle relaxation, hypotensive/antihypertensive responses, and, in some studies, the suppression of Ca<sup>2+</sup> influx. For example, carvacrol has been shown to induce dose-dependent blood pressure reduction in normotensive rats, and its ability to diminish calcium channel currents has been suggested<sup>13,14</sup>. Thymol has demonstrated both *in vivo* hypotensive effects<sup>15</sup> and attenuated Ca<sup>2+</sup>-dependent contraction in vascular smooth muscle<sup>16</sup>. Eugenol facilitates vasodilation in cerebral and mesenteric arteries, primarily by inhibiting smooth muscle contraction and/or sensory neuron calcium influx<sup>17</sup>. Olive leaf/oleuropein preparations, on the other hand, have exhibited antihypertensive effects in experimental models, with CCB-like mechanisms proposed<sup>18</sup>. These preliminary findings warrant a comparative assessment of the capacity of these natural compounds to bind to the same receptor pockets as a clinically established CCB (amlodipine). In this context, our study systematically compared the binding propensities of four phytochemicals (carvacrol, thymol, eugenol, oleuropein) with amlodipine in cardiac/vascular L-type CaV1.2 and neuronal T-type CaV3.2 channels via AutoDock Vina-based molecular docking; the binding free energies for the best binding conformations were summarized, and key contacts were qualitatively discussed via 2D interaction diagrams. The current version of Vina is widely adopted for hypothesis generation because of its multiple scoring functions and flexible usage options<sup>19</sup>. This study, while acknowledging the established clinical value of CCBs, aims to compare the rational binding potential of plant-derived small molecules to the same targets *in silico*.

## Materials and Methods

### Data Sources and Software/Environment

The CaV1.2 (L-type, Protein Data Bank (PDB): 8WE8) and CaV3.2 (T-type, PDB: 9AYG) structures were obtained from the RCSB Protein Data Bank<sup>20</sup>. Ligands (amlodipine, carvacrol, thymol, eugenol, and oleuropein) were downloaded from PubChem in 3D-SDF format<sup>21</sup>. All operations were performed on Windows 10; the software and versions utilized were Open Babel 3.1<sup>22</sup>, CB-Dock2 (web server)<sup>23</sup>, AutoDock Vina v1.2.7<sup>24</sup>, and BIOVIA Discovery Studio Visualizer (DSV, 21.1)<sup>25</sup>.

### Target protein preparation

From the downloaded PDB files, water/cofactor and complex ligand HETATM (heteroatom) records were removed, retaining only protein ATOM records (representing single-chain receptors). Polar hydrogen atoms were added to the receptors via Open Babel, Gasteiger partial charges were assigned, and the files were converted to PDBQT format (rigid receptor)<sup>22</sup>.

### Ligand Preparation

Ligands were retrieved from PubChem records (3D-SDF); protonation settings were adjusted to pH 7.4 via Open Babel; Gasteiger charges were assigned, and they were converted to PDBQT format<sup>21,22</sup>.



Determination of Binding Pockets and Grid Setup

Potential binding cavities were automatically scanned via CB-Dock2, and the most favorable pockets for amlodipine were selected: C4 in CaV1.2 and C5 in CaV3.2 <sup>23</sup>. The center and box dimensions from CB-Dock2 outputs were transferred to Vina grid parameters; a +5 Å safety margin was added to each axis (e.g., for CaV1.2/C4, center 148/139/179 Å; dimensions 34/24/23 Å → 39/29/28 Å) <sup>23</sup>.

Molecular Docking Protocol

Docking procedures were executed via AutoDock Vina v1.2.7 (scoring: vina; exhaustiveness = 8, num\_modes = 10, seed = 12345; CPU = 4). The best binding mode was selected on the basis of the most negative ΔG (kcal·mol<sup>-1</sup>) value; Vina logs were preserved <sup>24</sup>.

Interaction Analysis of Best Poses

For each target–ligand pair, the best pose was converted into a 2D ligand–receptor interaction diagram via DSV; hydrogen bond, hydrophobic, and π-interactions were depicted (H-bond distance threshold ≤3.6 Å; hydrophobic ≤4.5 Å; π-π/π-alkyl detections enabled).

Results

Prescreening (CB-Dock2)

Multiple cavities were identified in both targets via CB-Dock2; for amlodipine, the CaV1.2-C4 and CaV3.2-C5 pockets emerged as the most favorable locations (Figure 1 & 2). This screening provided approximate volume/geometry information and qualitative rankings of the pockets; detailed energy comparisons were conducted with AutoDock Vina docking simulations performed on a fixed grid (Table 1).

In CaV1.2/C4, oleuropein yielded the best score (−8.217), surpassing the reference amlodipine (−6.929); eugenol, carvacrol, and thymol exhibited weaker binding (−5.78...−5.20) (Figure 3). In CaV3.2/C5, oleuropein (−6.833) and carvacrol (−6.167) produced relatively strong values, while those of amlodipine were −6.084, thymol −5.670, and eugenol −5.116 (Figure 4). The complete set of data can be found in Table 2.

Table 1. Best binding free energy values (kcal/mol) of ligands on CaV1.2 (C4) and CaV3.2 (C5).

| Ligand     | CaV1.2 (C4) | CaV3.2 (C5) |
|------------|-------------|-------------|
| Amlodipin  | −6.5        | −6.8        |
| Carvacrol  | −5.8        | −5.4        |
| Thymol     | −5.1        | −5.2        |
| Eugenol    | −5.5        | −4.8        |
| Oleuropein | −8.1        | −6.5        |

Table 2. Vina binding free energy (ΔG, kcal·mol<sup>-1</sup>)

| Ligand     | CaV1.2 | CaV3.2 |
|------------|--------|--------|
| Amlodipine | −6.929 | −6.084 |
| Carvacrol  | −5.880 | −6.167 |
| Thymol     | −5.195 | −5.670 |
| Eugenol    | −5.776 | −5.116 |
| Oleuropein | −8.217 | −6.833 |

### Comparison Relative to Amlodipine Reference ( $\Delta\Delta G$ and Binding Fold)

Differences in binding free energy differences ( $\Delta\Delta G$ ) and estimated binding fold values calculated relative to amlodipine are provided in Tables 3–4. Briefly, in CaV1.2/C4, oleuropein is predicted to be approximately  $8.8\times$  stronger with a  $-1.288\text{ kcal}\cdot\text{mol}^{-1}$  advantage (Figure 5); in CaV3.2/C5, it is approximately  $3.5\times$  stronger with a  $-0.749\text{ kcal}\cdot\text{mol}^{-1}$  advantage. Carvacrol is close to amlodipine in CaV3.2/C5 ( $\Delta\Delta G = -0.083$ ;  $\approx 1.15\times$ ) (Figure 6), whereas thymol and eugenol are inferior to the reference in both targets.

### Representative Binding Modes (2D Interaction Maps)

In the Discovery Studio 2D diagrams, amlodipine exhibited  $\pi$ -alkyl/ $\pi$ -donor contributions around the dihydropyridine core and H-bonds (Ser/Lys, etc.) in the side chain. Oleuropein displayed multiple H-bonds via numerous hydroxyl and ester/ether oxygens, along with  $\pi$ -interactions from aromatic surfaces; this profile is consistent with the negative  $\Delta G$  values. Carvacrol/Thymol/Eugenol predominantly presented hydrophobic and  $\pi$ -alkyl networks alongside 1–2 H-bonds. The relevant visuals are provided in supplementary figures 7 - 9 (with Vina  $\Delta G$  values indicated for each).

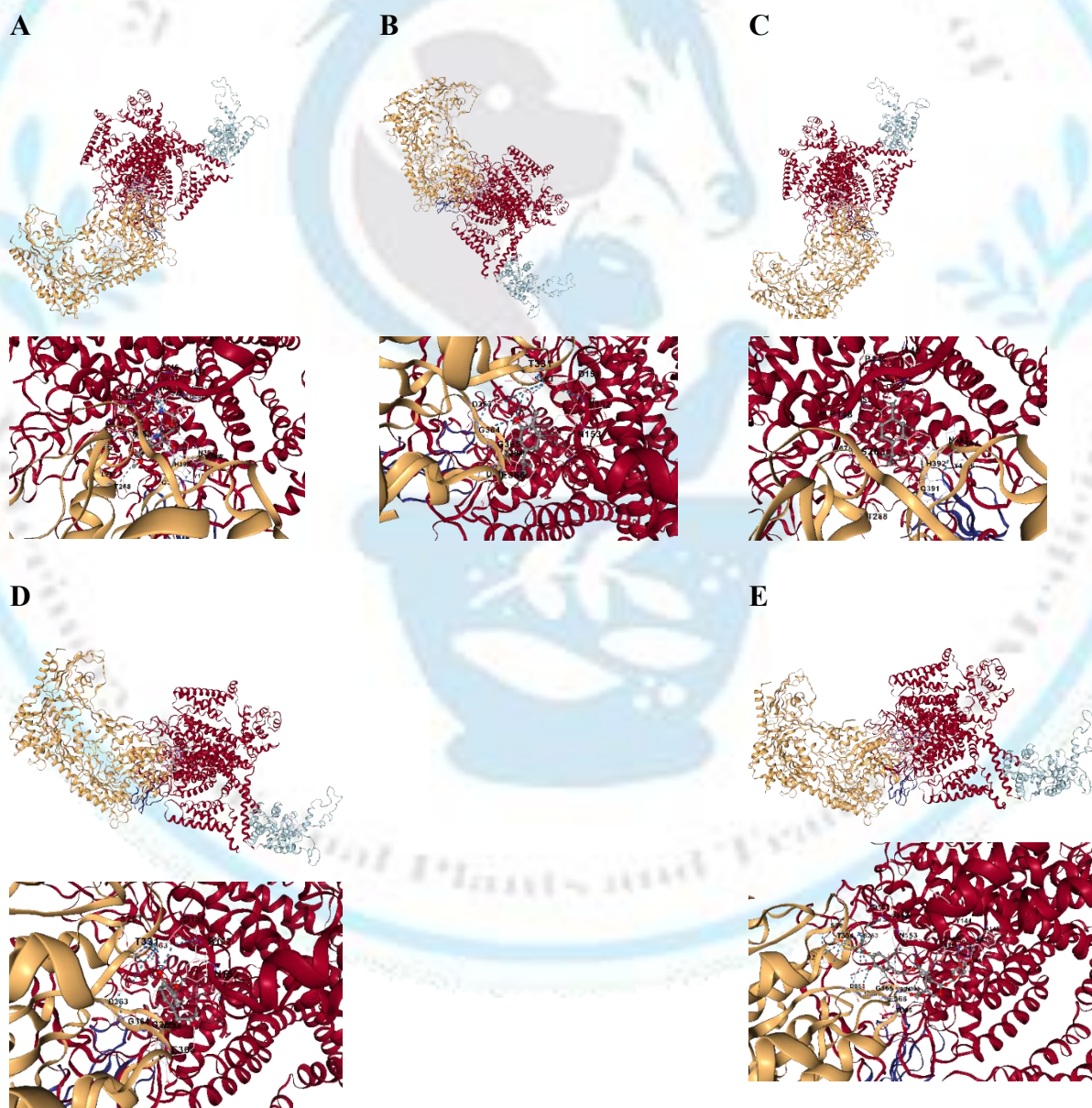


**Figure 1.** The best docking poses of (A) amlodipine, (B) carvacrol, (C) thymol, (D) eugenol, and (E) oleuropein on CaV1.2.



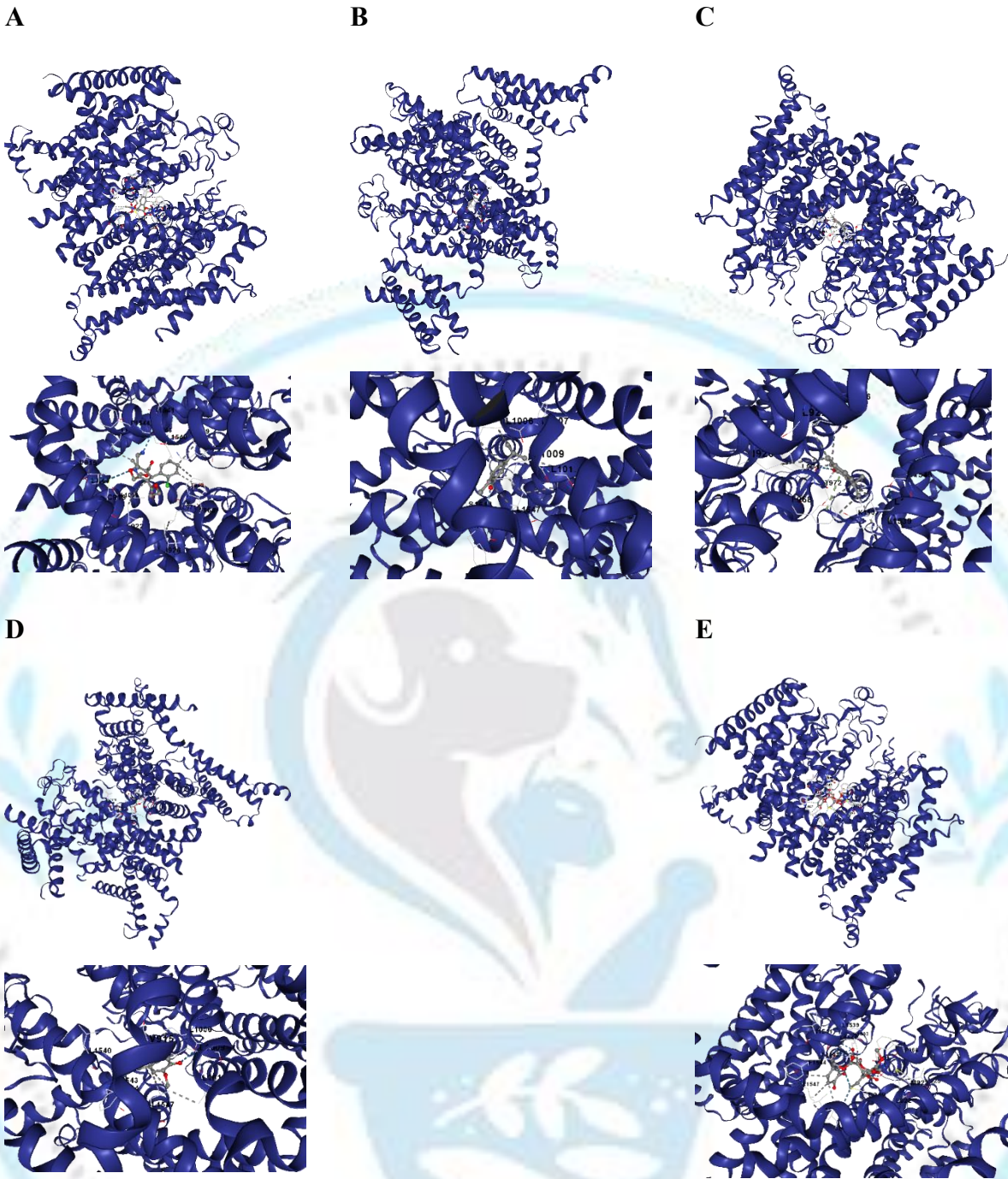


**Figure 2.** (A) Amlodipine, (B) carvacrol, (C) thymol, (D) eugenol, (E) oleuropein's best docking pose on CaV3.2.

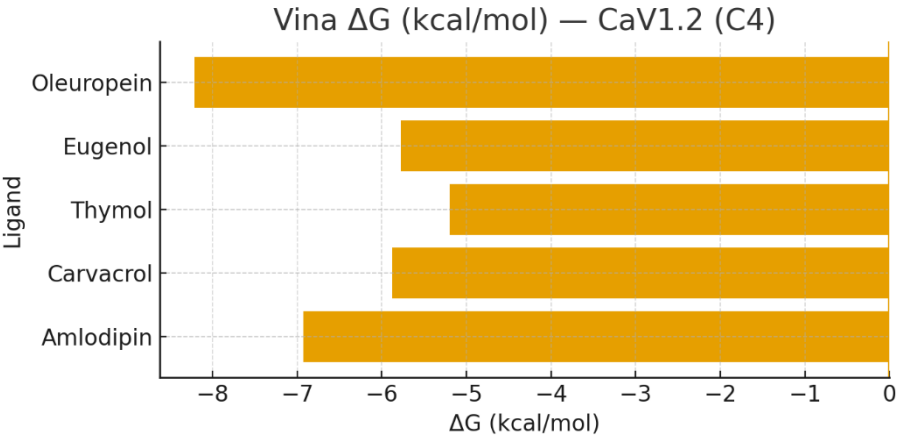


**Figure 3.** Vina binding free energy CaV1.2 ( $\Delta G$ , kcal/mol)

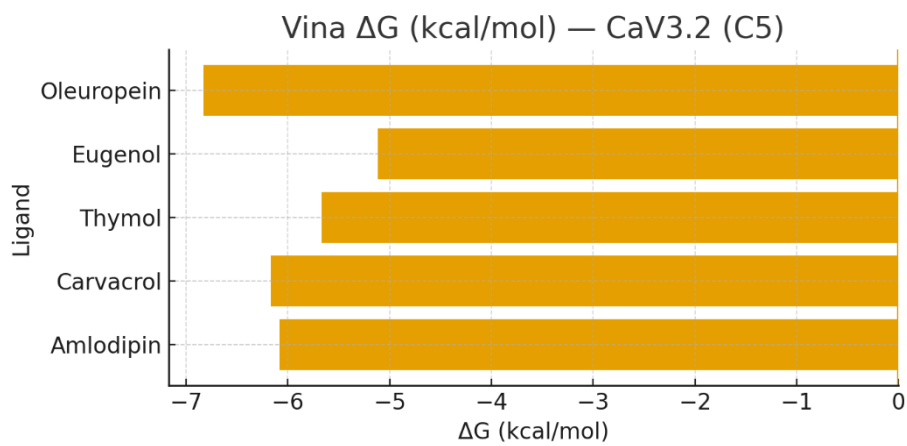


Figure 4. Vina binding free energy CaV3.2 (ΔG, kcal/mol)

Table 3. CaV1.2 ΔΔG and “fold” calculations (relative to amlodipine).

| Ligand     | ΔG (kcal/mol) | ΔΔG vs Amlodipin (kcal/mol) | Predicted binding (fold) | Rank (ΔG) |
|------------|---------------|-----------------------------|--------------------------|-----------|
| Oleuropein | -8.217        | -1.288                      | 8.790                    | 1         |
| Amlodipin  | -6.929        | 0.000                       | 1.000                    | 2         |
| Carvacrol  | -5.880        | 1.049                       | 0.170                    | 3         |
| Eugenol    | -5.776        | 1.153                       | 0.143                    | 4         |
| Thymol     | -5.195        | 1.734                       | 0.054                    | 5         |

Table 4. CaV3.2 ΔΔG and “fold” calculations (relative to amlodipine).

| Ligand     | ΔG (kcal/mol) | ΔΔG vs Amlodipin (kcal/mol) | Predicted binding (fold) | Rank (ΔG) |
|------------|---------------|-----------------------------|--------------------------|-----------|
| Oleuropein | -6.833        | -0.749                      | 3.541                    | 1         |
| Carvacrol  | -6.167        | -0.083                      | 1.150                    | 2         |
| Amlodipin  | -6.084        | 0.000                       | 1.000                    | 3         |
| Thymol     | -5.670        | 0.414                       | 0.498                    | 4         |
| Eugenol    | -5.116        | 0.968                       | 0.195                    | 5         |

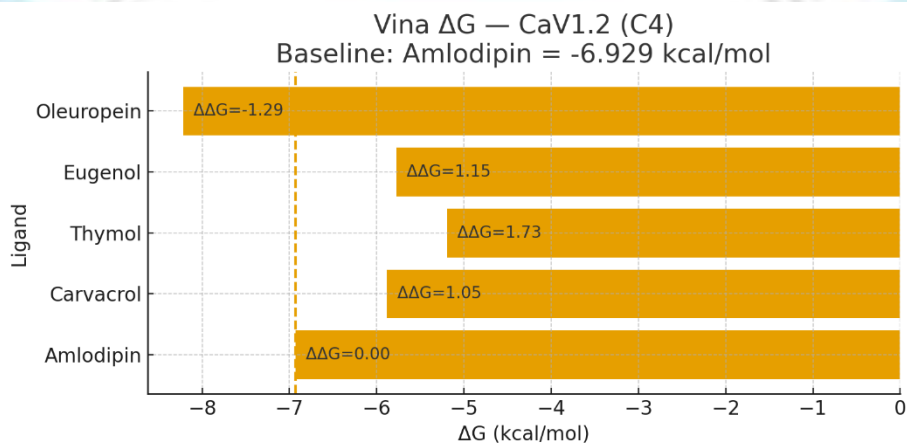


Figure 5. CaV1.2 ΔΔG calculations.



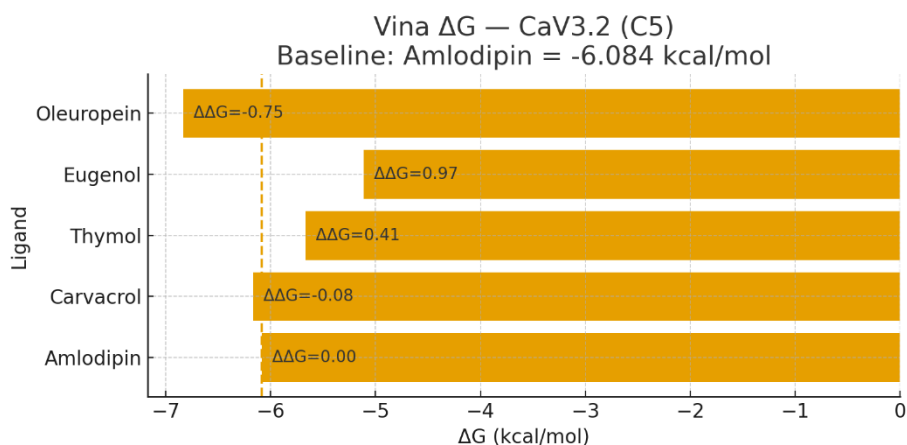


**Figure 6.** CaV3.2  $\Delta\Delta G$  calculations.

### Pocket volume and chemical compatibility

According to the geometry derived from CB-Dock2, the C4 (CaV1.2) pocket ( $\sim 2.9 \times 10^3 \text{ \AA}^3$ ) is larger than the C5 (CaV3.2;  $\sim 1.4 \times 10^3 \text{ \AA}^3$ ) pocket; this explains the superior performance of bulky/multifunctional ligands such as oleuropein and amlodipine in C4, whereas smaller phenolics remain competitive in C5.

### Robustness Check

The root mean square deviation (RMSD) (lb/ub) values in the Vina output table ranged from 1–6 Å for most complexes; the high RMSD values for carvacrol and thymol in CaV1.2/C4 suggest the possibility of alternative orientations.

### Discussion

This study, which employs docking simulations conducted under identical receptor pocket and fixed grid conditions, demonstrated that oleuropein significantly outperforms amlodipine in CaV1.2/C4 ( $\Delta G = -8.217 \text{ kcal} \cdot \text{mol}^{-1}$ ) and surpasses the reference in CaV3.2/C5 ( $-6.833$  vs.  $-6.084$ ). The observed energy differences are consistent with the combination of multiple H-bonds and  $\pi$ /hydrophobic packing evident in the 2D interaction maps; notably, oleuropein appears to establish a distributed polar network through its phenolic and ester/ether oxygens while enhancing packing via its aromatic surfaces. This pattern aligns with cryo-EM findings indicating that dihydropyridines are stabilized in L-type channels by polar anchor points within a hydrophobic cavity<sup>26</sup>.

The observed trends for phytochemicals also correlate with reported vasodilator/antihypertensive effects in veterinary and human medicine: eugenol has been shown to dilate cerebral arteries by inhibiting voltage-dependent  $\text{Ca}^{2+}$  channels in smooth muscle cells<sup>27</sup>, carvacrol to induce dose-dependent hypotension in normotensive rats<sup>28</sup>, thymol to reduce blood pressure<sup>29</sup>, and olive leaf/oleuropein to exhibit channel antagonism-like effects via calcium channel blockade<sup>30</sup>. This preliminary pharmacology rationalizes the prominence of oleuropein in our computational findings and the near-reference position of carvacrol in CaV3.2/C5.

Pocket volume and geometric differences aid in explaining the direction of the results: CB-Dock2 scans suggest that C4 ( $\sim 2.9 \times 10^3 \text{ \AA}^3$ ) is larger and richer in polar nodes than C5 ( $\sim 1.4 \times 10^3 \text{ \AA}^3$ ); consequently, multifunctional and bulky scaffolds (oleuropein/amlodipine) are more favorably positioned in C4, whereas smaller aromatic cores (carvacrol/thymol) can remain relatively competitive in C5<sup>31</sup>. In parallel,  $\Delta\Delta G$  differences calculated relative to the amlodipine reference indicate an advantage for oleuropein of  $\sim -1.29 \text{ kcal} \cdot \text{mol}^{-1}$  ( $\approx 8.8\times$ ) in CaV1.2/C4 and  $\sim -0.75 \text{ kcal} \cdot \text{mol}^{-1}$  ( $\approx 3.5\times$ ) in CaV3.2/C5.

However, the interpretation of molecular docking results inherently requires caution. Docking relies on a static receptor representation; protein flexibility, membrane dielectric, and aqueous/ionic environments are covered in a limited manner; scoring functions provide approximate free energy estimations<sup>32,33</sup>. In particular, the high RMSD values observed for carvacrol and thymol in CaV1.2/C4 ( $\approx 12\text{--}16\text{ \AA}$ ) suggest the presence of alternative orientations and necessitate rescoring across the pose ensemble (e.g., smina/AD4) and validation with MM/GBSA or more advanced chemical free energy methods<sup>32,34</sup>. Furthermore, in absolute  $\Delta G$  comparisons, the magnitude effect (the tendency of larger molecules to yield more negative  $\Delta G$ ) is well known; therefore, reporting normalized metrics such as ligand efficiency (LE) and especially lipophilic ligand efficiency (LLE) is recommended<sup>35,36</sup>.

In conclusion, the model proposed herein demonstrates that oleuropein possesses a distinct advantage in the L-type channel, predicated on a balance of multiple H-bonds and  $\pi$ -packing; conversely, the carvacrol scaffold can achieve a near-reference position in the compact/hydrophobic pocket of the T-type channel. These hypotheses warrant experimental validation via patch-clamp and site-directed mutagenesis, as well as computational corroboration through MD/MM-GBSA.

### Conclusion

Comparative docking simulations performed under identical pocket conditions revealed that oleuropein has a  $\Delta G$  profile that significantly surpasses that of amlodipine, particularly in CaV1.2/C4, while also maintaining strong binding in CaV3.2/C5. 2D interaction maps revealed that this superiority is attributable to the synergy of multiple H-bonds and  $\pi$ /hydrophobic contributions. These findings suggest that an oleuropein-centric pharmacophore can be directed toward L-type selectivity through the modulation of volume/lipophilicity, whereas the carvacrol scaffold offers a rational starting point for optimization in the T-type channel. Computational inferences necessitate support from independent scoring and dynamic/free energy methods, alongside electrophysiological validation, to ensure translational validity<sup>26,31-35</sup>.

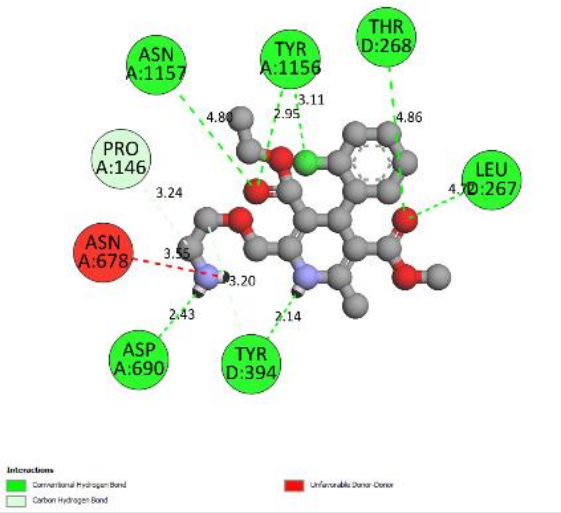
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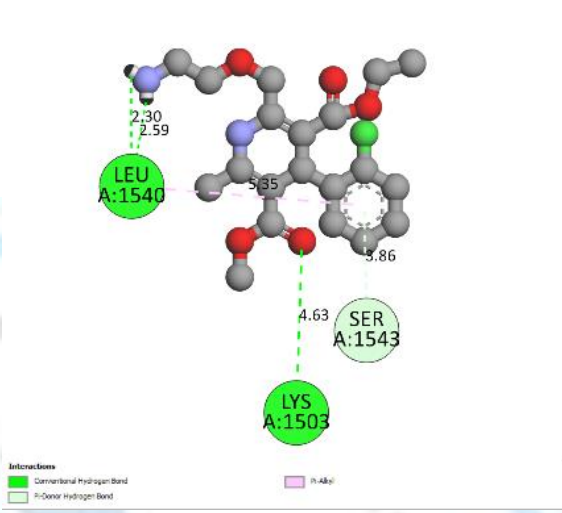


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A

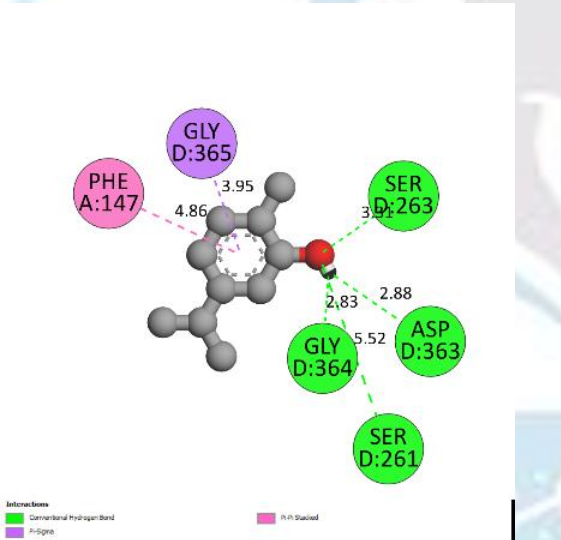


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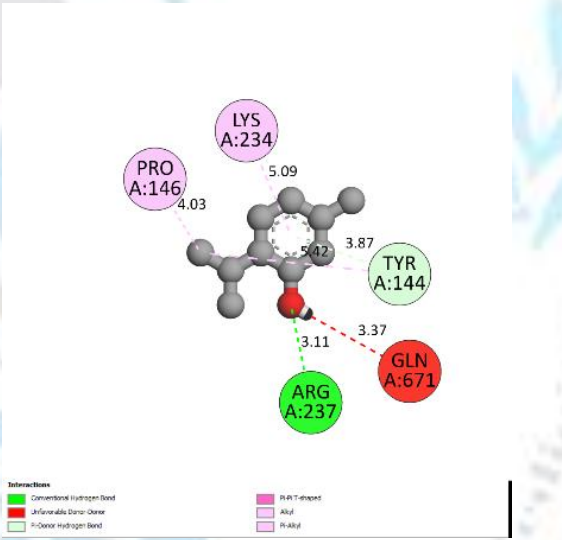


Supplementary Figure 7. (A) CaV1.2/C4 (B) CaV3.2/C5 interaction schemes for amlodipine.

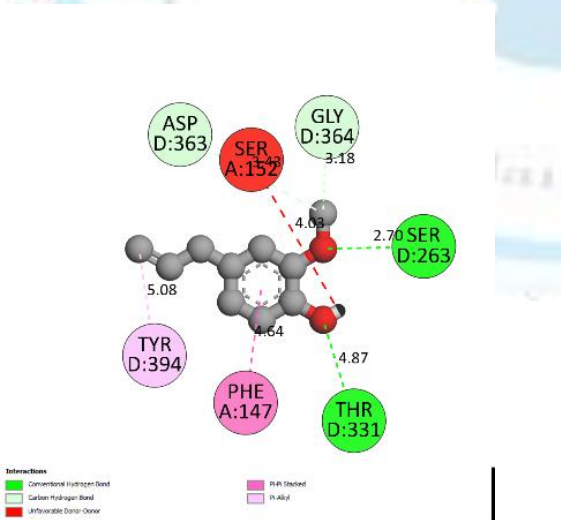
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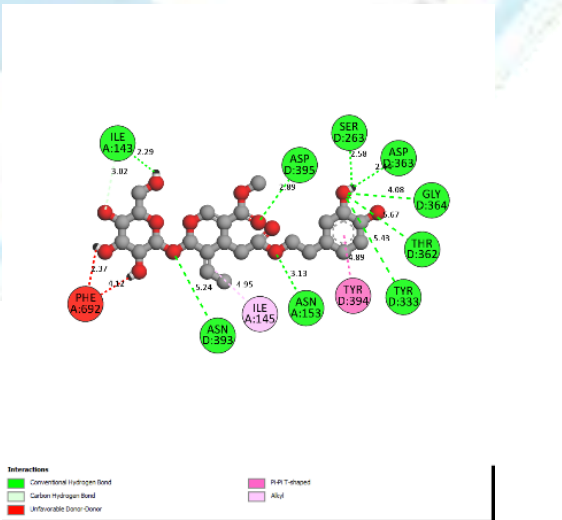
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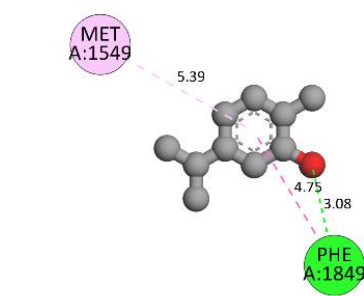
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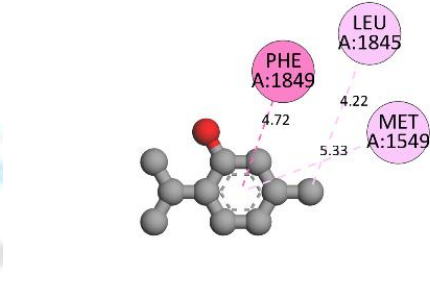
Supplementary Figure 8. CaV1.2/C4 interaction schemes for (A) carvacrol, (B) thymol, (C) eugenol, and (D) oleuropein 2B.



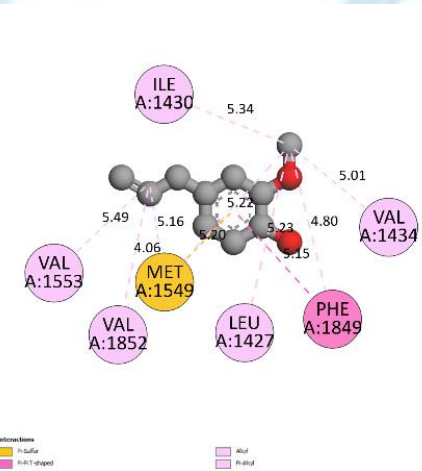
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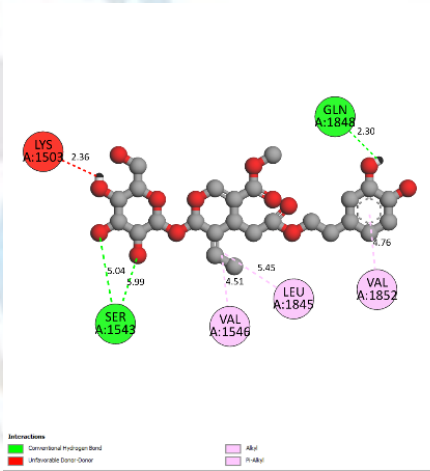
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**Supplementary Figure 9.** CaV3.2/C5 interaction schemes for (A) carvacrol, (C) thymol, (D) eugenol, and (E) oleuropein 2B.

## EPIGENETICS AND AGING: FROM EXPERIMENTAL RAT MODELS TO EPIGALLOCATECHIN GALLATE (EGCG)-SUPPORTED PHYTOTHERAPEUTIC GERIATRIC STRATEGIES

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### Abstract

Aging is a complex process characterized by a decline in biological functions, increased frailty, and a greater prevalence of age-related diseases. Genetic, environmental, and particularly epigenetic mechanisms play crucial roles in this process. DNA methylation, histone modifications, and noncoding RNAs are at the core of aging biology and serve both as biomarkers of aging and potential causal factors of age-related disorders. The concept of the epigenetic clock has enabled the estimation of biological age independently of chronological age, representing a breakthrough in aging research. Experimental animal models, especially the D-galactose-induced aging model, reproduce key mechanisms such as oxidative stress, mitochondrial dysfunction, and chronic inflammation, facilitating the study of epigenetic alterations. Among phytotherapeutic agents, epigallocatechin gallate (EGCG), a major polyphenol in green tea, stands out not only for its potent antioxidant properties but also for its epigenetic regulatory potential through effects on DNA methyltransferases and histone deacetylases. EGCG has also been reported to modulate microRNA expression, thereby interfering with molecular pathways involved in aging, apoptosis, and cell proliferation. Epigenetic biomarkers and modulatory drugs appear promising for the development of personalized therapeutic strategies in geriatric medicine. In conclusion, targeting epigenetic mechanisms may contribute to prolonging healthspan and preventing age-related diseases, providing a strategic approach to healthy aging.

**Keywords:** Aging, Epigallocatechin gallate (egcg), Experimental rat models, Geriatric strategies, Phytotherapy

### Introduction

Aging is an inevitable biological process characterized by a decline in biological functions and physical performance due to changes at the tissue and organ levels that occur with increasing chronological age.<sup>1</sup> As chronological age increases, molecular and cellular damage gradually accumulates under the influence of environmental, genetic, and epigenetic factors, leading to anatomical and functional changes in many organ systems. With increasing age, the incidence of diseases such as dementia, cardiovascular disorders, diabetes, and cancer increases, as does the number of elderly patients admitted to hospitals due to adverse health outcomes associated with these conditions.<sup>2</sup> Moreover, age-related loss of functional capacity and frailty increase dependency rates, emphasizing the need for long-term care.<sup>3</sup> In addition to causing health problems for individuals, aging imposes a significant burden on families and society, increasing healthcare costs and imposing a considerable economic burden on countries. In Türkiye and around the world, both the number and proportion of the elderly population are rapidly increasing. According to the World Health Organization, by 2050, individuals aged 65 and over constitute 16% of the global population, making the understanding of aging biology



a strategic public health priority.<sup>4</sup> In Türkiye, the elderly population reached 10.6% as of 2024, placing age-related health issues among the top priorities in national health policies.<sup>5</sup>

### Overview of the Role of Epigenetics in Aging

Epigenetics is the field that studies mechanisms regulating gene expression and inducing heritable changes without altering the DNA sequence. DNA methylation, histone modifications, and noncoding RNAs play central roles in this process.<sup>6</sup> Epigenetic regulation is crucial for maintaining cellular identity, directing developmental processes, and adapting to environmental factors. These mechanisms undergo systematic changes with age, leading to disruptions that play a decisive role in the development of age-related diseases. These alterations are considered both indicators and potential drivers of aging, forming the foundation of the concept of "epigenetic aging".<sup>7</sup>

The history of epigenetics dates back to the 1940s with Conrad Waddington's "epigenetic landscape" theory and gained momentum in the late 20th century with advances in molecular biology.<sup>8</sup> Research on epigenetic aging began in the 1960s when Wilson and colleagues demonstrated global DNA hypomethylation in aging mouse tissues. In the 2000s, Fraga et al.'s study on monozygotic twins revealed significant epigenetic divergence over time.<sup>9</sup> The concept of the "epigenetic clock," developed by Horvath and Hannum in 2013, enabled highly accurate measurement of biological age and marked a turning point in aging research.<sup>10</sup> Studies have shown that epigenetic changes are not merely biomarkers of aging but also play critical roles in the pathogenesis of age-related diseases such as Alzheimer's disease, Parkinson's disease, metabolic syndrome, sarcopenia, and immunosenescence.<sup>11,12</sup>

Today, epigenetic aging is considered highly important in understanding the pathogenesis of age-related diseases and in developing new therapeutic approaches. A growing number of recent studies have prioritized the clinical application of epigenetic biomarkers and the development of geroprotective therapies. This article discusses age-related epigenetic changes, explains core mechanisms, explores disease associations, and evaluates translational implications and future perspectives.

### Aging and Epigenetic Mechanisms

#### DNA Methylation

DNA methylation, which involves the addition of a methyl group to the 5th carbon of cytosine nucleotides, is one of the fundamental epigenetic mechanisms that regulate gene expression. It is one of the most well-characterized epigenetic changes in aging. DNA methylation is responsible for silencing oncogenes, regulating genes involved in cell differentiation and proliferation, genomic imprinting, and X chromosome inactivation.<sup>13</sup> In the human genome, DNA methylation predominantly occurs at CpG dinucleotides, which are often clustered in CpG islands located in gene promoter regions. Methylation of CpG islands in promoters and the loss of methylation in repetitive regions can activate these regions and cause genomic instability. During aging, epigenetic information changes sporadically in response to exogenous and endogenous factors. Two main patterns are observed during aging: global DNA hypomethylation and locus-specific hypermethylation.<sup>14</sup> Global hypomethylation leads to genomic instability and chromosomal abnormalities, whereas regional hypermethylation in CpG islands results in the silencing of tumor suppressor genes.<sup>15</sup>

As a heritable and stable repressive epigenetic marker, DNA methylation is catalyzed by the DNA methyltransferase (DNMT) enzyme family. Mutations in DNMT1 have been associated with neurodegenerative

disorders such as hereditary sensory autonomic neuropathy with dementia and hearing loss.<sup>16</sup> In cancer, DNA hypomethylation activates oncogenes and initiates chromosomal instability, whereas hypermethylation silences tumor suppressor genes. Hypermethylated CpG islands have been identified in various tumor tissues, including bladder, breast, endometrial, gastric, glioblastoma, hepatocellular, lung, ovarian, pancreatic, prostate, and renal cell cancers; leukemia; melanoma; duodenal adenocarcinoma; adrenocortical carcinoma; and neuroblastoma.<sup>17-19</sup> Studies in elderly individuals have shown hypomethylation in repetitive DNA regions such as LINE-1 s, whereas hypermethylation of promoter regions (e.g., p16<sup>INK4a</sup>) suppresses genes involved in cell cycle regulation.<sup>20</sup> This epigenetic reprogramming is considered both a marker and a potential driver of aging.

### Histone Modifications

Gene regulation involves not only DNA methylation but also histone proteins, which are crucial for genome organization and dynamics. Posttranslational modifications of the N-terminal tails of histones are involved in gene transcription, chromatin organization, DNA repair, and replication and represent another key epigenetic mechanism. Histone acetylation, methylation, and phosphorylation regulate chromatin structure and gene expression. During aging, redistribution of histone modifications, loss/gain of heterochromatin domains, and chromatin condensation occur. Aged tissues exhibit a more repressive chromatin state characterized by condensed heterochromatin, widespread transcriptional silencing, and reduced long-range chromatin interactions.<sup>21</sup> Histone acetylation levels on H3 and H4 have been reported to decrease or increase depending on the region.<sup>22</sup> Increased histone deacetylase (HDAC) activity contributes to transcriptional repression and impaired cellular stress responses. Alterations in histone H3K27 trimethylation shape age-associated gene expression profiles.<sup>23</sup> Reprogramming these modifications significantly impacts cellular renewal and regenerative capacity.

### Noncoding RNAs (miRNAs, lncRNAs)

MicroRNAs (miRNAs) and long noncoding RNAs (lncRNAs) are epigenetic regulators at the posttranscriptional level that play critical roles in stem cell differentiation, the cell cycle, gene expression, chromatin remodeling, cancer, apoptosis, and age-related diseases.<sup>24,25</sup> The expression patterns of miRNAs and lncRNAs change systematically with age. For example, increased expression of miR-34a has been associated with apoptosis.<sup>26</sup> Conversely, decreased levels of myogenic miRNAs in muscle cells have been linked to sarcopenia.<sup>27</sup> lncRNAs also play significant roles in chromatin remodeling and transcriptional control. For example, increased expression of HOTAIR lncRNA has been associated with age-related fibroblast dysfunction.<sup>28</sup>

### Epigenetic Clock Concept

The epigenetic clock refers to the use of DNA methylation patterns to estimate biological age. Models developed by Horvath and Hannum can accurately predict chronological age and quantitatively measure biological aging.<sup>10</sup> The pace of epigenetic aging has been associated with cardiovascular disease risk, mortality, and functional limitations.<sup>29</sup> Therefore, the epigenetic clock is a valuable biomarker in both aging biology and clinical applications.

### Experimental Aging Models

Experimental animal models play a key role in studying the biology of aging. Since examining natural aging processes is time-consuming and costly, induced aging models are often preferred in research. These models can rapidly induce aging-specific mechanisms such as oxidative stress, mitochondrial dysfunction, chronic inflammation, and epigenetic alterations.<sup>30</sup>



### **Aging models in rats**

Rats are among the most commonly used rodents in aging studies. The primary reasons include their short life cycles, well-characterized genetics, and physiological and biochemical similarities to those of humans. The main approaches for modeling aging in rats include the following:

Natural aging model: Monitoring animals until advanced age.

Pharmacological/chemical induction: Increased aging through oxidative agents or metabolic stressors.

Genetic models: Animals carrying mutations in genes associated with progeroid syndromes.<sup>31</sup>

### **D-Galactose-Induced Aging Model**

D-galactose (D-gal) is one of the most commonly used pharmacological agents in aging research.<sup>32</sup> Chronic administration of D-gal induces changes in animals that closely resemble natural aging.<sup>33-35</sup> In high amounts, D-gal cannot be fully metabolized due to the limited activity of D-galactokinase or galactose-1-phosphate uridyltransferase. The unmetabolized D-gal accumulates in cells and is converted into galactitol, a compound that causes osmotic stress and generates reactive oxygen species (ROS).<sup>33-36</sup>

Increased ROS levels cause somatic mutations in mitochondrial DNA (mtDNA), leading to errors in polypeptides encoded by mtDNA. These errors are stochastically transmitted during cell division and may impair the mitochondrial respiratory chain. This can result in further ROS production, creating a vicious cycle.<sup>33,35,37,38</sup> As a result, D-gal administration causes oxidative stress, inflammation, neurodegeneration, and immunosenescence—key phenotypes of aging. This model is widely used for understanding the molecular mechanisms of aging and testing potential therapeutic strategies.<sup>39</sup>

### **Phytotherapeutic Approaches**

#### **Antioxidant Plant-Based Compounds**

Epigallocatechin gallate (EGCG), the major polyphenol in green tea, is a prominent phytochemical in aging studies because of its strong antioxidant properties. EGCG not only directly neutralizes free radicals but also enhances the activity of endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase, and glutathione peroxidase, thereby reducing oxidative stress.<sup>40</sup> In D-gal-induced aging models, EGCG has been shown to inhibit lipid peroxidation, improve mitochondrial functions, and support cognitive performance.<sup>41</sup> Additionally, EGCG reduces the expression of inflammatory cytokines, helping to alleviate chronic inflammation.<sup>42</sup>

#### **Epigenetic Regulatory Potential**

EGCG not only is an antioxidant but also acts as an epigenetic modulator. It has been shown to inhibit DNA methyltransferases (DNMTs), thereby facilitating the re-expression of tumor suppressor genes.<sup>43</sup> EGCG also affects histone acetyltransferase (HAT) and histone deacetylase (HDAC) activity, contributing to chromatin remodeling.<sup>41</sup> Moreover, EGCG modulates the expression of certain microRNAs, playing a role in cellular aging, apoptosis, and proliferation at the epigenetic level.<sup>44</sup> These findings suggest that EGCG is a multifunctional phytotherapeutic agent capable of targeting both oxidative damage and epigenetic mechanisms involved in aging.

## **Epigenetic Alterations in Aged Rats**

### **Epigenetic Regulation in Neurodegenerative Processes such as Alzheimer's and Parkinson's Disease**

Epigenetic changes in neurodegenerative diseases play roles in disease pathogenesis and offer potential therapeutic targets. In Alzheimer's disease, methylation changes have been detected in the promoter regions of the APP and BACE1 genes, which can increase amyloid-beta production and accelerate neurodegeneration.<sup>45</sup> Additionally, reduced histone acetylation limits neuronal plasticity and contributes to cognitive decline.<sup>46</sup> BDNF gene methylation in Alzheimer's disease patients has been shown to predict treatment response with 71% sensitivity.<sup>47</sup>

In Parkinson's disease, hypomethylation of the alpha-synuclein (SNCA) gene has been reported to promote pathological protein aggregation and disease progression.<sup>48</sup> Modulating these epigenetic alterations with specific drugs is emerging as a novel therapeutic strategy for treating neurodegenerative diseases.

### **Epigenetics is linked with sarcopenia, metabolic syndrome, and immune aging**

Age-related metabolic changes are regulated via epigenetic modifications. Sarcopenia is a geriatric syndrome characterized by progressive, generalized loss of muscle mass and function.<sup>49</sup> Studies have demonstrated that changes in DNA methylation and dysregulation of miRNAs in genes responsible for myogenesis contribute to muscle aging.<sup>50</sup> In metabolic syndrome, epigenetic alterations have been reported in genes related to insulin resistance and obesity. Hypermethylation of the PGC-1 $\alpha$  gene has been shown to reduce mitochondrial biogenesis.<sup>51</sup> In immune aging (immunosenescence), meta-epigenetic changes in T cells underpin age-related immune dysfunction.<sup>52</sup> Telomere shortening and aberrant DNA methylation in T cells are associated with immunosenescence, promoting proinflammatory states and increasing susceptibility to infections in elderly individuals.<sup>53</sup>

### **Translational Implications and Clinical Reflections**

Animal models are fundamental tools for understanding aging biology. Studies in rats have revealed age-related changes in DNA methylation patterns similar to those in humans.<sup>54</sup> The rat epigenetic clock reflects phenotypic aging and is localized with histone modifications associated with heterochromatin.<sup>14</sup> However, some components of the epigenetic clock vary between species, emphasizing the need for validation with human data in translational approaches.

### **Epigenetic Modulator Drugs and Their Geroprotective Potential**

The reversible nature of epigenetic changes offers therapeutic opportunities for slowing aging or preventing diseases. Histone deacetylase (HDAC) inhibitors and DNA methyltransferase (DNMT) inhibitors are among the most extensively studied drugs in this field.<sup>55</sup>

HDAC inhibitors have been shown to enhance neuronal plasticity and support cognitive functions, whereas DNMT inhibitors normalize gene expression by correcting aberrant methylation patterns. DNMT inhibitors such as azacitidine (5-azacytidine) and decitabine (5-aza-2'-deoxycytidine) have already been approved for the treatment of myelodysplastic syndromes.<sup>56-58</sup>

Other DNMT-inhibiting agents (e.g., zebularine, procaine, hydralazine, and genistein) are being investigated in preclinical and clinical studies.<sup>59</sup> Furthermore, HDAC inhibitors (trichostatin A, valproic acid,



SAHA) and histone methyltransferase (HMT) inhibitors (Bix-01294) represent a new generation of compounds with significant potential in drug discovery.<sup>60</sup>

### Personalized Medicine and the Use of Epigenetic Biomarkers in Geriatric Clinics

Epigenetic biomarkers are used to determine an individual's biological age and disease risk. Blood, one of the most accessible tissues in the human body, is frequently utilized in studies exploring genetic–epigenetic models of disease risk. Circulating cell-free DNA (cfDNA), circulating tumor DNA (ctDNA), and mitochondrial DNA (mtDNA), which are released into the bloodstream through apoptosis, necrosis, autophagy, necroptosis, and other physiological processes, have gained attention. Recent studies suggest that quantitative analysis of these circulating molecules can help assess tumor progression and predict treatment response in various cancer types.<sup>61</sup> The mitochondrial DNA content has been significantly associated with conditions such as Ewing sarcoma, trauma, and microbial infections.<sup>62-64</sup> Circulating free mitochondrial DNA (cfmtDNA) levels in cerebrospinal fluid have also been shown to vary in Parkinson's disease.<sup>65</sup> The epigenetic clock offers a more accurate health indicator than chronological age does and allows the development of personalized treatment strategies.<sup>66</sup> Second-generation epigenetic clocks and aging pace metrics are associated with frailty and age-related disease risk.<sup>67</sup> These biomarkers have potential for identifying high-risk individuals and tracking preventive strategies. Widespread use of epigenetic profiling in geriatric practice will enable targeted therapies in older adults.

### Conclusion and Future Perspectives

Epigenetic modifications are crucial both as biomarkers and as potential therapeutic targets of aging. Changes in DNA methylation, histone modifications, and noncoding RNAs lie at the core of aging biology and are associated with neurodegenerative, metabolic, and immunological diseases. The epigenetic clock stands out as a robust biomarker for measuring biological age and predicting disease risk.

In model systems, epigenetic aging has been shown to be partially reversible. These findings suggest that interventions targeting epigenetic factors may yield clinical benefits. However, mechanistic and longitudinal intervention studies are needed to determine whether specific epigenetic changes are causal drivers or integrative markers of tissue aging.

Future research should focus on the clinical efficacy of epigenetic modulator drugs, demonstrate whether altering epigenetic age translates into meaningful clinical benefits, and explore the role of epigenetic biomarkers in personalized medicine. In this context, targeting epigenetic mechanisms may contribute to extending the healthy lifespan of older individuals.

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# THE EFFECT OF BETA-CARYOPHYLLENE ON MDA LEVELS AND SOD, GSH, AND GPX ACTIVITIES AGAINST LEAD ACETATE-INDUCED NEPHROTOXICITY

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## Abstract

In this study, an experimental nephrotoxicity model was created in rats via lead acetate (PbAc) to evaluate the effects of PbAc on oxidative stress and damage to kidney tissue. In addition, the potential protective role of beta-caryophyllene (BCP), a natural sesquiterpene, against this toxic effect was investigated. The experiment used a total of 50 adult male Sprague–Dawley rats. The animals were divided into five groups: the control group (physiologically saline), the BCP group (400 mg/kg BCP, intragastric), the PbAc group (30 mg/kg PbAc, oral), the BCP200+PbAc group (200 mg/kg BCP + 30 mg/kg PbAc), and the BCP400+PbAc group (400 mg/kg BCP + 30 mg/kg PbAc). At the end of the experiment, kidney tissue samples were collected from the animals, and malondialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH), and glutathione peroxidase (GPx) levels were measured. In the PbAc-treated group, a significant increase in the MDA content was detected, whereas a marked decrease in the SOD, GSH, and GPx activities was detected. BCP administration reduced MDA levels and increased SOD, GSH, and GPx activities. These findings suggest that BCP may exert a protective effect against PbAc-induced nephrotoxicity by suppressing oxidative stress and supporting antioxidant defense systems.

**Keywords:** BCP, Nephrotoxicity, Oxidative stress, PbAc

## Introduction

Lead (Pb) is a toxic heavy metal that is widely found in nature and can be absorbed into humans and animals through industrial and environmental sources. It is released into the environment from numerous sources, including paints, batteries, water pipes, fuel products, agricultural pesticides, and electronic waste; it easily accumulates in biological systems and can cause irreversible damage to various organ systems over time.<sup>1,2</sup> Pb toxicity causes significant damage, particularly to the hematopoietic, hepatic, neurological, and renal systems.<sup>3-6</sup> The kidneys, as the primary organs responsible for excreting Pb from the body, are the structures most affected by the accumulation of this toxic element and are thus the most affected.<sup>7</sup>

The toxic effects of Pb on renal tissue are multifaceted. The main mechanism is the destructive effect of Pb on oxidative stress. Pb ions disrupt the oxidative balance by increasing the production of reactive oxygen species (ROS), inhibiting endogenous antioxidant defense systems, and ultimately causing oxidative damage at the lipid, protein, and DNA levels.<sup>8</sup> During this process, the levels of MDA, a biomarker of lipid peroxidation, increase, whereas the levels of key antioxidant enzymes such as SOD, GSH, and GPx decrease significantly.<sup>9</sup> These biochemical alterations result in morphological changes in kidney cells, including apoptosis, tubular necrosis, and glomerular damage.<sup>8</sup>

In recent years, studies on the protective effects of natural antioxidant compounds against heavy metal toxicity have increased. One such compound, BCP, is a lipophilic sesquiterpene derivative found in plants that contain essential oils, such as black pepper (*Piper nigrum*) and clove (*Syzygium aromaticum*). The antioxidant, anti-inflammatory, and cell-protective properties of BCP have been demonstrated in various experimental models



in the literature, and this compound may have regulatory effects on oxidative stress and tissue damage.<sup>10,11</sup> However, information on the potential protective effects of BCP against PbAc-induced nephrotoxicity is quite limited. In this context, the present study aimed to evaluate the effects of BCP on the level of oxidative stress in kidney tissue in an experimental PbAc-induced nephrotoxicity model. In this context, the oxidative balance status was assessed by measuring the MDA, SOD, GSH, and GPx levels in kidney tissue, and the potential antioxidant effects of BCP were investigated at the biochemical level. These findings are expected to reveal the therapeutic potential of BCP in Pb-induced kidney damage.

### Materials and methods

Lead acetate (PbAc) ( $\geq 99\%$ , CAS No: 6080-56-4) and beta-caryophyllene (BCP, CAS No: 87--44--5) were obtained from Sigma–Aldrich Co. (St. Louis, MO, USA). Biochemical analyses were performed via ELISA kits produced by A.B.T. Laboratory Industry. The kits used were as follows: MDA (catalog no: ABT10165Ra), SOD (catalog no: ABT1626Ra), GSH (catalog no: ABT10155Ra), and GPx (catalog no: ABT2557Ra).

A total of 50 adult male Sprague Dawley rats (220–250 g) were obtained from the Atatürk University Experimental Research and Application Centre (ATADEM) (Ethics Committee Decision No: 2025/03, Decision No: 59). The experimental animals were housed in a controlled environment with a constant temperature of 25 °C °C, relative humidity of 60 %  $\pm$  10 %, and a 12-h light/12-h dark cycle. The animals were provided with standard laboratory chow and drinking water ad libitum throughout the experiment.

Before the experiment, the body weights of all the rats were recorded, and the animals were randomly divided into five groups (n=10):

Group I (Control): 7 days of intragastric saline.

Group II (PbAc): 7 days of intragastric PbAc (30 mg/kg).<sup>12</sup>

Group III (BCP200+PbAc): 7 days of intragastric PbAc (200 mg/kg) (I.G.). Thirty min later, 30 mg/kg PbAc (I.G.) was administered.<sup>13</sup>

Group IV (BCP400+PbAc): 7 days of intragastric PbAc (400 mg/kg) (I.G.). After 30 min, 30 mg/kg PbAc was administered i.g.<sup>13</sup>

Group V (BCP400): Only 400 mg/kg BCP was administered i.g. for 7 days.

Twenty-four h after the last PbAc administration, the rats were euthanized under light sevoflurane anesthesia. Kidney tissues were then quickly removed and stored at -80 °C °C for biochemical analyses.

Equal amounts of kidney tissue were weighed and placed in screw-cap tubes. A total of 1500  $\mu$ L of PBS was added, and the samples were homogenized at 5000 rpm for 10 min via a Magna Lyser device. The samples were then centrifuged at 5000 rpm for 10 min, and the supernatant was carefully removed, transferred to Eppendorf tubes and stored at -80 °C for analysis.<sup>14</sup>

### Analysis of Oxidative Markers and Antioxidant Enzyme Levels

MDA levels and GSH, SOD, and GPx activities were determined via the relevant ELISA kits, and measurements were performed via an ELISA reader (Bio-Tek, Winooski, VT, USA) capable of reading absorbance at a wavelength of 450 nm.<sup>14</sup>

One-way analysis of variance (ANOVA) was performed to determine differences between groups in the statistical analysis of the ELISA data. Multiple comparisons between groups with significant differences were performed via the Tukey post hoc test. Statistical analyses were performed via GraphPad Prism software, and a p value of  $<0.05$  was considered to indicate statistical significance.

Results

Biochemical Findings Related to Oxidative Stress and Antioxidant Activity in Kidney Tissue

Compared with those in the control group, the MDA levels in the PbAc group were significantly greater ( $p < 0.0001$ ). The administration of different doses of BCP with PbAc resulted in statistically significant decreases in MDA levels. The most pronounced decrease was observed in the BCP400 + PbAc group ( $p < 0.0001$ ). Additionally, the MDA levels in the group treated with BCP400 alone were similar to those in the control group. The statistical results obtained indicate that there were significant differences between the groups ( $p < 0.0001$ ).

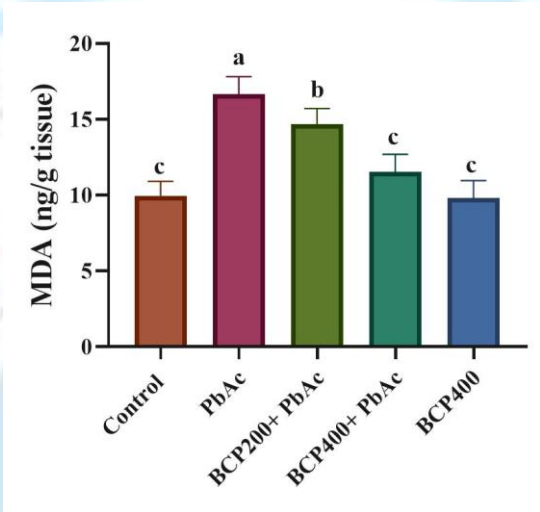


**Figure 1.** MDA levels in kidney tissues. The results are expressed as the means  $\pm$  standard deviations (SDs). Different letters indicate statistically significant differences between groups ( $P < 0.05$ ,  $n = 6$ ). SD: standard deviation

SOD activity in the PbAc group was significantly lower than that in the control group ( $p < 0.0001$ ). The administration of different doses of BCP with PbAc resulted in a statistically significant increase in SOD activity. The most pronounced increase was observed in the BCP400 + PbAc group ( $p < 0.0001$ ). The highest SOD activity was detected in the BCP400 group. Statistical analysis revealed significant differences in SOD activity between the groups ( $p < 0.0001$ ).

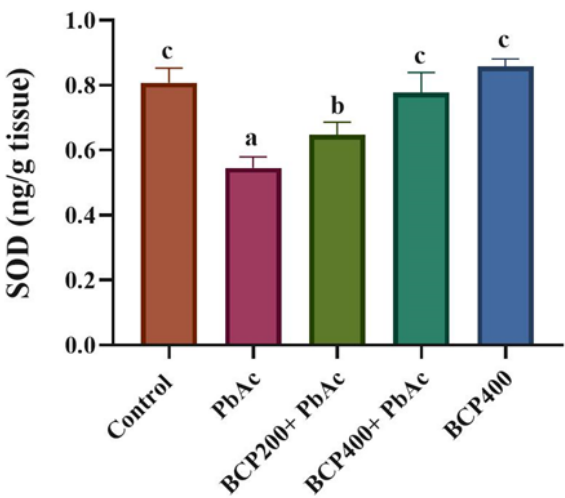


**Figure 2.** Demonstration of SOD activity in kidney tissues. The results are expressed as the means  $\pm$  standard deviations (SDs). Different letters indicate statistically significant differences between groups ( $P < 0.05$ ,  $n = 6$ ). SD: standard deviation



Compared with that in the control group, the GSH activity in the PbAc group decreased significantly ( $p < 0.0001$ ). The administration of different doses of BCP with PbAc resulted in a statistically significant increase in GSH activity. The most pronounced increase was observed in the BCP400 + PbAc group ( $p < 0.0001$ ). The highest GSH activity was detected in the BCP400 group. Statistical analysis revealed significant differences in GSH activity between the groups ( $p < 0.0001$ ).

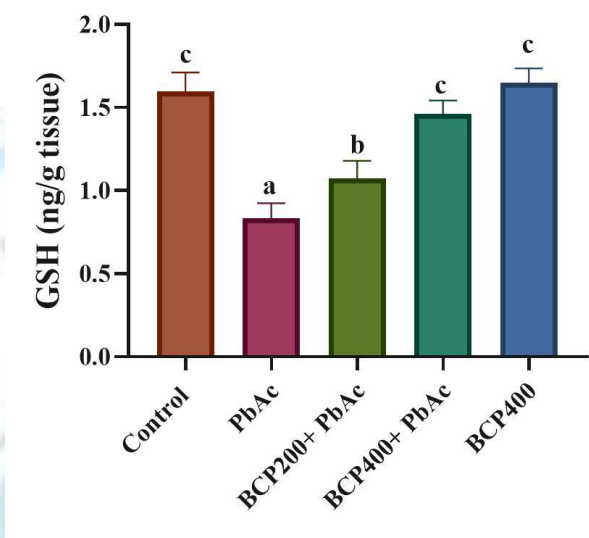


**Figure 3.** Demonstration of GSH activity in kidney tissues. The results are expressed as the means  $\pm$  standard deviations (SDs). Different letters indicate statistically significant differences between groups ( $P < 0.05$ ,  $n = 6$ ). SD: standard deviation

Compared with that in the control group, GPx activity in the PbAc group decreased significantly ( $p < 0.0001$ ). The administration of different doses of BCP with PbAc resulted in a statistically significant increase in GPx activity. The most pronounced increase was observed in the BCP400 + PbAc group ( $p < 0.0001$ ). The highest GPx activity was detected in the BCP400 group. Statistical analysis revealed significant differences in GPx activity between the groups ( $p < 0.0001$ ).

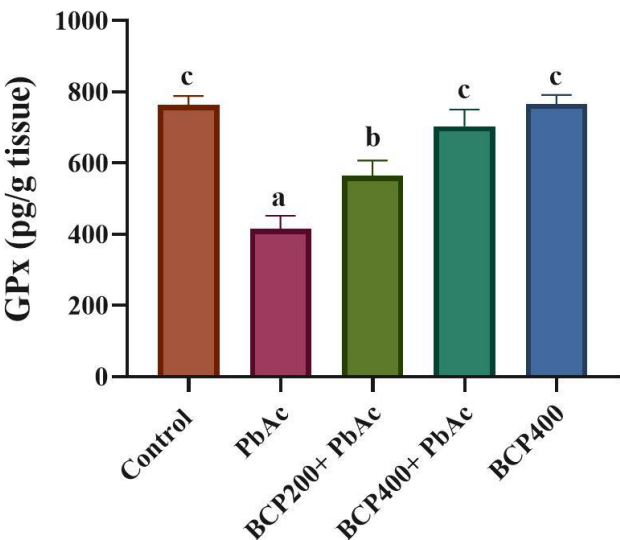


**Figure 4.** Demonstration of GPx activity in kidney tissues. The results are expressed as the means  $\pm$  standard deviations (SDs). Different letters indicate statistically significant differences between groups ( $P < 0.05$ ,  $n = 6$ ). SD: standard deviation.

## Discussion

Pb is a persistent heavy metal that accumulates in living organisms due to environmental exposure and industrial use, causing systemic toxicity.<sup>15</sup> The kidney, which is the primary excretory organ for Pb, is one of the tissues most exposed to the toxic effects of this metal.<sup>16</sup> Pb exposure causes structural and functional impairments in the proximal tubular epithelium, particularly due to oxidative stress.<sup>17</sup> In this study, oxidative stress markers (MDA, SOD, GSH, and GPx) were evaluated in an experimental nephrotoxicity model induced by PbAc, and the protective effects of BCP on these parameters were investigated.

Malondialdehyde (MDA), the end product of lipid peroxidation, is an important biomarker of oxidative stress and indicates disruption of cell membrane integrity.<sup>18</sup> In our study, a significant increase in MDA levels was observed in the PbAc group, indicating that lead triggers peroxidation damage in kidney tissue by increasing reactive oxygen species. Similarly, lead exposure increases MDA accumulation in kidney tissue, leading to oxidative damage to the cell membrane.<sup>8,19</sup> On the other hand, BCP, particularly at a dose of 400 mg/kg, significantly reduced MDA levels; these findings support the inhibitory effects of BCP on lipid peroxidation. Ullah et al. (2021) similarly reported that BCP can protect membrane stability by scavenging free radicals.<sup>20</sup> These findings suggest that BCP may reduce oxidative stress-induced damage by supporting the antioxidant defense system and could be considered a potential protective agent against lead-induced kidney toxicity.

Significant decreases in the levels of superoxide dismutase (SOD), glutathione (GSH) and glutathione peroxidase (GPx), which are enzymes belonging to the antioxidant defense system, were detected in the PbAc group. These findings indicate that lead inhibits endogenous defense mechanisms and weakens free radical detoxification capacity. The SOD enzyme forms the first line of defense against oxidative stress by converting superoxide radicals into the less harmful hydrogen peroxide.<sup>21</sup> GSH acts as an antioxidant that directly neutralizes reactive oxygen species (ROS) and is also a necessary substrate for the GPx enzyme. GPx, on the other hand, inactivates hydrogen peroxide and lipid peroxides via GSH.<sup>22</sup> The decrease in these three parameters in the PbAc group indicates the spread of oxidative stress and the suppression of the antioxidant system. These findings are consistent with the Pb-induced antioxidant system inhibition reported by Ortega et al. (2021) and Tian et al. (2021).<sup>23,24</sup>

BCP treatment resulted in significant increases in these antioxidant parameters and contributed to the restoration of redox balance. In particular, high-dose BCP treatment increased SOD, GSH, and GPx levels to values close to those of the control group. These findings suggest that BCP both supports antioxidant enzyme expression and enhances the cellular defense capacity by limiting ROS production. Refaat et al. (2022) reported that BCP reduced oxidative damage by increasing GSH levels in kidney tissue toxicity models.<sup>25</sup> Additionally, Ricardi et al. (2024) reported that BCP activates cellular defense genes via CB2 receptors and exerts regulatory effects on inflammation and oxidative stress.<sup>26</sup> The biochemical findings obtained in our study are highly consistent with the literature.

In conclusion, this study demonstrated that PbAc causes oxidative stress and structural damage in kidney tissue, whereas BCP significantly reduces these effects and protects the tissue at both the biochemical and histological levels. These effects of BCP may be related to mechanisms such as reducing lipid peroxidation, activating endogenous antioxidant systems, and maintaining tissue integrity. These results support the potential of BCP as a therapeutic agent against PbAc-induced nephrotoxicity.

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## PROTECTIVE ROLE OF B-CARYOPHYLLENE AGAINST LEAD ACETATE- INDUCED CARDIOTOXICITY IN RATS: EFFECTS ON OXIDATIVE STRESS AND ANTIOXIDANT RESPONSE

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### Abstract

In this study, an experimental cardiotoxicity model was established in rats by administering lead acetate (PbAc), and the effects of PbAc on oxidative stress and tissue damage in the heart were investigated. Furthermore, the potential protective role of  $\beta$ -caryophyllene (BCP), a natural plant-derived compound, against this toxic effect was evaluated. A total of 50 adult male Sprague–Dawley rats (220–290 g) were divided into five groups: control (physiological saline), BCP (400 mg/kg BCP, intragastric), PbAc (30 mg/kg PbAc, oral), BCP200+PbAc (200 mg/kg BCP + 30 mg/kg PbAc), and BCP400+PbAc (400 mg/kg BCP + 30 mg/kg PbAc). BCP, dissolved in olive oil, was administered intragastrically 30 min before PbAc exposure. At the end of the experimental period, heart tissue samples were collected for biochemical and histopathological analyses. Oxidative stress was assessed by measuring malondialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH), and glutathione peroxidase (GPx) levels. The PbAc group presented significant increases in MDA levels and marked decreases in SOD, GSH, and GPx activities. In contrast, the BCP-treated groups presented reduced MDA levels and increased antioxidant enzyme activities. Histopathological evaluation revealed pronounced myocardial degeneration, structural damage, and inflammatory cell infiltration in the PbAc group, whereas BCP administration mitigated these changes and better preserved tissue integrity. These findings indicate that PbAc induces severe cardiotoxicity at both the biochemical and histological levels and that BCP exerts a substantial protective effect by reducing oxidative stress and enhancing antioxidant defense systems.

**Keywords:**  $\beta$ -caryophyllene, cardiotoxicity, GPx, GSH, MDA, PbAc, SOD

### Introduction

Lead (Pb) is a widespread and persistent heavy metal released into the environment from various sources, such as industrial activities, vehicle exhaust emissions, paints, water pipes, and batteries<sup>1</sup>. Owing to its tendency to accumulate in biological systems, its toxic effects progressively increase over time, causing serious health problems in both humans and animals. Pb can induce toxicity in the hematopoietic, renal, hepatic, neurological, and particularly cardiovascular systems<sup>2-5</sup>. Clinical and experimental studies have demonstrated that chronic Pb exposure leads to cardiac dysfunction, myocardial cell damage, and elevated oxidative stress levels<sup>6</sup>. One of the primary mechanisms underlying Pb-induced cardiac injury is increased production of reactive oxygen species (ROS), accompanied by a weakened antioxidant defense system<sup>7</sup>.

In cardiomyocytes, ROS imbalance results in oxidative damage to cellular lipids, proteins, and DNA, as well as increased inflammatory responses and cell death<sup>8</sup>. This process is characterized by an increase in malondialdehyde (MDA), a key indicator of lipid peroxidation, along with a decrease in the activities of antioxidant defense components such as superoxide dismutase (SOD), glutathione (GSH), and glutathione



peroxidase (GPx). These biochemical alterations indicate that Pb induces oxidative damage in cardiac tissue, potentially leading to structural and functional impairments in cardiac performance<sup>7</sup>. In addition to these biochemical effects, evaluating the morphological alterations caused by Pb toxicity at the tissue level is also highly important. Histopathological changes in the heart may include myocardial fiber degeneration, cellular infiltration, edema, capillary damage, and necrotic areas<sup>5,9</sup>. Microscopic examination of these alterations enables a more comprehensive understanding of Pb-induced cardiotoxicity.

In recent years, the potential protective effects of natural antioxidants against cardiac injury caused by toxic agents have emerged as an important area of research. One such compound is  $\beta$ -caryophyllene (BCP), a sesquiterpene widely found in plant-derived essential oils<sup>10</sup>. In addition to its antioxidant, anti-inflammatory, and cytoprotective effects, BCP has phytocannabinoid properties, making it a promising therapeutic candidate for various toxicity models<sup>11</sup>. While numerous studies have investigated the hepatoprotective, neuroprotective, and renoprotective effects of BCP, its influence on Pb-induced cardiac injury remains largely unexplored.

In this study, the effects of BCP on oxidative stress and histopathological alterations in the heart were investigated via an experimental cardiotoxicity model induced by PbAc administration. Cardiac MDA, SOD, GSH, and GPx levels were measured to evaluate the antioxidant defense status, and histopathological examinations were performed to assess PbAc-induced tissue damage and the potential protective effects of BCP via light microscopy. The findings from this study are expected to contribute to the understanding of the therapeutic potential of BCP against PbAc-induced cardiotoxicity.

## Materials and methods

### Chemicals

Lead acetate (PbAc) ( $\geq 99\%$ , CAS No: 6080--56--4) and  $\beta$ -caryophyllene (BCP, CAS No: 87--44--5) were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Biochemical analyses were performed via ELISA kits supplied by A.B.T. Laboratory Industry. The kits used were as follows: MDA (Cat. No: ABT10165Ra), SOD (Cat. No: ABT1626Ra), GSH (Cat. No: ABT10155Ra), and GPx (Cat. No: ABT2557Ra).

### Animals

A total of 50 adult male Sprague-Dawley rats (220–250 g) were obtained from the Atatürk University Experimental Research and Application Center (ATADEM) (Ethics Committee Approval No: 2025/09, Decision No: 185). The animals were housed under controlled environmental conditions at 25 °C and 60  $\pm$  10 % relative humidity with a 12-h light/12-h dark cycle. Standard laboratory chow and drinking water were provided ad libitum throughout the experimental period.

### Experimental Design

Prior to the experiment, all the rats were weighed and randomly assigned to five groups (n=10 per group):

Group I (Control): Received intragastric (i.g.) physiological saline for 7 days.

Group II (PbAc): PbAc was received at a dose of 30 mg/kg i.g. for 7 days<sup>13</sup>.

Group III (BCP200+PbAc): BCP at 200 mg/kg i.g. for 7 days, followed 30 min later by PbAc at 30 mg/kg i.g.<sup>12</sup>.

Group IV (BCP400+PbAc): BCP at 400 mg/kg i.g. for 7 days, followed 30 min later by PbAc at 30 mg/kg i.g. <sup>12</sup>.

Group V (BCP400) received BCP at 400 mg/kg i.g. for 7 days only.

Twenty-four h after the last PbAc administration, all the animals were euthanized under mild sevoflurane anesthesia. Heart tissues were rapidly excised and stored at  $-80^{\circ}\text{C}$  until biochemical and histopathological analyses.

### **Preparation of Heart Tissue Homogenates**

Equal amounts of heart tissue were weighed and placed into screw-cap tubes. A total of 1500  $\mu\text{L}$  of PBS was added, and the tissues were homogenized via a Magna Lyser at 5000 rpm for 10 min. The homogenates were centrifuged at 5000 rpm for 10 min, and the supernatants were carefully collected into Eppendorf tubes and stored at  $-80^{\circ}\text{C}$  until analysis<sup>14</sup>.

### **Biochemical Analyses**

#### **Oxidative Stress Markers and Antioxidant Enzyme Activities**

MDA levels and GSH, SOD, and GPx activities were determined via specific ELISA kits, and measurements were carried out at a wavelength of 450 nm via a microplate reader (Bio-Tek, Winooski, VT, USA)<sup>14</sup>.

#### **Histopathological examination**

Heart tissue samples were fixed in 10 % buffered formalin for 48 h, processed routinely, and embedded in paraffin blocks. Sections of 4  $\mu\text{m}$  thickness were prepared, stained with hematoxylin–eosin (H&E), and examined under a light microscope (Leica Flexacam i5). Histopathological evaluation focused on myocardial degeneration, necrosis, and myocarditis. The severity of the observed lesions was scored as absent (–), mild (+), moderate (++) , or severe (+++) <sup>15</sup>.

### **Statistical analysis**

For ELISA data, differences between groups were assessed via one-way analysis of variance (ANOVA). When a significant difference was detected, Tukey’s post hoc test was performed for multiple comparisons. Statistical analyses were conducted via GraphPad Prism software, and  $p < 0.05$  was considered statistically significant.

For histopathological results, the Shapiro–Wilk test was used to assess normality. Nonparametric Kruskal–Wallis tests were applied to determine group effects, and Mann–Whitney U tests were used for pairwise comparisons. Statistical significance was set at  $p < 0.05$ .

### **Results**

#### **Biochemical Findings Related to Oxidative Stress and Antioxidant Activity in Cardiac Tissue**

Compared with those in the control group, the MDA levels in the PbAc group were significantly elevated ( $p < 0.0001$ ). Coadministration of BCP at different doses with PbAc significantly reduced the MDA level, with the most pronounced decrease observed in the BCP400+PbAc group ( $p < 0.0001$ ). The MDA levels in the BCP400 group were comparable to those in the control group. Overall, the statistical analyses revealed significant differences among the groups ( $p < 0.0001$ ).

SOD activity was significantly lower in the PbAc group than in the control group ( $p < 0.0001$ ). The administration of BCP in combination with PbAc significantly increased SOD activity, with the greatest improvement observed in the BCP400+PbAc group ( $p < 0.0001$ ). The highest SOD activity was recorded in the BCP400 group. Statistical analyses confirmed significant differences in SOD activity among the groups ( $p < 0.0001$ ).



Similarly, GSH activity was markedly lower in the PbAc group than in the control group ( $p < 0.0001$ ). BCP at both doses in combination with PbAc significantly increased GSH activity, with the most prominent increase observed in the BCP400+PbAc group ( $p < 0.0001$ ). The highest GSH activity was found in the BCP400 group. Group comparisons revealed statistically significant differences in GSH activity ( $p < 0.0001$ ).

GPx activity was also significantly lower in the PbAc group than in the control group ( $p < 0.0001$ ). Cotreatment with BCP led to significant increases in GPx activity, with the most pronounced effect observed in the BCP400+PbAc group ( $p < 0.0001$ ). The highest GPx activity was detected in the BCP400 group. Statistical analyses revealed significant intergroup differences in GPx activity ( $p < 0.0001$ ).

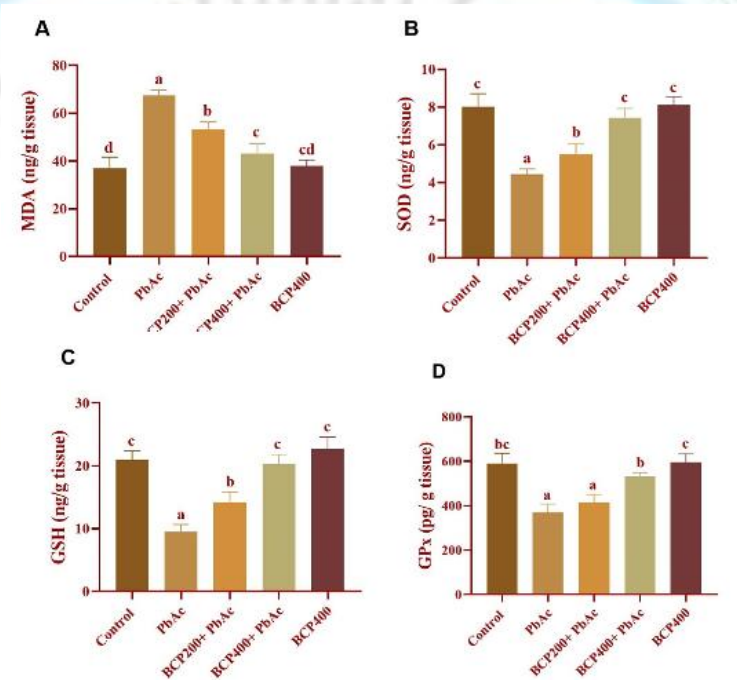


**Figure 1.** Levels of oxidative stress and antioxidant defense parameters in cardiac tissue: (A) MDA levels, (B) SOD activity, (C) GSH levels, and (D) GPx activity. The data are presented as the means  $\pm$  standard deviations (SDs) ( $n = 6$ ). Different letters indicate statistically significant differences between groups ( $p < 0.05$ ). SD, standard deviation.

### Histopathological findings

Histopathological examination of cardiac tissues revealed no remarkable findings in the control and BCP400 groups. Severe myocyte degeneration and necrosis, along with myocarditis, were observed in the PbAc group. Compared with those in the PbAc group, histopathological lesions were significantly reduced in a dose-dependent manner in the BCP200+PbAc and BCP400+PbAc groups ( $p < 0.05$ ) (Figure 2). The scoring of histopathological findings in cardiac tissue and the corresponding statistical analyses are presented in Figure 3.

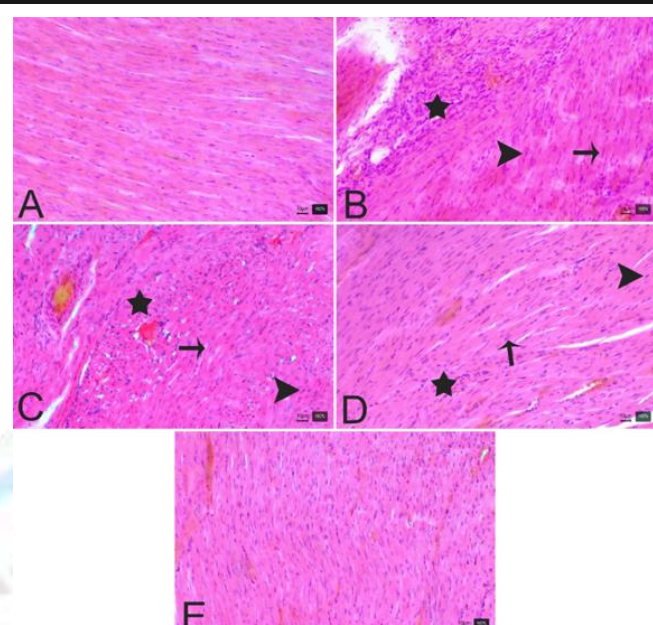


**Figure 2.** Cardiac tissue: Control (A), PbAc (B), BCP200+PbAc (C), BCP400+PbAc (D), and BCP400 (E) groups. Myocyte degeneration is indicated by arrows, necrosis by arrowheads, and myocarditis by asterisks. Staining: H&E; scale bar: 10 µm; objective: 20×; zoom: 100 %.

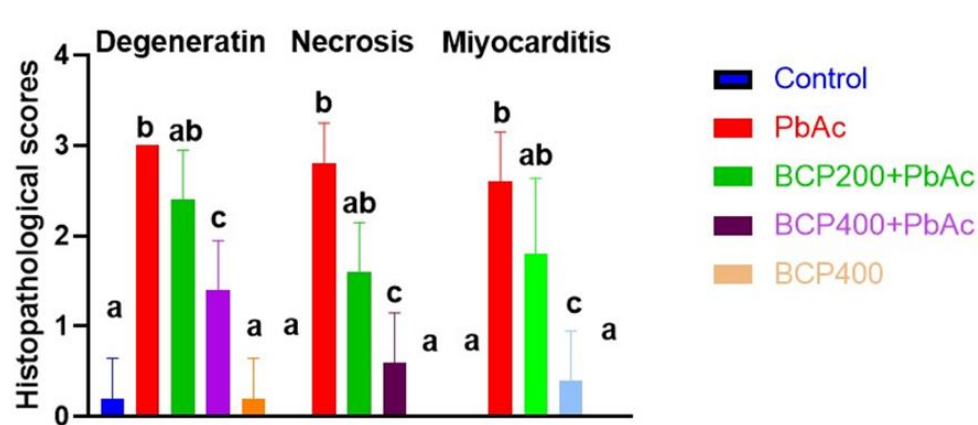


**Figure 3.** Scoring and statistical analysis of histopathological findings in cardiac tissue. The evaluation of the staining results was performed via the Kruskal–Wallis test followed by the Mann–Whitney U test. Different letters (b and c) indicate statistically significant differences compared with the control group (a) ( $p < 0.05$ ). The data are presented as the means  $\pm$  SDs.

### Discussion and Conclusion

Lead (Pb) is a widely distributed environmental pollutant that bioaccumulates in organisms and induces toxic effects in various organs <sup>16</sup>. Exposure to lead acetate (PbAc) has been shown to increase oxidative stress in cardiac tissue, leading to associated cellular damage. In the present study, oxidative stress markers—malondialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH), and glutathione peroxidase (GPx)—were evaluated, and PbAc exposure was found to induce a marked redox imbalance in the heart. Importantly, the administration of the natural phytochemical  $\beta$ -caryophyllene (BCP) significantly attenuated PbAc-induced oxidative injury and histopathological alterations.

MDA, the end product of lipid peroxidation, is formed through the interaction of polyunsaturated fatty acids in cell membranes with reactive oxygen species (ROS) and is considered a direct indicator of oxidative stress<sup>17</sup>. In our study, MDA levels were significantly elevated in the PbAc group, reflecting the destructive impact



of lead on membrane lipids and the initiation of oxidative processes. Consistent with our findings, Qu et al. (2019) and Lopes et al. (2016) reported that lead exposure promotes MDA accumulation and exacerbates lipid peroxidation in cardiac tissue. Treatment with BCP, particularly at the 400 mg/kg dose, markedly suppressed the increase in MDA, suggesting the potent free radical-scavenging and membrane-stabilizing properties of BCP.

In contrast, the levels of the antioxidant defense components SOD, GSH, and GPx were significantly reduced following PbAc exposure. SOD plays a pivotal role by converting superoxide radicals into less reactive species, thus interrupting the initial steps of oxidative damage<sup>20</sup>. GSH acts both as a direct antioxidant and as a cofactor for enzymes such as GPx, which detoxifies peroxides and protects cells from oxidative injury<sup>21</sup>. The observed decline in these antioxidants in the PbAc group suggests that lead impairs enzymatic functions and severely compromises the cellular defense capacity. Similarly, previous studies have demonstrated that heavy metal toxicity can inhibit antioxidant enzymes, thereby facilitating the progression of oxidative stress<sup>22-24</sup>. In the present study, BCP supplementation significantly restored antioxidant enzyme activities, with the high-dose BCP group showing values approaching those of the control group. Ricardi et al. (2024) reported that BCP, a CB2 receptor agonist, exerts modulatory effects on inflammation and oxidative stress. Similarly, Rahimi et al. (2023) demonstrated that BCP stimulates the antioxidant defense system and re-establishes redox homeostasis, findings that are consistent with our biochemical results.

Histopathological analysis supported the biochemical data, revealing severe myocardial degeneration, inflammatory cell infiltration, and necrosis in the PbAc group. These pathological features likely result from mechanisms such as mitochondrial dysfunction, ionic imbalance, and apoptosis in cardiomyocytes. Our findings align with those of Küçükler et al. (2021) and Zahara et al. (2024), who reported prominent degenerative, inflammatory, and structural damage in rat hearts following PbAc exposure. Notably, BCP treatment—particularly at higher doses—substantially reduced these lesions, preserved tissue architecture, and minimized inflammatory infiltration. Similarly, Li et al. (2024) demonstrated that BCP confers histological protection against cardiotoxicity, whereas Ahmad et al. (2025) reported that BCP mitigates structural cardiac damage induced by various toxicants.

In conclusion, our findings demonstrate that PbAc induces significant oxidative stress and histological damage in cardiac tissue, whereas BCP effectively counteracts these deleterious effects at both the biochemical and structural levels. The cardioprotective action of BCP is likely mediated through the enhancement of endogenous antioxidant defenses and the suppression of lipid peroxidation. These results support the potential application of BCP as a protective agent against heavy metal-induced cardiotoxicity.

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## THE APPLICATION OF HERBAL AND TRADITIONAL MEDICINE IN TREATMENT OF LAMENESS IN HORSES

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### Abstract

Equine lameness represents a significant welfare, performance and economic challenge within the equestrian industry, often necessitating prolonged and costly management strategies. While conventional veterinary medicine offers a range of effective treatments, including nonsteroidal anti-inflammatory drugs (NSAIDs), corticosteroids, and regenerative therapies, these approaches can be associated with side effects, limitations in long-term use, or substantial financial burden. Consequently, there is a growing interest among horse owners and veterinarians in complementary and alternative medicine (CAM), particularly herbal and traditional medicine, to provide adjunctive or alternative therapeutic options for musculoskeletal conditions. This review explores the scientific rationale, current applications, and future potential of herbal and traditional medicinal approaches for managing equine lameness. We investigated specific botanicals and traditional systems (e.g., traditional Chinese medicine, Ayurveda) known for their anti-inflammatory, analgesic, chondroprotective, and tissue-regenerative effects. Key herbal agents such as *Boswellia serrata*, *Curcuma longa*, *Harpagophytum procumbens*, and *Salix alba* are discussed in detail regarding their mechanisms of action and evidence of their efficacy. Furthermore, the present review addresses critical considerations, including quality control, potential drug interactions, regulatory challenges and the importance of an evidence-based, integrative approach to ensure patient safety and optimize therapeutic outcomes. The aim is to bridge the gap between historical wisdom and contemporary scientific understanding, fostering a more holistic and sustainable paradigm for equine lameness management.

**Keywords:** Herbal medicine, Traditional medicine, Lameness, Horse

### Introduction

Equine lameness, defined as any alteration in horse gait or stance, is arguably the most common and economically impactful health issue affecting horses across all disciplines, from companion animals to elite athletes (Ross & Dyson, 2011). It can stem from a myriad of causes, including traumatic injuries, degenerative joint disease, infectious processes, developmental orthopedic diseases, and metabolic disorders. The debilitating nature of lameness not only compromises performance and longevity but also raises significant animal welfare concerns due to associated pain and discomfort (Mills & McDonnell, 2005). The economic ramifications are substantial, encompassing veterinary costs, lost training time, reduced market value, and potential premature retirement or euthanasia (Anderson et al., 2004).

For decades, conventional veterinary medicine has been the cornerstone of lameness diagnosis and treatment. Diagnostic modalities have advanced significantly, incorporating sophisticated imaging techniques such as radiography, ultrasonography, scintigraphy, computed tomography (CT), and magnetic resonance

imaging (MRI) to precisely identify the anatomical source of pain (Wright & McIlwraith, 2015). Therapeutic strategies typically involve a multimodal approach, which may include rest, controlled exercise, physical therapy, farriery modifications, and pharmacological interventions (Wright & McIlwraith, 2015). Nonsteroidal anti-inflammatory drugs (NSAIDs), such as phenylbutazone, flunixin meglumine, and firocoxib, are widely used for their potent analgesic and anti-inflammatory effects, primarily by inhibiting cyclooxygenase (COX) enzymes (Wright & McIlwraith, 2015). While effective, chronic NSAID use can lead to adverse effects such as gastrointestinal ulceration, renal dysfunction, and right dorsal colitis, particularly in susceptible individuals (Wright & McIlwraith, 2015). Corticosteroids, administered intra-articularly or systemically, offer powerful anti-inflammatory benefits but carry risks of laminitis, immunosuppression, and potential chondrotoxicity with repeated use (Wright & McIlwraith, 2015). In addition to anti-inflammatories, conventional treatments include disease-modifying osteoarthritis drugs (DMOADs), such as polysulfated glycosaminoglycans (PSGAGs) and hyaluronic acid (HA), which aim to protect cartilage and improve joint fluid viscosity (Todhunter & Wooldridge, 2004). More recently, regenerative therapies, including platelet-rich plasma (PRP), autologous conditioned serum (ACS or IRAP), and mesenchymal stem cells (MSCs), have gained prominence for their potential to promote tissue repair and reduce inflammation, although their long-term efficacy and cost-effectiveness are still the subjects of ongoing research (Lombana et al, 2015). Surgical interventions, such as arthroscopy for joint debridement, fracture repair, or tenoscopy for soft tissue injuries, are reserved for specific conditions (Merchán et al., 2022). Despite advancements in conventional veterinary care, several factors have driven the exploration of complementary and alternative medicine (CAM) in equine practice (Lange et al., 2018). These include a desire for more "natural" or holistic approaches, concerns about the side effects of conventional drugs, the limitations of conventional medicine in chronic or refractory cases, and the increasing cost of advanced treatments (Lange et al., 2018). Owners often seek CAM therapies to provide additional support, improve overall well-being, or reduce reliance on pharmaceuticals (Gyles., 2020). Among the various CAM modalities, herbal medicine involves the use of plant-derived materials for therapeutic purposes, and traditional medicine systems, such as traditional Chinese medicine (TCM) and Ayurveda, which employ comprehensive philosophies and practices, have a long history of use in both human and animal health (Mangan et al., 2022). The historical use of plants for medicinal purposes predates modern pharmacology, with evidence of herbal remedies for pain and inflammation dating back thousands of years across diverse cultures (Bullitta et al., 2018). In equine practice, traditional remedies were once the primary form of treatment and were passed down through generations of horsemen and early veterinarians (Gyles et al., 2020). Today, this ancient wisdom is increasingly being scrutinized through the lens of modern scientific research, seeking to validate efficacy, elucidate mechanisms of action, and ensure safety (Wynn and Fougère., 2009).

The present review aims to provide a comprehensive overview of the application of herbal and traditional medicine in the treatment of lameness in horses. This review explores the pharmacological basis of commonly used herbal remedies; discusses their potential roles as anti-inflammatory, analgesic, chondroprotective, and tissue-supportive agents; and highlights the principles of traditional medicine systems relevant to musculoskeletal health (Wynn and Fougère., 2009). Furthermore, critical aspects such as quality control, potential drug interactions, regulatory considerations, and the importance of responsibly integrating these therapies into an evidence-based veterinary practice will be addressed (Wynn and Fougère., 2009). By synthesizing the current



scientific understanding with traditional knowledge, this review seeks to inform veterinarians and horse owners about the judicious and effective incorporation of herbal and traditional medicine into a holistic management plan for equine lameness. The growing interest in herbal and traditional medicines for equine lameness stems from their potential to offer multifaceted therapeutic benefits, often with fewer side effects than conventional pharmaceuticals (Wynn and Fougère., 2009). These benefits typically revolve around modulating inflammatory pathways, providing analgesia, supporting tissue repair, and improving overall physiological balance (Gyles., 2020). A detailed exploration of specific herbal agents and traditional systems reveals their mechanisms and applications:

### Anti-inflammatory and analgesic herbs

Inflammation is a fundamental component of lameness and contributes to pain, swelling, and tissue damage. Many herbal remedies possess potent anti-inflammatory and analgesic properties, often targeting pathways similar to those of NSAIDs but with different pharmacokinetic profiles or broader effects (Wynn and Fougère., 2009).

1- ***Boswellia serrata*** (Frankincense): Extracts from the gum resin of *Boswellia serrata* contain boswellic acids, particularly acetyl-11-keto- $\beta$ -boswellic acid (AKBA), which are potent inhibitors of 5-lipoxygenase (5-LOX), an enzyme crucial in the synthesis of leukotrienes, key mediators of inflammation. This mechanism differs from that of NSAIDs, which primarily target cyclooxygenase (COX) enzymes, suggesting a complementary role (Sharma et al., 2004). *Boswellia* has been shown to reduce pain and improve mobility in human and animal models of osteoarthritis (Beghelli et al., 2023).

2- ***Curcuma longa*** (turmeric): The active compounds in turmeric, known as curcuminoids, especially curcumin, exhibit strong anti-inflammatory effects by inhibiting NF- $\kappa$ B, a transcription factor that regulates the expression of proinflammatory cytokines, and by modulating the COX and LOX pathways (Beghelli et al., 2023). Curcumin also acts as an antioxidant, scavenging free radicals that contribute to tissue damage during inflammation (Ak & Gulcin, 2008). Its poor bioavailability in its raw form necessitates formulations with piperine or liposomal preparations to increase its absorption (Anand et al., 2007).

3- ***Harpagophytum procumbens*** (Devil's Claw): The primary active constituents are iridoid glycosides, particularly harpagoside (Brendler et al., 2021). *Harpagophytum* is widely used for musculoskeletal pain and inflammation, with studies suggesting its efficacy in treating osteoarthritis and low back pain (Chrubasik et al., 2002). Its mechanism involves modulating the production of cytokines (TNF- $\alpha$  and IL-1 $\beta$ ) and potentially inhibiting COX-2. Concerns about gastrointestinal irritation exist, although they are generally less severe than NSAIDs are (Chrubasik et al., 2002).

4- ***Salix alba*** (white willow bark): Salicin, which is metabolized in the body to salicylic acid, the precursor to aspirin. It exerts anti-inflammatory and analgesic effects through COX inhibition, although its action is slower and generally milder than that of synthetic aspirin, potentially with fewer gastrointestinal side effects (Shara & Stohs, 2015).

5- ***Zingiber officinale*** (Ginger): Gingerols and shogaols, the active components, possess anti-inflammatory properties by inhibiting the COX and LOX pathways, reducing prostaglandin and leukotriene synthesis, and modulating proinflammatory cytokines. Ginger can also act as an antioxidant and improve circulation (Grzanna et al., 2005).

### Chondroprotective and Joint Health Herbs

Maintaining cartilage integrity and joint fluid health is crucial for long-term soundness (McIlwraith, 2015). Some herbs are recognized for their ability to support joint structures and mitigate cartilage degradation.

1- ***Yucca schidigera***: Contains steroidal saponins and polyphenols that may reduce inflammation and pain by inhibiting urease activity and acting as antioxidants. While direct chondroprotective effects are less established, its anti-inflammatory action can indirectly benefit joint health by reducing cartilage-damaging enzymes (Cheeke et al., 2006).

2- ***Equisetum arvense*** (Horsetail): Rich in silica, a mineral essential for collagen synthesis and bone and connective tissue formation. While not directly chondroprotective, its role in supporting the structural integrity of connective tissues makes it relevant for overall joint health (Gründemann et al., 2014).

3- ***Symphytum officinale*** (Comfrey): *Symphytum officinale* is traditionally used topically for sprains, strains, and fractures because of its allantoin content, which promotes cell proliferation and tissue repair (Tsintzas., 2016). However, systemic use is controversial because of the presence of hepatotoxic pyrrolizidine alkaloids. Topical application, under veterinary guidance, can be beneficial for superficial lameness conditions (Mei et al., 2010).

### Herbs in Bone Health

While less direct in acute lameness, long-term bone health is vital, particularly in conditions such as navicular syndrome or metabolic bone disease (Leung et al., 2013).

1- ***Trifolium pratense*** (Red clover): This class contains isoflavones, which are plant estrogens that may have a positive effect on bone density, particularly in postmenopausal women, potentially offering analogous benefits for bone remodeling in horses (Krebs et al., 2021).

2- ***Medicago sativa*** (alfalfa): A nutrient-dense herb rich in vitamins (K, C), minerals (calcium, magnesium), and phytoestrogens, all of which are important for bone metabolism and overall skeletal health (Goraya et al., 2013).

### Muscle and Soft Tissue Support Herbs

Lameness often involves muscle soreness, strains, or ligamentous injuries (Dyson, 2011).

1- ***Arnica montana***: Primarily used topically as a liniment or cream for bruising, swelling, and muscle soreness due to its anti-inflammatory and analgesic properties. It is thought to work by inhibiting NF-κB and reducing leukocyte extravasation. Internal use is generally not recommended due to toxicity (ESC LABORATORY 45 route de Chaluzuy 58000 Saint-Eloi).

2- ***Capsicum frutescens*** (Cayenne): *Capsicum frutescens* contains capsaicin, which acts as a counterirritant and selectively desensitizes nerve fibers by depleting substance P, a neurotransmitter involved in pain transmission (Chrubasik et al., 2007). When used topically, it can provide localized pain relief, but caution is needed to avoid skin irritation.

### Circulation Enhancers

Improved blood flow can aid in nutrient delivery, waste removal, and healing, which are particularly relevant in conditions such as laminitis (van Eps and Pollitt, 2009).



1- ***Ginkgo biloba***: Known for its ability to improve peripheral circulation and act as an antioxidant. While direct studies on equine lameness are limited, its vasodilatory effects could theoretically benefit conditions where blood flow is compromised (ESC LABORATORY 45 route de Chaluzzy 58000 Saint-Eloi).

2- ***Crataegus oxyacantha*** (Hawthorn): Traditionally used for cardiovascular support, it can improve coronary blood flow and myocardial contractility. Its general circulatory benefits may indirectly support tissue health and repair in lameness cases (Wynn and Fougère., 2009).

### Traditional Medicine Systems

Traditional systems offer a holistic framework, treating the whole animal rather than just the symptoms (Gyles, 2020).

1- **Traditional Chinese Veterinary Medicine (TCVM)**: TCVM is based on the principles of Qi (life force), Blood, Yin, Yang, and the Five Elements (Munsterman, 2023). Lameness is viewed as a disruption of Qi and blood flow and is often categorized as "Bi Syndrome" (painful obstruction) due to external pathogenic factors (wind, cold, dampness) or internal imbalances (kidney/liver deficiency). Herbal formulas in TCVM are complex, synergistic combinations designed to address the underlying pattern of imbalance (e.g., Du Huo Ji Sheng Tang for chronic pain with weakness and San Bi Tang for acute painful obstruction). Acupuncture, often used in conjunction with herbal medicine, stimulates specific points to restore energy flow and alleviate pain (Munsterman, 2023).

2- **Ayurveda**: An ancient Indian system focused on balancing the three Doshas (Prasad et al., 2016). Lameness is often associated with imbalances in Vata (responsible for movement and the nervous system) and Kapha (responsible for lubrication and structure). Ayurvedic herbs such as *Withania somnifera* (Ashwagandha), which has adaptogenic and anti-inflammatory effects, and *Commiphora wightii* (Guggul), which has anti-inflammatory and lipid-lowering properties, are commonly used. Ayurvedic principles emphasize diet, lifestyle, and detoxification alongside herbal remedies (Prasad et al., 2016).

### Safety, Quality Control and Regulatory Considerations

The use of herbal and traditional medicines is not without risk (Harkins et al., 2012).

1- **Adverse effects and drug interactions**: Although often perceived as natural, herbs contain pharmacologically active compounds that can cause adverse effects (e.g., pyrrolizidine alkaloids in Comfrey, which can cause liver toxicity) (Gyles, 2020). Interactions with conventional drugs are a significant concern; for example, *Harpagophytum* and *Salix alba* can potentiate the effects of anticoagulants such as warfarin, increasing the risk of bleeding (Gyles, 2020). *Ginkgo biloba* can also affect blood clotting (Gyles, 2020).

2- **Quality Control and Standardization**: The lack of tough regulations in many countries means that the potency, purity, and consistency of herbal products can vary widely (European Medicines Agency, 2014). Adulteration with synthetic drugs, contamination with heavy metals, pesticides, or microbes, and misidentification of plant species are serious issues. Reputable suppliers and standardized extracts are essential to ensure consistent efficacy and safety. (European Medicines Agency, 2014).

3- **Doping Regulations**: Many herbs and their active constituents are considered prohibited substances by equestrian governing bodies (e.g., FEI) because of their analgesic or anti-inflammatory properties (FEI, 2023). Veterinarians and owners must be acutely aware of withdrawal times and testing protocols to avoid inadvertent doping violations (Hartgens, 2008).

3- **Veterinary Oversight**: Due to the complexities of diagnosis, potential drug interactions, and the need for individualized treatment plans, herbal and traditional medicine should always be administered under the

guidance of a qualified veterinarian (Wynn, 2009). Self-medication can lead to misdiagnosis, delayed appropriate treatment, or adverse outcomes.

### Integration with Conventional Medicine

The most effective approach for treating equine lameness often involves an integrative strategy that combines the best conventional and complementary therapies (Gyles, 2020). Herbal medicine can serve as an invaluable adjunctive therapy to reduce reliance on NSAIDs for chronic conditions, minimize side effects (Wynn, 2009), provide long-term support for joint health and cartilage metabolism (Wright & McIlwraith, 2015), enhance recovery postinjury or surgery by supporting tissue repair and reducing inflammation (Wright & McIlwraith, 2015) and address underlying systemic imbalances contributing to lameness, as emphasized by traditional medicine systems (Parsad et al., 2016). A truly holistic approach considers the entire well-being of horses, including their nutrition, exercise, environment, and mental state, alongside targeted treatments (Mills & McDonnell, 2005).

### Future Research Directions

Despite promising anecdotal evidence and some preliminary studies, robust, large-scale, randomized, controlled clinical trials in horses are critically needed to scientifically validate the efficacy and safety of many herbal and traditional remedies for specific lameness conditions (Wynn and Fougère., 2009).

Future research should focus on the following:

- 1- **Pharmacokinetics and Pharmacodynamics:** Understanding how herbal compounds are absorbed, distributed, metabolized, and excreted in horses is crucial for accurate dosing and predicting effects (Wynn and Fougère., 2009).
- 2- **Mechanism of action:** Further elucidation of the specific cellular and molecular mechanisms by which these herbs exert their effects.
- 3- **Standardization and Quality Control:** Developing industry standards for herbal extracts to ensure consistent potency and purity (European Medicines Agency, 2014).
- 4- **Long-term Safety Studies:** Assessing the long-term effects of chronic drug administration, including potential cumulative toxicities and drug interactions (Wynn and Fougère., 2009).
- 5- **Comparative studies:** Direct comparisons of herbal therapies with conventional treatments or as adjunctive therapies to establish their relative and combined benefits (Robinson, 2012).

### Conclusion

Equine lameness remains a multifaceted challenge, demanding a comprehensive and adaptable approach to management (Ross & Dyson, 2011). Herbal and traditional medicine offers a valuable and expanding toolkit for veterinarians and horse owners seeking to address pain, inflammation, and tissue degeneration associated with musculoskeletal conditions (Wynn and Fougère., 2009). Owing to their diverse pharmacological properties, ranging from targeted anti-inflammatory effects to holistic systemic balancing, these modalities hold significant promise as complementary or alternative therapies. However, the effective and safe integration of herbal and traditional medicine into mainstream equine practice hinges upon a commitment to evidence-based principles. Rigorous scientific research, particularly well-designed clinical trials in horses, is imperative to validate efficacy, elucidate mechanisms, and establish optimal dosing and long-term safety profiles. Furthermore, strict attention to product quality, potential drug interactions, and adherence to doping regulations are paramount (European Medicines Agency, 2014; FEI, 2023). A collaborative approach in which conventional veterinary expertise is synergistically combined with judiciously applied, scientifically supported herbal and traditional therapies is



recommended. This integrative paradigm promises to enhance animal welfare, optimize performance, and provide more sustainable and holistic solutions for the complex challenges associated with equine lameness.

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# THE EFFECT OF THE PROBIOTIC PREPARATION "LACTOBACT-ARZ" ON THE DYNAMICS OF CERTAIN MORPHOLOGICAL AND BIOCHEMICAL BLOOD INDICATORS IN CALVES

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## Abstract

This article presents the results of a study on the use of the probiotic preparation "Lactobact-ARZ" in Holstein calves. The study was conducted on a total of 30 newborn calves divided equally into control and experimental groups (15 calves each). The use of lactobact-ARZ improved clinical and biochemical blood parameters; the Hb concentration tended to increase in the experimental groups of calves. Specifically, the hemoglobin level (HGB) increased by 11.2 % compared with that in the control groups, and this difference was statistically significant ( $p < 0.05$ ). When the erythrocyte group of red blood cells, erythrocytes (RBCs), which are responsible for hemoglobin transport and metabolic processes in tissues and organs, was analyzed, the number of erythrocytes in the experimental group %was significantly greater (14.6 %) than that in the control group ( $p < 0.05$ ). The hematocrit level on the 15th day of the experiment in the calves of the experimental groups was 30.14 %, which was 9.2 % higher than that in the control group (27.85 %,  $p < 0.05$ ). The number of leukocytes (WBCs) decreased by 5.2 % compared with that in the control groups, whereas in the control group, the lymphocyte content increased to  $78.7 \pm 4.50$  %, i.e., by 5.5 %. Compared with those in the control groups, the level of IgG immunoglobulin increased by 13.6 %, and the level of IgM increased by 2.7 %. The increase in total protein concentration compared with that of the control was 11.3 %. A decrease in ALT and AST indicators was observed, which may indicate an improvement in the general condition of the calves in the experimental group compared with those in the control group during the experiment: ALT by 9.4 % and AST by 9.3 %.

**Keywords:** Calves, Lactobacillus-ARZ probiotic, Hematological status

## Introduction

The use of intensive technologies in industrial livestock farming leads to increased stress sensitivity in animals, a decrease in their immune status, and the development of pathological conditions. To increase the survival rate of young animals, including by reducing their morbidity and mortality from diseases, feed additives containing probiotics, prebiotics, and/or other components that stimulate the immunological resistance of the animal's body, growth, and productivity are being developed. Probiotics are stabilized cultures of microbes that are symbiotic with normal gastrointestinal microflora or their fermentation products. They suppress the activity of pathogenic and opportunistic intestinal bacteria, increase the resistance of the animal's body, improve the assimilation of nutrients from the diet, and activate metabolic processes. Therefore, they are widely used to treat and prevent gastrointestinal diseases in animals, such as dysbacteriosis of various etiologies, dyspepsia,



gastroenteritis, etc. [1, 2, 3]. Probiotics are capable of activating the relationship between the digestive tract microflora and the state of the organism, inhibiting the development of pathogenic and opportunistic strains, having a growth-stimulating effect, and enhancing the body's immune system and the survival rate of calves [4,5]. Antibiotics, which are used as therapeutic agents in preparations to stimulate the growth and development of young animals, can accumulate in products obtained from animals, which may lead to allergies and dysbiosis in humans [6]. When used in excessive amounts, disorders of the normal function of the gastrointestinal tract in animals are observed, which affect their further growth and development [7, 8]. Young cattle of early age often experience digestive problems, leading to a weakening of the immune system, growth retardation, and decreased resistance to diseases [9, 10, 11]. The use of probiotic preparations is primarily aimed at improving the microbiological balance of the rumen and intestines, increasing immunoresistance, and enhancing the realization of the genetically determined potential for productivity in calves [12, 13, 14]. The qualitative and quantitative composition of blood is the most labile indicator of an organism's physiological state, accurately and quickly responding to sufficiently strong influences. Blood reflects changes occurring in the body; therefore, determining the quantitative and qualitative content of blood components has not only substantial diagnostic but also prognostic importance [15]. The purpose of this work was to study the influence of probiotics on the hematological status of newborn calves in the early postnatal period of development. The aim of this study was to investigate the influence of probiotic preparations on the dynamics of hematological indicators in calves under the conditions of the "Bayserke-Agro" LLP.

### Materials and Methods

To conduct the experiment according to the principles of analogs, newborn Holstein calves were selected and divided into two groups, which were kept under identical housing and feeding conditions. The calves in the experimental groups were given probiotic preparations orally with milk twice a day for 15 days after birth. The first control group did not receive probiotics. The second experimental group received a probiotic at a dose of 6 grams of lyophilized probiotic with milk. The calves in the control and experimental groups were observed for 15 days. All animals were continuously monitored throughout the study (temperature, pulse, and respiration).

During the study, an AbakusJunior hematological analyzer (Austria, 2013 model) was used. A BK1200 biochemical analyzer (China, 2024 model) was also utilized. A STATFAX-2100 spectrophotometer (reader) (verified until 19.05.2024) was used. The biochemical reagents used were produced by HUMAN (Germany). Immunological IgG, IgM and IgE test systems were produced by "VECTOR-BEST" CJSC (Novosibirsk, Russia), "IMMUNOTEX" CJSC (Moscow, Russia), and "Alkor BIO" CJSC (Saint Petersburg, Russia).

To study immune status, as well as other physiological indicators, blood samples were taken from all experimental calves via gel vacuum tubes from the jugular vein (Wu et al., 2021) at 3 days of age and after 15 days. The hematological blood test was conducted at the "T-Helper" laboratory of the "Omicron 3D" Limited Liability Partnership.

Independent t tests were used to identify statistically significant differences between the control and experimental groups. The results were considered significant at a level of  $p < 0.05$ . The levels of significance indicated for differences were  $p < 0.05$ ,  $p < 0.01$ , and  $p < 0.001$ . The analysis was performed via Microsoft Excel software.

*Ethical approval.* The methodology for conducting research on laboratory animals and birds was approved by the Bioethics Commission of the Non-Profit Joint-Stock Company "Kazakh National Agrarian Research University" (protocol dated November 11, 2022). All procedures complied with the biosafety requirements and ethical principles outlined in the European Convention for the Protection of Vertebrate Animals Used for Experimental and Scientific Purposes (Strasbourg, 1987).

**Results and Discussion**

This study aims to update the use of domestic probiotics in livestock farming to improve herd survival during the critical period from birth to the first month of life and to overcome the period of active intestinal infections from birth to the first month of life. In the first five days, the calf may contract a viral intestinal infection, which can cause death during this difficult period in the life of newborn calves.

In Table 1, the results of the morphological and biochemical blood indicators of calves raised in the "Bayserke-Agro" LLP, located in the Almaty region, Talgar district, Arkabay village (15 calves in the experimental group, 15 calves in the control group), are presented.

**Table 1.** Morphological and biochemical blood parameters of calves raised in the "Bayserke-Agro" LLP.

| Indicators                     | Research timeframe                              |              |                                              |               |
|--------------------------------|-------------------------------------------------|--------------|----------------------------------------------|---------------|
|                                | Background level at the start of the experiment |              | At the end of the experiment on the 15th day |               |
|                                | control                                         | experimental | control                                      | experimental  |
| Hemoglobin (HGB)               | 85,6±7,54                                       | 95,6±5,62    | 94,42±10,36**                                | 105,77±10,32* |
| Red blood cells (RBC)          | 5,92±2,35                                       | 6,416±2,69   | 6,11±3,88                                    | 8,95±3,54     |
| Hematocritis (HCT)             | 24,18±6,02                                      | 25,44±6,25   | 27,85±4,34**                                 | 30,14±2,52*   |
| Platelets (PLT)                | 125,6±2,2                                       | 99,2±2,54    | 265,71±65,87                                 | 118,44±82,51  |
| Leukocytes (WBC)               | 12,86±5,01                                      | 11,78±4,52   | 17,60±4,65**                                 | 9,19±2,98*    |
| Granulocytes (GRA %)           | 35,6±12,35                                      | 33,6±10,58   | 23,71±5,98                                   | 43,88±15,24   |
| Eosinophils (EO %)             | 2,8±1,02                                        | 2,3±0,9      | 4,14±1,26                                    | 3,44±1,04     |
| Monocytes and others (MID %)   | 3,0±1,25                                        | 2,9±1,65     | 9,28±2,36                                    | 8,88±2,54     |
| Lymphocytes (LIM %)            | 57,2±13,25                                      | 61,0±10,24   | 78,7±4,5                                     | 43,66±13,25*  |
| Erythrocyte sedimentation rate | 1                                               | 1            | 1                                            | 1             |
| Glucose (Glu)                  | 4,7±0,42                                        | 5,10±0,52    | 2,25±0,84                                    | 5,5 0,64      |
| Alanine aminotransferase (ALT) | 37,34±6,54                                      | 42,06±4,25   | 33,65±5,42                                   | 31,84±4,01    |



|                                  |              |              |              |              |
|----------------------------------|--------------|--------------|--------------|--------------|
| Aspartate aminotransferase (AST) | 29,54±5,14   | 26,31±4,52   | 28,51±4,69   | 26,65±4,25   |
| Urea (UREA)                      | 4,91±1,85    | 8,70±3,54    | 3,6±0,98     | 3,96±2,14    |
| Creatinine (CREAT)               | 68,53±6,58   | 66,98±5,47   | 64,53±6,21   | 67,89±5,87   |
| Total Protein (Total Pro)        | 68,98±3,36   | 67,30±4,25   | 78,3±5,26    | 68,81±3,54   |
| Bilirubin (Bil)                  | 7,44±1,02    | 7,35±0,89    | 7,01±0,82    | 7.02±1,01    |
| Alkaline phosphatase (ALP)       | 248,56±23,54 | 244,65±23,65 | 233,97±23,69 | 226,6±23,41* |
| IgG                              | 10,36±1,35   | 10,25±1,20   | 11.42±2,10   | 15,54±1,52*  |
| IgM immunoglobulin               | 0,99±0,09    | 0,90±0,09    | 0.91±0,78    | 1,89±0,07    |
| IgE immunoglobulin               | 14,81±6,24   | 14,85±6,54   | 15.39±5,47   | 15.21±5,01   |

Note: \*P<0.05; \*\*P<0.01; \*\*\*P<0.001-Statistical significance for the compared indicators.

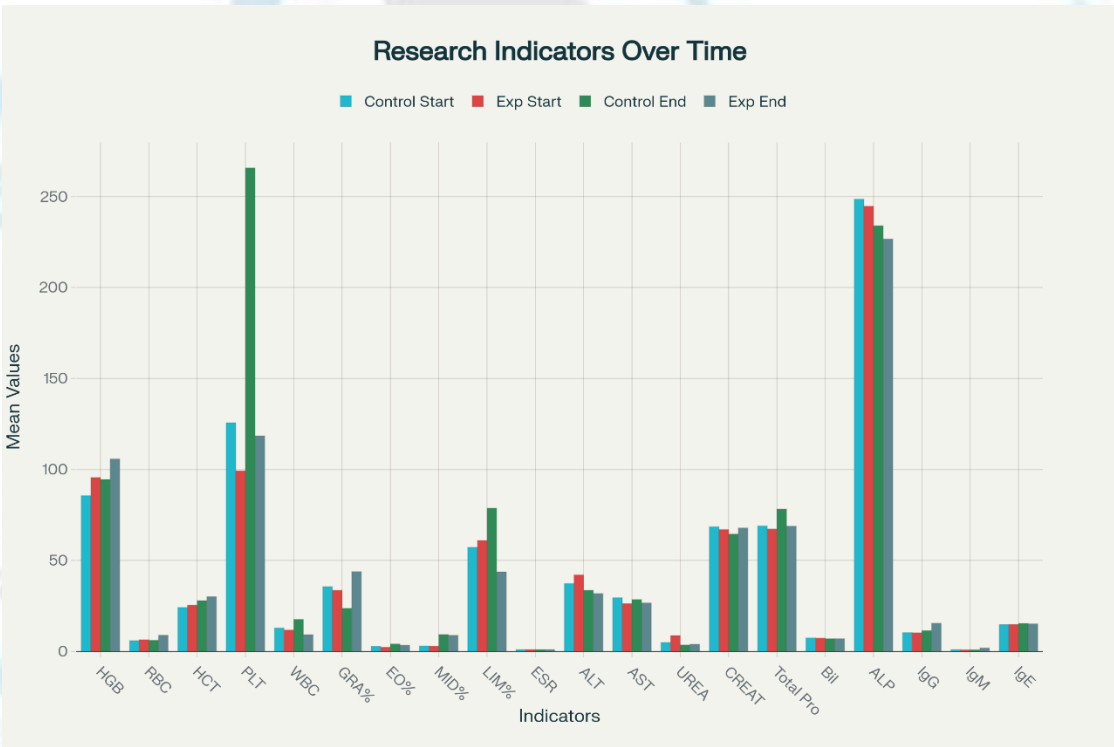


**Figure 1.** Grouped bar chart of blood parameters in the control and experimental groups at the beginning and end of the experiment

As shown in Figure 1, the results of the present study revealed that the Hb concentration tended to increase in the experimental groups of calves. Thus, the hemoglobin level (HGB) increased by 11.2 % compared with that in the control groups ( $P<0.05$ ). The erythrocyte group of red blood cells, erythrocytes (RBCs), which are responsible for hemoglobin transport and tissue and organ metabolic processes, increased by 14.6 % in the experimental group % compared with the control group ( $P<0.05$ ). The hematocrit level on the 15th day of the experiment in the experimental groups was 30.14 %, which was 9.2 % higher than that in the control group (27.85 %). The number of leukocytes (WBCs) decreased by 5.2 % compared with that in the control groups ( $P<0.05$ ). In the control group, the lymphocyte content increased to  $78.7\pm4.50$  %, i.e., by 5.5 % ( $p<0.01$ ). Compared with

those in the control groups, the level of IgG immunoglobulin increased by 13.6 %, and the level of IgM increased by 2.7 % ( $P<0.05$ ). A more pronounced increase in hemoglobin levels was observed in the experimental groups than in the control groups, which undoubtedly led to higher indicators of the total oxidative capacity of blood cells. Compared with that in the control group, the leukocyte count in the experimental group tended to decrease to within the range of normal physiological values. Compared with the control calves, the calves in the experimental group were less prone to intestinal infections and adapted faster to the animal housing conditions.

A slight increase in the level of total protein in the blood serum of the experimental group compared with the control group was observed, which, in general, had a positive effect on the immune status of the calves. Overall, a positive trend was observed in the experimental groups compared with the control groups. Thus, the increase in the concentration of total protein compared with that of the control was 11.3 %.

A decrease in ALT and AST indicators was observed, which can indicate an improvement in the general condition of the calves in the experimental group compared with those in the control group during the experiment: ALT by 9.4 % and AST by 9.3 %. The reduction in the hepatic enzymes ALT and AST in the experimental calves is indicative of improved liver function and general health status, which has also been documented in animal probiotic studies [5,7]. ALP practically does not change, which is associated with the formation of bone tissue.

Our results support the findings of a wider literature review emphasizing the benefits of adding probiotics to improve intestinal health, nutrient absorption, and immune function in young animals [9,12]. These improvements likely contributed to the observed positive trends in clinical and biochemical blood parameters. Overall, we noted a positive trend in general clinical, biochemical, and immunological laboratory indicators, which was more pronounced in the experimental group than in the control group.

### Conclusion

Notably, under the influence of the probiotic preparation lactobact-ARZ, positive changes in hematological and biochemical blood parameters were observed in calves. This is evidenced by an increase in the immune status of young animals during the early postnatal period of development.

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## PHYTOTHERAPY IN VETERINARY MEDICINE: PERSPECTIVES FOR THE TREATMENT OF INFECTIOUS DISEASES

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### Abstract

Infectious diseases of farm animals remain among the most pressing problems in modern veterinary medicine, causing significant economic damage to animal husbandry and posing a potential risk to public health. The widespread use of antibiotics has contributed to the development of resistant strains of microorganisms, which has become a global challenge. In this context, phytotherapy is being actively studied as an alternative and complementary approach in veterinary practice. The present review summarizes international and national research on the use of medicinal plants in the prevention and treatment of infectious diseases in animals. Particular attention has been given to the pharmacological effects of herbal preparations, including their antimicrobial, anti-inflammatory, immunomodulatory, and regenerative properties. A comparative analysis of phytotherapy and antibiotic therapy demonstrated the strengths and limitations of both approaches and substantiated the prospects of their combined use to increase treatment efficiency and reduce the risks associated with antibiotic resistance. For Kazakhstan, phytotherapy represents a promising direction, given the country's rich plant resources and the growing need for ecologically safe livestock technologies. This article emphasizes the necessity of clinical trials, standardization of phytopreparations, and their integration into national veterinary protocols.

**Keywords:** Phytotherapy, Veterinary medicine, Infectious diseases, Antimicrobial resistance, Medicinal plants, Livestock health, Kazakhstan

### Introduction

Infectious diseases in animals remain among the most urgent challenges in contemporary veterinary medicine, causing considerable economic losses to livestock production and posing a potential risk to public health through zoonotic infections and the food chain. The widespread prevalence of bacterial and viral diseases reduces animal productivity, increases the costs of treatment and prevention, and necessitates the development of innovative therapeutic approaches. In the context of intensified animal husbandry and the globalization of the livestock trade, the risk of transboundary spread of infections is increasing, which makes their control a priority task of modern veterinary science [1].

For decades, antibiotics and synthetic chemotherapeutics have been the principal tools for combating bacterial infections. Its use has significantly decreased animal mortality, improved livestock profitability, and contributed to food security. However, the extensive and often uncontrolled application of antibiotics has led to the emergence of resistant microbial strains, which has become a global concern. According to the World Health Organization (WHO) and the World Organization for Animal Health (WOAH), antimicrobial resistance constitutes a serious threat to human and animal health as well as to the global economy [2]. Veterinary medicine plays a critical role in this context, since excessive antibiotic use in animal husbandry not only promotes the transmission of resistant strains in animals but also facilitates their transmission to humans [3]. In addition to the challenge of resistance, antibiotic usage is associated with several further limitations. These include the risk of



drug residues in meat, milk, and other animal-derived products, which endangers consumers and undermines the export potential of livestock production. Adverse effects on the intestinal microbiota, hepatotoxicity and nephrotoxicity, as well as environmental consequences such as contamination of soil and water with antibiotics, reinforce the urgency of seeking alternative methods for the treatment and prevention of infectious diseases [4]. Against this background, growing attention is being directed toward phytotherapy—an approach based on the use of medicinal plants and their biologically active compounds. In contrast to synthetic pharmaceuticals, herbal preparations demonstrate multifactorial activity: they can suppress pathogenic microflora, exert anti-inflammatory and immunomodulatory effects, and stimulate the body's natural defense mechanisms. Their key advantages include ecological safety, minimal side effects, and the possibility of long-term use without the risk of inducing microbial resistance [5]. International experience confirms that phytotherapy has been successfully applied in various regions of the world. Thus, the global problem of antimicrobial resistance and the urgent need for safe and environmentally friendly approaches for the treatment of animal infectious diseases determine the relevance of studying phytotherapy. Assessing its potential in comparison with traditional methods of therapy makes it possible to identify both the advantages and the limitations of herbal medicines and to outline the prospects for their application in modern veterinary medicine [6].

The aim of this review is to summarize current research on the application of phytotherapy in the treatment of infectious animal diseases and to determine its role within the integrated system of veterinary therapy.

### Materials and methods

In preparing this review, a comprehensive analysis of national and international scientific sources published between 2000 and 2024 was conducted, covering data on the use of phytotherapy in veterinary medicine, its effectiveness, and its limitations. The database included international scientific platforms such as PubMed, Scopus, and Web of Science, as well as dissertations, monographic works, and regulatory-methodological documents published within the post-Soviet space. Source selection was carried out via the following keywords: phytotherapy, veterinary medicine, infectious diseases, medicinal plants, and antimicrobial resistance.

### Results

**International experience in the use of phytotherapy.** In European countries, phytotherapy has established a strong position in veterinary practice, particularly in organic livestock farming where the use of antibiotics is restricted [7]. In Germany, herbal preparations are used for the treatment of mastitis in dairy cows as well as for gastrointestinal and respiratory diseases in calves [8]. In France, phytotherapy is used to alleviate stress conditions and enhance the immune resistance of animals, whereas in Switzerland, it has been officially included in national recommendations for organic farms, contributing to a reduction in antimicrobial usage. Thus, the European experience demonstrates a consistent trend toward ecologically sustainable livestock production and a reduction in antimicrobial resistance [9]. In the United States and other developed countries, phytotherapy is increasingly considered an adjunct to conventional therapy, particularly in the context of antibiotic resistance, and as a means of reducing adverse side effects of treatment [10]. Herbal complexes containing garlic, echinacea, and turmeric are commonly used for their immunomodulatory and antimicrobial properties. Clinical studies conducted by American researchers are primarily aimed at dosage standardization, the development of safety protocols, and the evaluation of economic efficiency in the use of plant-based preparations in animal husbandry

[11]. In Asia, phytotherapy is an integral element of veterinary medicine that is largely shaped by the long-standing traditions of folk healing. In China, traditional remedies, including ginseng, ginger, garlic, and turmeric, are widely employed and exhibit pronounced antibacterial and immunostimulatory effects [12]. In India, phytotherapy is applied in the treatment of infectious diseases in cattle, goats, and poultry, with state-level initiatives directed toward incorporating herbal remedies into preventive and therapeutic veterinary programs. These countries demonstrate rich ethnoveterinary heritage based on the systematic use of medicinal plants [13]. In Africa and other developing regions, phytotherapy occupies a special place in veterinary practice as an affordable alternative to synthetic drugs. Small- and medium-scale farms rely on indigenous medicinal plants for the treatment of parasitic and infectious diseases. Ethnoveterinary knowledge is transmitted across generations; however, the absence of standardized protocols and unified dosages remains a challenge. Nevertheless, studies indicate that the use of essential oils and herbal mixtures can successfully control gastrointestinal infections and improve animal resistance [14]. In Russia, phytotherapy in veterinary medicine is considered a promising direction, particularly in the context of combating antimicrobial resistance and improving the quality of livestock production. A number of studies have focused on the use of herbal preparations in cattle and poultry diseases. Domestic researchers have demonstrated the effectiveness of phytobiotics derived from garlic, onion, and echinacea in stimulating immune responses and reducing the bacterial load in respiratory infections. In poultry farming, herbal supplements such as sage, thyme, and wormwood are actively being investigated for their antimicrobial and antioxidant properties, which contribute to improved productivity and flock survival. The application of phytotherapy in Russia is still limited by the scale of research and the absence of large-scale production of standardized herbal preparations, but the field is gradually developing within agricultural universities and research institutes [15]. In Kazakhstan, phytotherapy has not yet achieved widespread implementation in veterinary practice. However, with the growth of organic livestock farming and the increasing need to reduce antibiotic use, interest in this approach is steadily increasing. In practice, herbal preparations are mainly applied in the treatment of gastrointestinal infections in young animals and skin diseases and as part of complex therapy for respiratory disorders. Native plants such as wormwood (*Artemisia*), St. John's wort (*Hypericum*), and calendula (*Calendula officinalis*) show considerable potential due to their antimicrobial, anti-inflammatory, and immunomodulatory effects. Several Kazakhstani studies have demonstrated the potential of plant extracts for the treatment of gastrointestinal diseases in calves and sheep, as well as their use in poultry prophylaxis. Further development in this field requires the standardization of herbal preparations and their incorporation into national veterinary guidelines [16]. Thus, international experience highlights the importance of phytotherapy as an effective tool for the prevention and treatment of infectious animal diseases. The use of medicinal plants reduces the reliance on antibiotics, lowers the risk of antimicrobial resistance, and supports the advancement of ecologically sustainable livestock production technologies [17].

**Infectious Diseases Amenable to Phytotherapy.** Phytotherapy in veterinary practice is most widely applied in the management of mild to moderately severe infectious diseases in animals. One of the primary areas of its use is respiratory disorders, such as bronchopneumonia and catarrhal inflammation of the respiratory tract. Herbal preparations based on liquorice (*Glycyrrhiza glabra*), thyme (*Thymus vulgaris*), echinacea (*Echinacea purpurea*), and garlic (*Allium sativum*) demonstrate expectorant, anti-inflammatory, and antimicrobial properties, thereby alleviating clinical symptoms and accelerating recovery. Clinical observations indicate that phytotherapy



may be employed as an adjunct to conventional therapy for respiratory infections, enhancing its efficacy and reducing treatment duration [18]. Considerable attention has also been given to the use of Phyto preparations in gastrointestinal diseases of infectious etiology. Extracts of oak (*Quercus robur*) and St. John's wort (*Hypericum perforatum*), as well as garlic and onion, possess pronounced antimicrobial and astringent properties, making them effective at reducing the severity of diarrhea in calves and lambs. Some studies note that herbal supplements containing tannins contribute to the restoration of the intestinal microbiota and reduce the risk of secondary bacterial complications, which is of particular importance for young farm animals [19]. Skin diseases and inflammatory conditions, such as dermatitis and wound infections, also represent targets for phytotherapeutic intervention. Infusions and ointments based on calendula (*Calendula officinalis*), chamomile (*Matricaria chamomilla*), and aloe (*Aloe vera*) exhibit strong antiseptic and regenerative effects, promoting tissue repair and preventing the development of secondary infections. Experimental studies have confirmed that herbal preparations shorten skin recovery periods and reduce the need for local antibiotic treatments [20]. Phytotherapy also plays an important role in combating parasitic diseases, including helminthiases. Preparations derived from wormwood (*Artemisia absinthium*), tansy (*Tanacetum vulgare*), and garlic have traditionally been used for animal deworming and have demonstrated significant antiparasitic activity. Contemporary research has confirmed that plant-based remedies can reduce the intensity of infestations without inducing resistance in pathogens, which is a key advantage over chemical anthelmintics. Furthermore, such phytopreparations show immunomodulatory activity, enhancing the natural resistance of animals to reinfection [21]. Thus, phytotherapy exerts multifactorial effects on infectious diseases in animals by combining antibacterial, antiviral, anti-inflammatory, and immunomodulatory effects. The use of herbal preparations not only alleviates the clinical course of diseases but also improves the efficacy of conventional therapy and reduces the risk of antimicrobial resistance, making this approach particularly promising in modern veterinary medicine [22].

**Mechanisms of infection and the role of phytotherapy** The infectious process in animals involves a complex cascade of biological reactions that includes pathogen entry, active replication, disruption of metabolic pathways, development of inflammatory responses, and the formation of intoxication syndromes. These pathological alterations lead to reduced productivity, weakened immune function, and increased susceptibility to secondary infections. In this context, phytotherapy is of particular interest, as it acts simultaneously on several key stages of pathogenesis [23]. Flavonoids and alkaloids contained in medicinal plants exert pronounced antimicrobial effects by inhibiting the growth and replication of pathogenic bacteria and viruses. Essential oils, which are rich in phenolic compounds, suppress pathogenic microflora and prevent the establishment of resistant microbial colonies in the gastrointestinal and respiratory systems of animals. Polysaccharides and vitamins present in many plants demonstrate immunomodulatory properties: they activate nonspecific host resistance, increase the phagocytic activity of leukocytes, and increase serum antibody levels. Tannins, in turn, have anti-inflammatory and astringent effects, which are particularly important in infectious diarrheal syndromes, as they reduce the severity of inflammation and accelerate the restoration of the intestinal mucosa [24].

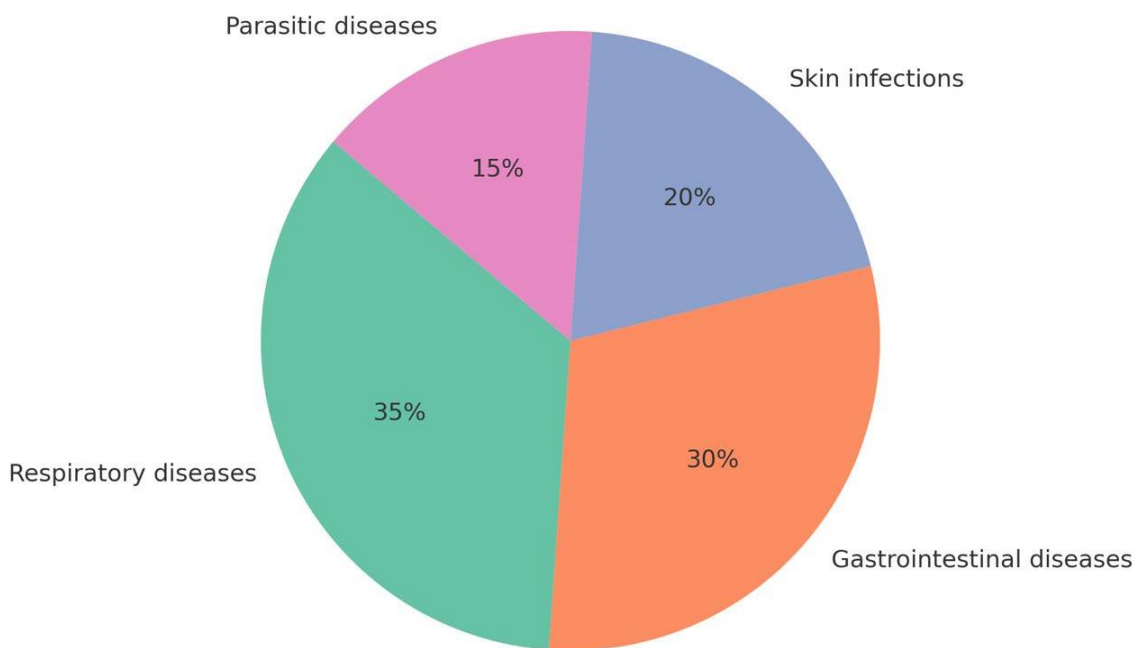


**Figure 1.** Application of phytotherapy for groups of diseases in farm animals (review-based assessment).

The largest share of phytotherapy use is associated with respiratory diseases (35 %). This is explained by the high prevalence of respiratory disorders—such as bronchitis, pneumonia, and catarrhal inflammation—particularly in young animals, which require mild antiseptic and anti-inflammatory remedies to alleviate symptoms and prevent complications. Herbal preparations containing active phytochemical components, such as essential oils and flavonoids, have demonstrated potential in slowing or preventing the spread of respiratory pathogens [25]. Gastrointestinal diseases ranked second (30 %). Diarrhea and other infections of the digestive tract constitute major causes of productivity losses in livestock. Ethnographic and field studies have shown that herbal remedies, including locally available plants, are employed to restore the microbiota balance, reduce inflammation, and prevent secondary bacterial complications [26]. Skin infections and inflammatory conditions account for approximately 20 % of cases. In this category, a wide range of regional practices report the use of ointments and tinctures based on calendula (*Calendula officinalis*), chamomile (*Matricaria chamomilla*), St. John’s wort (*Hypericum perforatum*), and other plants, which exhibit antiseptic, antimicrobial, and regenerative properties, thereby reducing inflammation and accelerating tissue repair [27]. Parasitic diseases represent a smaller share (15 %), reflecting the limited number of experimental and clinical studies. Nevertheless, *in vitro* investigations have demonstrated the activity of plant extracts such as *Azadirachta indica* (neem) and *Tithonia diversifolia* against parasites of the genus *Hemonchus contortus*, underscoring the potential of plants as anthelmintic agents [28].

### Comparison of Phytotherapy with Other Treatment Methods.

Phytotherapy possesses a number of distinctive features that make it advantageous compared with antibiotics and synthetic pharmaceuticals. It ensures high ecological safety, minimal side effects, and the possibility of long-term administration without the risk of toxic accumulation. Furthermore, medicinal plants exert a multifactorial influence: they suppress the growth of pathogenic microflora, stimulate immune responses,



and accelerate regenerative processes, thereby serving as a valuable tool in the prevention and treatment of infectious diseases [29]. At the same time, herbal preparations have certain limitations. Their effects develop more slowly than those of antibiotics do, and their efficacy may vary depending on the quality of the raw plant material, storage conditions, and methods of preparation. An additional barrier is the lack of standardized dosages, which hinders the widespread introduction of phytotherapy into veterinary practice. In contrast, antibiotics provide a rapid and reproducible therapeutic effect; however, their use is associated with the risk of developing resistant strains of pathogens and with serious adverse side effects [30]. The most promising direction is the integration of phytotherapy with conventional treatment methods. This combined approach makes it possible to reduce antibiotic dosages, preserve their therapeutic efficacy, and simultaneously minimize the likelihood of resistant strain formation. The concurrent use of plant-based and synthetic agents enhances the overall resistance of animals, decreases the burden on the immune system, and renders therapy more biologically safe. From a long-term perspective, this approach may represent an effective strategy for combating antimicrobial resistance [31].

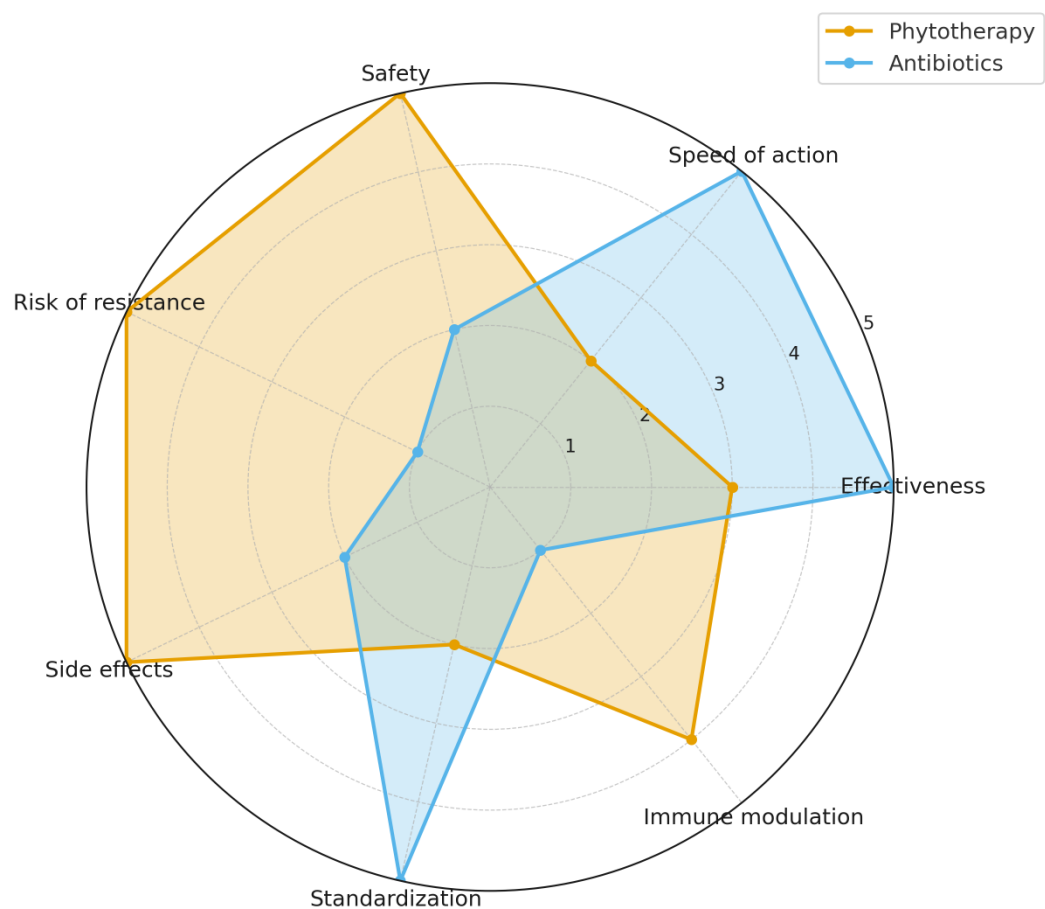


**Figure 2.** Comparative characteristics of phytotherapy and antibiotics in veterinary medicine (radar chart).

In Figure 2, a radar chart is presented that illustrates the relative strengths and weaknesses of two therapeutic approaches—phytotherapy and antibiotic therapy. Seven key parameters were selected for analysis to determine the effectiveness and applicability of these methods in veterinary practice: efficacy, speed of action, safety, risk of resistance development, side effects, degree of standardization, and immunomodulatory effects [32]. Antibiotics occupy leading positions in terms of efficacy and speed of action, which is explained by their targeted antimicrobial activity and the rapid clinical response they provide in cases of acute infection. In addition, their high level of standardization is ensured by industrial quality control and unified dosing regimens. However,

significant limitations of antibiotic therapy remain: pronounced side effects (including disruption of the microbiota and toxic effects on the liver and kidneys) and a high risk of resistance development in microorganisms, which make their long-term use unsafe [33]. In contrast, phytotherapy has stronger performance in aspects such as safety, absence of resistance risk, minimal side effects, and the presence of immunomodulatory properties. Herbal preparations act gently on animals, stimulate natural defense mechanisms, and rarely cause complications. Moreover, phytotherapy results in a lower speed of action and standardization, which is associated with variability in the content of biologically active compounds in raw materials and the relatively slow onset of therapeutic effects [34]. The differences visualized in the diagram highlight the necessity of an integrated approach: antibiotics remain indispensable for severe and acute infections, whereas phytotherapy represents a promising tool for prevention, supportive therapy, and reducing the overall antibiotic burden. The combined use of both methods may become an effective strategy for modern veterinary medicine, allowing high therapeutic efficacy to be maintained, resistance risks to be reduced, and the biological safety of livestock products to be ensured [35].

### Discussion and Conclusion

The analysis of the literature and international experience indicates that phytotherapy represents a significant and increasingly relevant direction in contemporary veterinary medicine, particularly in the context of the global challenge of antimicrobial resistance. Unlike traditional chemotherapeutic agents, medicinal plants exhibit a broad spectrum of pharmacological activities, including antimicrobial, antiviral, anti-inflammatory, immunomodulatory, and regenerative effects. These properties make phytotherapy a valuable tool for both the prevention and treatment of infectious diseases in farm animals. A comparative assessment of phytotherapy and antibiotic therapy demonstrated that herbal preparations are inferior in terms of the speed and predictability of therapeutic effects; however, they considerably outperform antibiotics in terms of parameters such as safety, ecological compatibility, and the absence of resistance development in microorganisms. Moreover, the use of phytotherapy reduces the toxic burden on animals and decreases the likelihood of residual drug contamination in food products. These factors are particularly relevant in the context of organic livestock farming, which is oriented toward the production of ecologically safe products. International experience from Europe, Asia, Africa, and the United States confirms the effectiveness of integrating phytotherapy into veterinary treatment regimens. Reported benefits include a reduction in antibiotic use, improvement in animal immune status, and an overall increase in productivity. Moreover, the need for standardization of phytopreparations, establishment of optimal dosages, and development of clinical application protocols remains evident. The availability of such studies in developed countries reflects the growing interest in herbal remedies as either alternatives or complementary measures to synthetic drugs. In Kazakhstan, phytotherapy remains insufficiently developed despite the considerable resource potential of native flora. Plants traditionally used in folk medicine—such as wormwood (*Artemisia*), calendula (*Calendula officinalis*), St. John's wort (*Hypericum perforatum*), and chamomile (*Matricaria chamomilla*)—possess proven antimicrobial and immunomodulatory properties and could be effectively integrated into veterinary practice. However, the implementation of phytotherapy in official preventive and therapeutic frameworks requires the conduction of clinical trials, the development of standardized methodologies, and its incorporation into the regulatory and legal framework of veterinary medicine. Therefore, phytotherapy in veterinary practice should be considered a promising complement to existing methods for managing infectious



animal diseases. Its application contributes to reducing antibiotic usage, enhancing animal resistance to infections, and fostering the development of ecologically sustainable livestock production systems. A particularly promising direction for future research is the development of combined treatment regimens that incorporate rational combinations of phytotherapeutic and synthetic agents, thereby improving overall therapeutic efficacy and minimizing the risk of microbial resistance.

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## DEVELOPMENT OF NANOEMULSION-BASED PATCH FORMULATIONS CONTAINING *PLANTAGO LANCEOLATA* AND *ALTHAEA OFFICINALIS* EXTRACTS FOR THE TREATMENT OF COUGH AND SORE THROAT

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### Abstract

In recent years, plant-based extracts have garnered considerable attention for their applications in healthcare, with a corresponding increase in the number of related studies. *Plantago lanceolata*, which possesses anti-inflammatory, antibacterial, and antitussive properties, has long been used to treat coughs and the common cold. *Althaea officinalis* is also traditionally used as an emollient and a protective agent against respiratory tract irritation caused by chronic cough. Nevertheless, the limited stability and poor solubility of these plant extracts frequently limit their therapeutic application. Nanoemulsions (NEs) offer a promising approach to overcome these limitations by increasing their solubility, stability, and bioactivity while protecting active compounds from environmental degradation. Moreover, the formulation of nanoemulsion-based mucoadhesive patches (NE-MPs) can prolong the residence time at the site of action, form a protective barrier on mucosal surfaces, and alleviate local symptoms such as pain and irritation. The objective of this study was to develop an NE-MP incorporating extracts of *P. lanceolata* and *A. officinalis* for the treatment of cough and sore throat. The extract-loaded nanoemulsion (E-NE) was prepared using ethyl oleate, Labrasol, Kolliphor RH40, Pluronic F68, Span 80, and ultrapure water. Moreover, NaCMC, gelatin, and glycerin were used to prepare mucoadhesive patches. *In vitro* characterization studies were performed for the NE and NE-MP formulations. The average droplet sizes of the blank and extract-loaded NEs were  $112.63 \pm 2.62$  nm and  $115.96 \pm 3.90$  nm, respectively, with PDI values  $<0.3$ , confirming a monodisperse distribution. Additionally, the NEs exhibited Newtonian flow behavior. FT-IR analysis demonstrated the molecular-level dispersion of extracts within the formulations. The pH values of both the NE and NE-MP formulations were suitable for mucosal application. Overall, NE and NE-MP systems containing *P. lanceolata* and *A. officinalis* extracts were successfully prepared, highlighting their potential as innovative dosage forms for the symptomatic treatment of cough and sore throat.

**Keywords:** *Althaea officinalis*, Cough, Mucoadhesive patch, Nanoemulsion, *Plantago lanceolata*

### Introduction

Respiratory disorders such as cough and sore throat remain among the most common health complaints and are often associated with infections or environmental irritants, and their effective management continues to draw scientific interest. In recent years, herbal extracts/active agents have gained increasing attention as alternative or complementary approaches for treating such conditions owing to their favorable safety profiles and multifaceted pharmacological activities.<sup>1</sup> *Plantago lanceolata*, commonly known as ribwort plantain, is an herbaceous species of the *Plantaginaceae* family and is widely distributed across Türkiye and many other regions. Traditionally, it has been used both externally and internally for a variety of ailments, including skin infections, wounds, asthma, bronchitis, and gastrointestinal disorders, and as an expectorant and anti-

inflammatory agent. Its anti-inflammatory, antibacterial, and antitussive properties have been reported in pharmacological research, highlighting its potential to reduce cough and cold symptoms. Clinical studies have also shown that *P. lanceolata* extracts significantly reduce respiratory symptoms in patients, confirming the traditional use of these plants. Furthermore, it has been reported that bioactive components such as acteoside and aucubin regulate inflammatory pathways and have antimicrobial activity against a wide range of microorganisms.<sup>2</sup>

Another medicinal plant with a long history of therapeutic use is *Althaea officinalis*, which belongs to the *Malvaceae* family. In Türkiye and other regions, its roots, leaves, and flowers are employed as emollients, demulcents, and protective agents in the treatment of cough, pharyngitis, gastritis, and mucosal irritation. *A. officinalis* has a high mucilage content that forms a protective film over mucosal surfaces, thereby reducing irritation and inflammation. Experimental and clinical studies have demonstrated its anti-inflammatory, antitussive, and gastroprotective effects, which are attributed mainly to its polysaccharide-rich extracts and flavonoid constituents. Notably, its polysaccharides attenuate cough reflexes in animal models with an efficacy comparable to that of nonnarcotic antitussive drugs.<sup>3</sup>

Although plant extracts have well-known therapeutic benefits, their clinical application is limited by poor water solubility and short-term stability, which hinders their effective delivery. NEs, colloidal systems formed by combining oil and water phases with the aid of surfactants, with droplet sizes typically ranging from 100 to 500 nm, offer a promising approach for overcoming these limitations. Depending on their droplet size, nanoemulsions may appear transparent or semitransparent. NEs are kinetically stable and resistant to variations in pH, temperature, and dilution. Brownian motion is the dominant force because of the nanoscale size of the droplets. This effectively prevents creaming and flocculation for extended periods. Consequently, nanoemulsions exhibit long-term physical stability. The very small droplet size of NEs increases the surface area, thereby enhancing absorption. NEs can contain hydrophilic and lipophilic compounds. NEs have been demonstrated to increase the solubility and bioavailability of active ingredients owing to their nanoscale droplets and the solubility-enhancing and absorption-enhancing components that are incorporated into their structure. NEs are suitable dosage forms for protecting plant extracts and active compounds from environmental factors, increasing their stability, solubility, and efficacy. Additionally, gel and mucoadhesive patch formulations can be used to maintain the NE at the application site for an extended period.<sup>4</sup>

The aim of this study was to create and characterize an NE-MP that contains extracts from *A. officinalis* and *P. lanceolata*, which are known to have antibacterial, antitussive, and anti-inflammatory properties for the treatment of cold.

## Materials and methods

### Preparation of Plant Extracts

The aerial parts of *P. lanceolata* and *A. officinalis* were separately powdered via a laboratory blender. For each plant, 10 g of powdered material was extracted with 250 mL of solvent (ethanol for *P. lanceolata* and methanol for *A. officinalis*) under continuous stirring on a magnetic stirrer at 600 rpm for 24 h at room temperature. Following extraction, the mixtures were filtered, and the solvents were removed via a rotary evaporator at 40 °C and 90 rpm. The obtained crude extracts were collected and stored in airtight containers at 2–8 °C, protected from light, until further use.



### Preparation of NEs

Blank nanoemulsions (B-NE, without extracts) and extract-loaded nanoemulsions (E-NE, containing *A. officinalis* methanolic extract and *P. lanceolata* ethanolic extract) were prepared using ethyl oleate (8 %), Labrasol (4 %), Span 80 (1 %), Kolliphor RH40 (1 %), Pluronic F68 (1 %), and ultrapure water. Initially, the oil phase (ethyl oleate and Span 80) and the aqueous phase (Kolliphor RH40, Pluronic F68, and ultrapure water) were prepared separately. The plant extracts were dissolved in Labrasol and subsequently incorporated into the oil phase, followed by magnetic stirring at 750 rpm for 5 min. The aqueous phase was then added dropwise to the

The oil phase was continuously stirred (750 rpm) to form a coarse emulsion. This coarse emulsion was further homogenized via a high-speed homogenizer (Ultra-Turrax; 27500 rpm, 6 min) and subjected to ultrasonication (55 % power, 18 min), resulting in a reduction in the droplet size to the nanoscale. B-NE was prepared via the same procedure but without the addition of plant extracts.

### Characterization of NEs

The NEs were first evaluated macroscopically to assess phase separation, followed by centrifugation at 3500 rpm for 15 min to further confirm their physical stability. The droplet size, polydispersity index (PDI) and zeta potential values of the NEs were determined via a Zetasizer Nano ZSP (Malvern Instruments, UK). The morphological characteristics of the NEs were examined via transmission electron microscopy (TEM). The pH values of the formulations were measured via a pH meter. Fourier transform infrared (FT-IR) spectroscopy was performed in the range of 4000–400  $\text{cm}^{-1}$  to analyze the functional groups of the components and possible interactions within the formulations. In addition, rheological analysis of the NEs was carried out at room temperature via a Brookfield RV DV2T viscometer.

### Preparation of NE-MPs

On the basis of preliminary studies, gelatin and NaCMC were selected as the polymeric components for patch preparation. The formulations were composed of 5 % (w/w) gelatin, 20 % (w/w) glycerin, and 1 % (w/w) NaCMC, with the remaining weight completed to 10 g via either B-NE or E-NE. Initially, NaCMC was dispersed into the NE under magnetic stirring (750 rpm) for 30 min. Separately, gelatin was dissolved in glycerin in a water bath at 70 °C and subsequently transferred to a magnetic stirrer. The NaCMC–NE mixture was then added to the gelatin solution under continuous stirring (750 rpm) and homogenized until a uniform mixture was obtained. The final formulation was cast into glass Petri dishes and allowed to dry at room temperature to form NE-MPs (B-NE-MPs and E-NE-MPs).

### Characterization of NE-MPs

NE-MPs were subjected to *in vitro* characterization to evaluate their physicochemical and mechanical properties. The patch thickness was determined by measuring 1  $\text{cm}^2$  samples via a digital caliper. For surface pH analysis, 1  $\text{cm}^2$  samples were immersed in flacons containing 4 mL of ultrapure water, and pH measurements were recorded after 15 min. Swelling studies were conducted by immersing 1  $\text{cm}^2$  patch samples in phosphate buffer (pH 6.8) at both room temperature and 37 °C. At predetermined time intervals, the samples were removed, blotted with filter paper to eliminate excess water, and weighed to calculate the swelling percentage. The test was continued until the patches underwent complete disintegration. FT-IR analysis was performed in the range of 4000–400  $\text{cm}^{-1}$  to identify functional groups and

potential interactions. Finally, the tensile strength and elasticity of the patches were assessed via a texture analyzer.

Statistical analysis

The statistical evaluation of the data was performed via SPSS Statistics 22.0 software (SPSS Inc., Chicago, USA). An independent t test was applied for the comparison of groups, and differences were considered statistically significant at  $p < 0.05$ .

Results

NEs were successfully prepared via high-energy methods, and their physicochemical characteristics were evaluated. The centrifugation test revealed no signs of creaming or phase separation in any of the formulations, indicating good physical stability. Droplet size analysis revealed that B-NE had an average size of  $112.63 \pm 2.62$  nm, whereas E-NE had a slightly larger size of  $115.96 \pm 3.90$  nm (Table 1). The polydispersity index (PDI) values were  $0.173 \pm 0.029$  and  $0.145 \pm 0.010$  for B-NE and E-NE, respectively. The zeta potential values were measured as  $-34.66 \pm 2.99$  mV for the B-NE and  $-23.79 \pm 0.92$  mV for the E-NE (Table 1).

**Table 1.** Droplet size, PDI and zeta potential values of the NEs (mean±SD; n=9)

| Formulation | Droplet size (nm) | PDI         | Zeta potential (mV) |
|-------------|-------------------|-------------|---------------------|
| B-NE        | 112.63±2.62       | 0.173±0.029 | -34.66±2.99         |
| E-NE        | 115.96±3.90       | 0.145±0.010 | -23.79±0.92         |

TEM images of the E-NE revealed spherical and uniformly distributed droplets at the nanoscale (Figure 1).

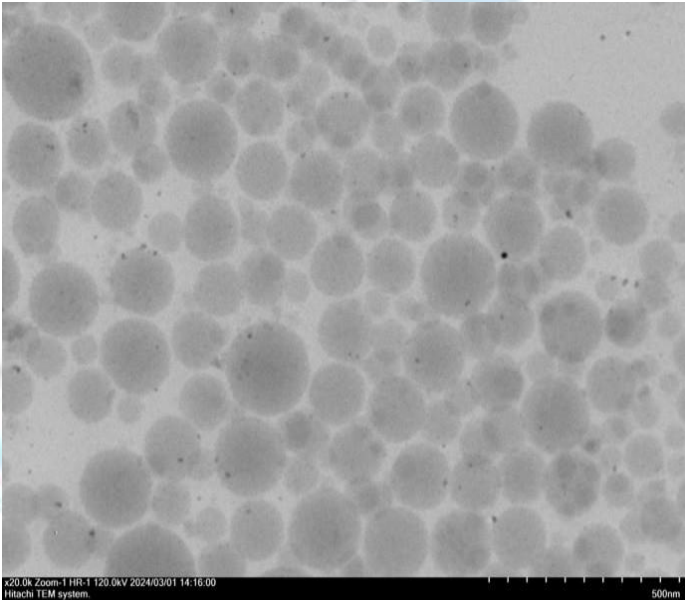


**Figure 1.** TEM image of E-NE.

The pH values of the formulations were  $5.14 \pm 0.02$  for B-NE and  $5.03 \pm 0.01$  for E-NE. The FT-IR spectra of the B-NE and E-NE are given in Figure 2.



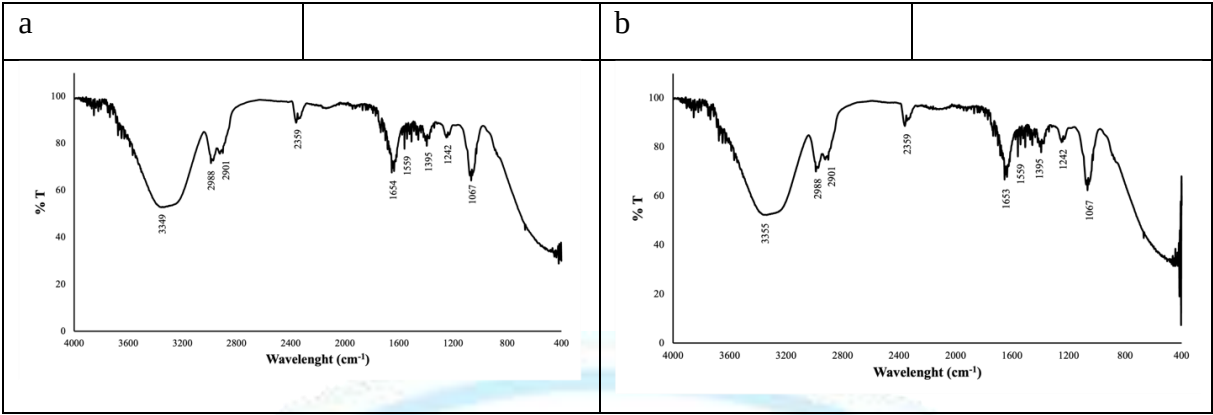


**Figure 2.** FT-IR spectra of B-NE and E-NE

The rheograms of B-NE and E-NE are shown in Figure 3. The viscosity values were  $3.01 \pm 0.05$  cP for the B-NE and  $2.81 \pm 0.13$  cP for the E-NE at a shear rate of  $1500 \text{ s}^{-1}$ .

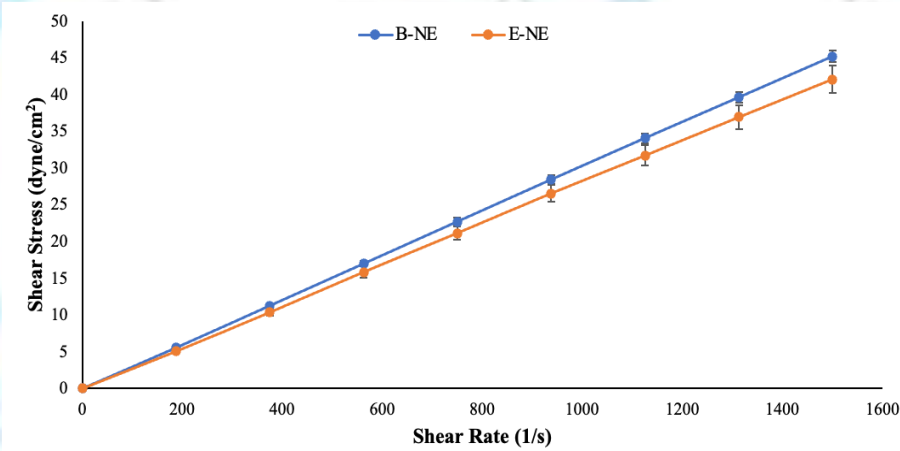


**Figure 3.** Rheograms of B-NE and E-NE

Digital photographs of the NE-MPs are presented in Figure 4, showing smooth and uniform surfaces.

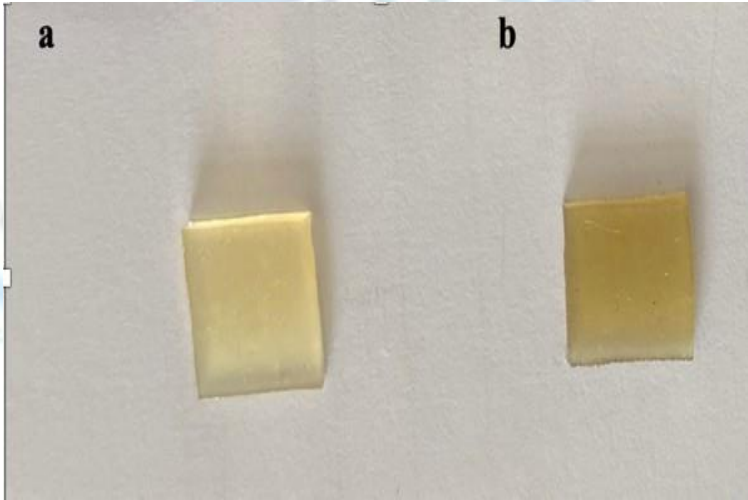


**Figure 4.** Digital photographs of B-NE-MP (a) and E-NE-MP (b)

The thickness measurements were  $0.39 \pm 0.01$  mm for the B-NE-MP and  $0.41 \pm 0.02$  mm for the E-NE-MP. B-NE-MP and E-NE-MP had surface pH values of  $6.21 \pm 0.03$  and  $6.25 \pm 0.07$ , respectively. The swelling profiles of NE-MPs at room temperature and  $37^\circ\text{C}$  are shown in Figure 5. The FT-IR spectra of the B-NE-MP and E-NE-MP are given in Figure 6. A tensile strength test was performed to determine the elasticity of the

patch formulations, and the % elasticity values were determined to be  $30.55 \pm 2.37$  and  $39.28 \pm 4.52$  for B-NE and E-NE, respectively.

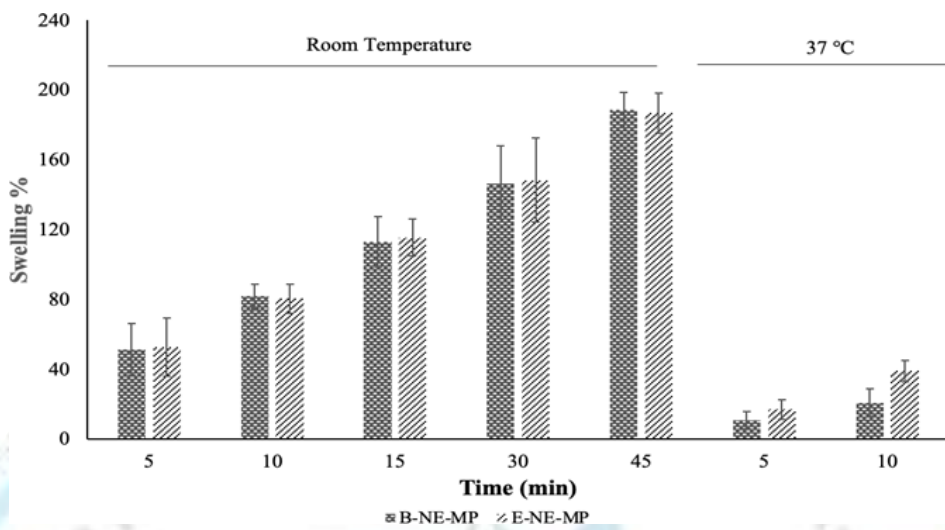


Figure 5. Swelling profiles of B-NE-MP and E-NE-MP.

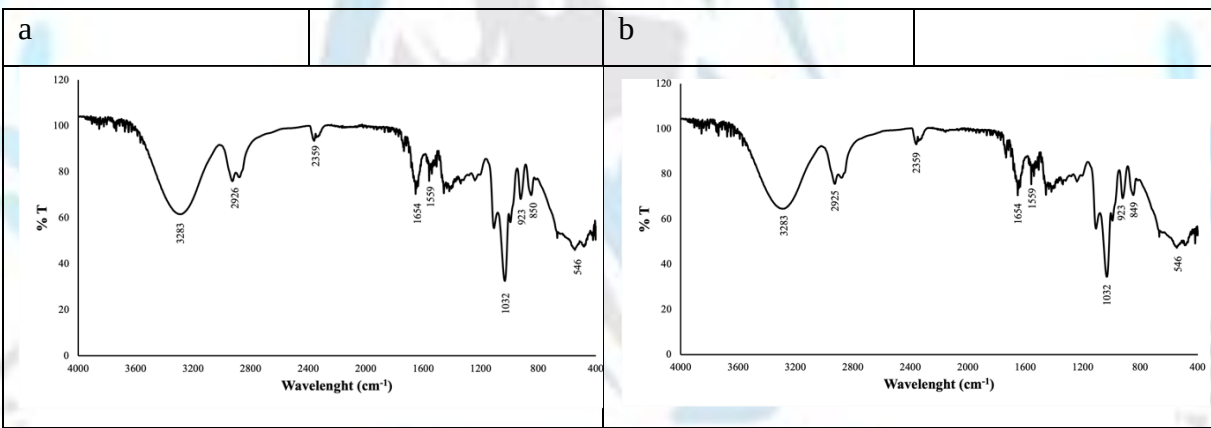


Figure 6. FT-IR spectra of B-NE-MP (a) and E-NE-MP (b).

### Discussion

The present study demonstrated that NE formulations containing extracts of *P. lanceolata* and *A. officinalis* could be successfully prepared via high-energy methods. Droplet size, PDI, and zeta potential measurements are essential for assessing the physical stability of NEs. The Brownian motion of nanosized droplets in NEs helps prevent physical destabilization caused by coalescence, creaming, and gravity separation.<sup>5</sup> The incorporation of the extracts into the NE did not result in a significant difference in droplet size ( $p > 0.05$ ). The mean droplet sizes of B-NE (112.63 nm) and E-NE (115.96 nm) and their associated low PDI values ( $<0.2$ ) indicated the homogeneity of the formulations. In drug delivery via lipid-based carriers, a PDI value of 0.3 or less indicates a homogeneous system.<sup>6</sup> Zeta potential is a crucial factor that has a direct effect on nanoemulsion stability. Higher values of zeta potential help ensure that droplets remain in Brownian motion for a longer time by repelling similar charges and separating the droplets farther apart. Zeta potential values between  $(\pm) 30$  mV provide good physical stability for emulsions. However, formulations that combine electrostatic stabilization with steric stabilization can achieve sufficient physical stabilization with zeta potential values of approximately  $(\pm) 20$  mV.<sup>6</sup> In our study, B-NE had a greater negative charge ( $-34.66$  mV) than did E-NE ( $-23.79$  mV), indicating that the addition of extracts decreased the electrostatic repulsion



between droplets ( $p < 0.05$ ). However, both formulations had sufficient zeta potential values to maintain colloidal stability. The TEM image of the NE formulation revealed that nanosized and nearly spherical droplets were obtained.<sup>7</sup> FT-IR analysis was conducted to evaluate the functional groups and potential interactions between the extracts and formulation components. The spectra of B-NE and E-NE were highly similar, with no distinct extract peaks observed in E-NE, confirming that the extract was molecularly dispersed within the NE (Figure 2).<sup>8</sup> Viscosity is a critical parameter for both the release of active compounds from the formulation and the overall stability of the system. To evaluate the flow behavior of the prepared nanoemulsions, the relationship between shear stress and shear rate was examined (Figure 3). At room temperature and a shear rate of 1500 s, the viscosity values of B-NE and E-NE were 3.01 and 2.81 cP, respectively. Furthermore, the flow behavior of the NEs was described via the power-law model, and the calculated “n” values were found to be approximately 1 for both formulations. An “n” value close to 1 indicates Newtonian flow behavior, confirming that NEs exhibit Newtonian fluid characteristics.<sup>9</sup>

The potential applicability of the developed NE-MPs was further evaluated. Thickness measurements confirmed the uniformity of the films, with values within the ideal range of 0.05–1 mm, as reported in the literature.<sup>10</sup> The surface pH of the NE-MPs was consistent with the physiological mucosal pH, indicating that the NE-MPs minimized tissue irritation.<sup>11</sup> In addition, the FT-IR spectra of the NE-MPs confirmed the presence of characteristic functional groups without the appearance of new peaks, suggesting that no undesirable chemical interactions occurred between the extracts and excipients and that the extracts were successfully dispersed within the formulation.<sup>12</sup> According to the swelling studies, NE-MPs exhibited more than 180 % swelling at room temperature after 45 min, indicating progressive hydration. This is important for prolonged contact with mucosal tissue and the sustained release of active compounds.<sup>13</sup> Lower swelling values were observed at 37 °C during the initial periods, which may be attributed to faster polymer relaxation and degradation at physiological temperature.<sup>14</sup> According to the results of the mechanical tests, the E-NE-MP exhibited greater elasticity than did the B-NE-MP. These results suggest that the phytoconstituents contributed to the improved flexibility of the polymeric films.<sup>15</sup>

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## PREVENTION AND MITIGATION OF AFLATOXIN EFFECTS THROUGH PHYTOBIOTICS IN POULTRY

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### Abstract

Mycotoxins, particularly aflatoxins, represent one of the most critical challenges to feed safety in poultry production. Aflatoxins, especially aflatoxin B<sub>1</sub>, can induce severe health problems even at low concentrations, including liver damage, increased oxidative stress, immunosuppression, and elevated mortality rates. Moreover, the continuous consumption of feed contaminated with aflatoxins poses a residue risk for public health through animal-derived products. Although traditional control methods such as physical, chemical, and biological detoxification strategies have been employed, their limited efficacy and potential side effects necessitate the search for safer and more sustainable alternatives. In recent years, phytobiotics—encompassing plant extracts, essential oils, and pure phytochemicals—have emerged as promising alternatives to antibiotic growth promoters owing to their antioxidant, anti-inflammatory, immunomodulatory, and antifungal properties in mitigating the detrimental effects of aflatoxicosis. Phytobiotics can suppress enzymes involved in aflatoxin biosynthesis, neutralize free radicals to reduce oxidative damage, and support immune function. Additionally, some components have been reported to directly inhibit aflatoxin formation. Through these multifaceted mechanisms, phytobiotics not only limit the harmful impacts of aflatoxins on animals but also contribute to safeguarding feed safety. In conclusion, current scientific evidence demonstrates that phytobiotics provide a reliable, natural, and sustainable strategy for reducing aflatoxin-related risks in poultry production.

**Keywords:** Aflatoxins, Feed safety, Phytobiotics, Poultry production

### Introduction

The poultry industry has undergone rapid growth and transformation worldwide in recent decades, driven by population increases and changing consumption patterns. On a global scale, the production of chicken meat and eggs continues to increase to meet the growing demand for animal protein, a trend particularly evident in developing countries. Poultry meat plays a critical role in public health, as it represents an affordable and easily accessible source of high-quality protein.<sup>1</sup> However, this rapid expansion of production has also introduced several challenges, with feed safety being among the most pressing concerns. High productivity in poultry requires diets that are balanced in terms of energy, protein, vitamins, and minerals. However, cereals and other feed ingredients commonly used in poultry nutrition are highly susceptible to contamination by toxigenic molds and fungi during cultivation, harvesting, processing, and storage. As a result, mycotoxins pose a major threat not only to poultry health but also to human health through residues in animal-derived food products.<sup>2,3,4</sup>

Mycotoxins are secondary metabolites produced by toxigenic fungi that can contaminate a wide range of agricultural commodities, including maize, wheat, barley, sorghum, oats, oilseeds, and nuts. The mycotoxins most frequently encountered in poultry feeds include aflatoxins, ochratoxin A, trichothecenes, fumonisins, and zearalenone. Among these, aflatoxins are of particular importance because of their high toxicity, wide occurrence,

and carcinogenic potential. Aflatoxin B1 (AFB<sub>1</sub>), the most potent and prevalent form, has been shown to cause growth retardation, immunosuppression, liver damage, and reduced egg and meat production in poultry, even at low concentrations. Furthermore, it has been classified by the International Agency for Research on Cancer (IARC) as a Group 1 carcinogen.

Among farm animals, poultry are considered to have one of the highest degrees of susceptibility to aflatoxins. Comparative grading indicates that rabbits, ducks, and Türkiyes are more sensitive than chickens are, whereas cattle, sheep, and fish are relatively more resistant. In poultry, susceptibility is influenced by multiple factors, such as genetics (species, breed, strain), physiology (age, nutrition, stress level), and environmental conditions (climate, farm management). Chicks at the brooding stage are vulnerable even to low levels of aflatoxins, whereas young and fast-growing birds are generally more affected than adults. Broilers are particularly sensitive compared with layers, and male birds often show greater susceptibility than females because of faster growth rates.<sup>5</sup>

Therefore, feed safety remains one of the most critical obstacles to sustainable poultry production. Various physical, chemical, and biological detoxification methods have been developed to counteract the harmful effects of mycotoxins, but these approaches often exhibit limited efficacy and economic drawbacks. As a result, there is an increasing demand for safer, natural, and more sustainable strategies. In this context, phytobiotics, with their antioxidant, anti-inflammatory, immunomodulatory, and antifungal properties, have recently gained attention as promising alternatives for mitigating the adverse impacts of mycotoxins in poultry nutrition.

### **Mycotoxins**

Mycotoxins are small molecules produced by fungi that contaminate various agricultural commodities, such as maize, wheat, barley, sorghum, oats, sunflower seeds, nuts, spices, soybeans, and coffee beans, and are considered secondary metabolites of these fungi. The mycotoxins most frequently encountered in cereals and related feed ingredients include \*\*aflatoxins (AFB<sub>1</sub>, AFB<sub>2</sub>, AFG<sub>1</sub>, and AFG<sub>2</sub>), ochratoxin A (OTA), citrinin, patulin, trichothecenes [particularly deoxynivalenol (DON), T-2 toxin (T2), and HT-2 toxin (HT2)], fumonisins (FB<sub>1</sub>, FB<sub>2</sub>, FB<sub>3</sub>), and zearalenone (ZEA). \*\*<sup>3,6</sup> Aflatoxins are synthesized mainly by fungal species such as *Aspergillus flavus* and *Aspergillus parasiticus*. Among these mycotoxins, AFB<sub>1</sub> is considered one of the most hazardous mycotoxins and is capable of inducing severe toxic effects in poultry even at low concentrations.<sup>7</sup>

Chronic exposure to low doses of AFB<sub>1</sub> in poultry can lead to \*\*liver damage, immunosuppression, growth retardation, and decreased egg production. \*\*<sup>8</sup> In addition, AFB<sub>1</sub> has been classified as a Group 1 carcinogen by the \*\*International Agency for Research on Cancer (IARC). \*\*<sup>9,10</sup> Therefore, controlling this toxin has become a primary objective in poultry production.

For decades, physical, chemical, and biological detoxification strategies have been employed to combat mycotoxins.<sup>11</sup> However, the economic and practical limitations of these approaches necessitate the development of more natural and sustainable alternatives. In this context, phytobiotics have recently gained considerable attention as promising candidates in poultry nutrition.

### **Definition and Importance of Phytobiotics**

Phytobiotics are bioactive compounds derived from plants that are incorporated into animal feeds as natural additives. In the literature, this term is often used interchangeably with “phytochemicals” or “phytogenic feed additives.”<sup>12,13</sup> The main attributes of phytobiotics include their antimicrobial, antioxidant,



immunomodulatory, and antifungal properties.<sup>14</sup> Through these properties, they contribute not only to the maintenance of animal health but also to the enhancement of feed safety.

Within the framework of the European Parliament and Council’s definition of feed additives, phytobiotics are classified as natural additives that improve animal nutrition and support production.<sup>15</sup> The concept of using phytobiotics as feed additives dates back centuries.<sup>16</sup> In particular, after the ban on the use of antibiotics as growth promoters, phytobiotics have gained prominence due to their safety, lack of harmful residues, and consumer acceptance.

The importance of phytobiotics in poultry nutrition can be highlighted in three main aspects. First, they support animal health by strengthening the immune system, thereby helping to build resistance against diseases. In addition, phytobiotics contribute to feed safety; by mitigating the toxic effects of mycotoxins, they not only protect animal health but also indirectly increase food safety. Finally, since they are of natural origin, phytobiotics pose fewer risks to the environment and public health than antibiotics do, thus providing a significant advantage for sustainable livestock production practices.

**Classification, Mechanisms, and Effects of Phytobiotics**

Phytobiotics are a diverse group of plant-derived bioactive compounds with different biochemical structures and modes of action. They are classified as essential oils, herbs and spices, oleoresins, or pure phytochemicals, each possessing unique mechanisms and beneficial effects.

| Category                           | Main Compounds/Examples     | Main Mechanisms                | Effects on Animals               | References  |
|------------------------------------|-----------------------------|--------------------------------|----------------------------------|-------------|
| Essential Oils                     | Oregano, rosemary, cinnamon | Antimicrobial, antioxidant     | Gut health, growth, immunity     | 17,18,19,20 |
| Herbs and Spices                   | Oregano, garlic, turmeric   | Antioxidant, anti-inflammatory | Immune support, stress reduction | 17,19,20,21 |
| Oleoresins and Pure Phytochemicals | Carvacrol, thymol, saponins | Strong antimicrobial activity  | Pathogen control, gut health     | 18,19,20,22 |

**Adverse effects of aflatoxins**

Diets containing aflatoxins can impair immune function and reduce stress tolerance in laying hens. Weakened immune responses may lead to a smaller egg size and, consequently, reduced egg production.<sup>23,24</sup> Studies have shown that feeding poultry diets containing high levels of aflatoxin mixtures results in a significant decrease in body weight but an increase in the relative weights of organs such as the liver and kidneys. Moreover, aflatoxin exposure has been reported to increase blood urea nitrogen levels and decrease serum total protein, albumin, and phosphorus concentrations.<sup>25</sup>

Aflatoxins are heat-resistant mycotoxins commonly found in poultry feeds and are produced mainly by *Aspergillus flavus* and *A. parasiticus*. Among them, AFB<sub>1</sub> is considered the most toxic due to its high potency and wide contamination potential.<sup>26,27</sup> AFB<sub>1</sub> is classified as a Group 1 carcinogen by the International Agency for Research on Cancer (IARC) and primarily exerts toxic effects on the liver. Chronic exposure may result in hepatic damage, cirrhosis, and hepatocellular carcinoma.<sup>28,29,30,31</sup> Liver toxicity often manifests as jaundice, hepatocyte degeneration, necrosis, and tissue congestion. In addition, AFB<sub>1</sub> can also exert harmful effects on other organs, such as the intestine, kidneys, and reproductive system.<sup>32,33,34</sup>

Oxidative stress plays a central role in the toxic effects of AFB<sub>1</sub>. Prolonged exposure leads to the activation of phagocytes and the release of reactive oxygen species (ROS), which reduce the tissue's repair capacity and exacerbate cellular damage.<sup>35,36</sup> In this process, antioxidant enzymes and the Nrf2 signaling pathway play crucial roles in cellular defense.<sup>37,38</sup> Poultry, particularly chicks, are highly sensitive to AFB<sub>1</sub>; dietary exposure has been associated with liver swelling, necrosis, and vacuolar degeneration in broilers.<sup>39,40</sup>

The intestine is the primary site of AFB<sub>1</sub> absorption and therefore holds special importance. Approximately 50 % of ingested AFB<sub>1</sub> from contaminated feed is absorbed in the gut. This toxin may trigger inflammatory responses, weaken the intestinal barrier, and suppress the Nrf2 pathway, thereby reducing the activity of antioxidant enzymes.<sup>41,42,43</sup> Consequently, AFB<sub>1</sub> induces damage to both the liver and the intestine, ultimately compromising broiler health.

### Effects of Phytobiotics on Aflatoxins

The use of phytobiotics to counteract the toxic effects of aflatoxins in poultry has recently become a subject of intense investigation in both experimental and field studies. Research has demonstrated that these natural compounds contribute significantly to maintaining intestinal morphology, reducing liver damage, enhancing immune responses, and improving feed safety through their antifungal properties. Phytobiotics strengthen cellular defense mechanisms by activating antioxidant pathways. For example, owing to its  $\beta$ -diketone structure, curcumin neutralizes free radicals, prevents lipid peroxidation, and activates the Nrf2 signaling pathway, thereby alleviating AFB<sub>1</sub>-induced damage to the liver and intestine.<sup>44,45,46</sup> Essential oils of plant origin possess antifungal and mycotoxin-detoxifying properties,<sup>47</sup> and some have been reported to directly inhibit aflatoxin biosynthesis.<sup>48</sup>

Conventional control strategies include good agricultural practices, improved storage conditions, the use of adsorbents (e.g., bentonite, yeast cell walls), and biological detoxification. However, phytobiotics serve as complementary natural additives with multitarget and strategic actions, thereby supporting both poultry health and feed safety.<sup>47</sup> The mechanisms by which plant-derived natural compounds inhibit aflatoxin formation have not yet been fully elucidated. Nonetheless, studies suggest that they may interfere with aflatoxin biosynthesis at various stages. The proposed mechanisms include the modulation of intracellular and extracellular factors regulating biosynthesis, the disruption of early signaling pathways, the inhibition of carbohydrate metabolism linked to aflatoxin production, and the suppression of specific genes or enzymes involved in the biosynthetic pathway.<sup>49</sup>

Silymarin, a mixture of flavonolignans extracted from milk thistle (*Silybum marianum*), has long been recognized for its hepatoprotective effects. In the context of AFB<sub>1</sub> exposure, silymarin has been reported to limit protein and DNA damage in hepatocytes, increase antioxidant enzyme activity, and contribute to the scavenging of free radicals.<sup>50</sup> Moreover, silymarin supports intestinal barrier function, thereby reducing toxin absorption. Cinnamaldehyde, a potent phenolic compound found in cinnamon bark, is well known for its antioxidant and antimicrobial properties. It has been shown to suppress the proliferation of pathogens such as *Salmonella* and *E. coli* in the intestine while enhancing digestive enzyme activity and feed utilization.<sup>51</sup> In studies on aflatoxin toxicity, cinnamaldehyde was shown to normalize liver enzyme function and reduce the levels of oxidative stress markers. Investigations on oregano, cinnamon, rosemary, clove, and lemon essential oils against *Aspergillus flavus* revealed that cinnamaldehyde and citral had the strongest inhibitory effects, reducing AFB<sub>1</sub> production by



more than 90 %.<sup>52</sup> Other phytobiotics, such as ginger, turmeric, anise, coriander, green tea, and anthocyanin-rich plants, also display antioxidant activity, protecting feed lipids from oxidative damage and acting similarly to antioxidant additives such as  $\alpha$ -tocopheryl acetate and BHT.<sup>53</sup> Ginger, in particular, is rich in phenolic compounds and essential oils; its antioxidant and anti-inflammatory activities support immunity in poultry. Studies on AFB<sub>1</sub>-induced hepatotoxicity have shown that ginger supplementation decreases lipid peroxidation and increases antioxidant enzyme activities in liver tissues.<sup>54</sup> Encapsulation of *Origanum majorana* essential oil (OmEO) into a chitosan nanoemulsion effectively suppressed *Aspergillus flavus* growth and AFB<sub>1</sub> production and prevented lipid peroxidation, suggesting its potential as a novel antifungal preservative for extending the shelf-life of food products.<sup>55</sup> The intestine, which is the primary site of aflatoxin absorption, is where initial toxic effects occur. Thus, intestinal integrity and microbial balance play critical roles in resistance against aflatoxicosis. Wang et al.<sup>56</sup> demonstrated that supplementation with cinnamon oil and cinnamaldehyde increased the expression of tight junction proteins such as zonula occludens-1 and occludin, thereby protecting the intestinal barrier from AFB<sub>1</sub>-induced disruption.

Previous studies have indicated that 1 mg/kg AFB<sub>1</sub> induces hepatic inflammation in broilers, whereas supplementation with 500 mg/kg CUR reduces oxidative stress in both the liver and intestine.<sup>40,57</sup> Zhang et al.<sup>46</sup> reported that while 1 mg/kg AFB<sub>1</sub> impaired growth performance and caused oxidative damage to the liver and intestine of broilers, supplementation with 500 mg/kg CUR significantly alleviated these adverse effects by activating the Nrf2 signaling pathway. The liver, as the main target organ of aflatoxins, suffers the greatest damage from their toxicity. The hepatoprotective role of phytobiotics is largely attributed to their ability to reduce oxidative stress. Fan et al.<sup>50</sup> reported that silymarin supplementation normalized serum alanine transaminase (ALT), aspartate transaminase (AST), and alkaline phosphatase (ALP) levels, decreased lipid peroxidation in hepatocytes, and increased glutathione levels under AFB<sub>1</sub> exposure. Importantly, phytobiotics not only mitigate the toxic effects of aflatoxins but also suppress toxin production. Chaudhari et al.<sup>48</sup> reported that active compounds in oregano and cinnamon oils directly inhibited *Aspergillus flavus* growth and AFB<sub>1</sub> biosynthesis. In addition to aflatoxins, studies also highlight the broad-spectrum effects of phytobiotics against other mycotoxins and pathogens. Hassan et al.<sup>58</sup>, in their study on White Leghorn breeder hens, investigated the tissue and egg residues of OTA and AFB<sub>1</sub> administered alone or in combination and reported that coexposure led to reduced residue levels compared with those resulting from single exposures.

## Conclusion

Aflatoxins remain a major threat to feed safety and poultry health. Among these toxins, aflatoxin B<sub>1</sub> can cause severe problems such as liver damage, immunosuppression, oxidative stress, and productivity losses, even at low levels. Therefore, there is an increasing need for sustainable and reliable solutions. Recent studies have shown that phytobiotics such as oregano, cinnamon, garlic, ginger, *aloe vera*, neem, moringa, and silymarin provide multifaceted protection. These compounds help maintain intestinal health, improve liver functions, strengthen the immune system, suppress the growth of *Aspergillus* species and aflatoxin biosynthesis, and offer protective effects against other pathogens. However, the effectiveness of phytobiotics depends on factors such as the plant species, active compound concentration, dosage, formulation, and physiological status of the animals. Moreover, most findings come from experimental conditions, and more large-scale field studies are needed. Overall, phytobiotics are not a stand-alone solution but should be considered as part of an integrated strategy

together with good agricultural practices, proper storage conditions, and the use of mycotoxin adsorbents. When applied in appropriate doses and combinations, phytobiotics represent a natural, safe, and sustainable alternative that can mitigate the toxic effects of aflatoxins while enhancing gut health, immune resilience, and overall welfare in poultry.

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## 18 $\beta$ -GLYCYRRHETINIC ACID: MECHANISMS OF ANTIOXIDANT AND ANTI-INFLAMMATORY ACTION

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### Abstract

18 $\beta$ -Glycyrrhetic acid (18 $\beta$ -GA), a bioactive triterpenoid from liquorice (*Glycyrrhiza glabra*), has a broad spectrum of pharmacological actions, including hepatoprotective, antiviral, anticancer, antioxidant, anti-inflammatory, and metabolic regulatory effects. Glycyrrhizin is metabolized in the intestine to its active form, 18 $\beta$ -GA, which exhibits improved absorption and tissue distribution, crosses the blood–brain barrier to exert effects on the central nervous system, and remains in plasma for an extended period with a half-life ranging from 8--20 h. 18 $\beta$ -GA facilitates the maintenance of cellular energy production by preventing mitochondrial damage and sustaining ATP generation, thereby providing neuroprotective effects. It exerts potent antioxidant activity in the brain by activating antioxidant enzymes, neutralizing free radicals, and modulating the Nrf2/HO-1 pathway. Consequently, 18 $\beta$ -GA is considered to have potential benefits in the prevention and treatment of neurodegenerative diseases. 18 $\beta$ -GA suppresses neuroinflammation by inhibiting the NF- $\kappa$ B and MAPK signaling pathways. It reduces the levels of proinflammatory cytokines, including IL-6 and TNF- $\alpha$ , and downregulates the expression of inflammatory enzymes such as COX-2 and iNOS. By modulating the inflammatory response, 18 $\beta$ -GA can mitigate inflammation-associated tissue damage and has potential therapeutic applications in various inflammatory diseases. In conclusion, 18 $\beta$ -GA has significant antioxidant and anti-inflammatory effects by protecting mitochondrial functions, regulating neuroinflammation, and modulating key signaling pathways. Animal studies have shown that this compound is effective in reducing oxidative stress and inflammation, thereby supporting the therapeutic potential of 18 $\beta$ -GA at the preclinical level. Consequently, 18 $\beta$ -GA is considered to have potential benefits in the prevention and treatment of neurodegenerative and other inflammation-related diseases.

**Keywords:** 18 $\beta$ -Glycyrrhetic acid, Inflammation, Oxidative stress, Therapeutic potential, Triterpenes

### INTRODUCTION

Natural products have long been recognized as valuable sources for the prevention and treatment of various diseases, and their accessibility remains relatively straightforward. When formulated into therapeutic agents, medicinal plants possess structurally diverse compounds with a wide range of biological activities. Owing to their relatively low incidence of adverse effects, high efficacy, favorable safety profiles, and broad public acceptance, these plant-derived products have gained increasing attention and popularity worldwide in recent years. <sup>1,2</sup> Currently used herbal products are not entirely devoid of risk. Many of these drugs may cause adverse effects, and undesirable interactions can occur when they are administered concomitantly with other medications. In this context, identifying and utilizing herbal medicines or products that exhibit relatively minimal adverse reactions and demonstrate better efficacy within the organism is critical and urgent. <sup>2</sup>



Licorice roots, derived from the dried roots and stems of *Glycyrrhiza uralensis*, *Glycyrrhiza inflata*, or *Glycyrrhiza glabra* L., contain a variety of beneficial and naturally occurring bioactive compounds. These constituents include glycyrrhizin (a triterpenoid saponin glycoside), flavonoids, coumarins, and several other phytochemicals. Among them, 18 $\beta$ -glycyrrhetic acid (18 $\beta$ -GA) is a pentacyclic triterpenoid metabolite obtained from liquorice. Pentacyclic triterpenes, which are naturally present in plants, fungi, aquatic invertebrates, and other organisms, represent one of the most common classes of secondary metabolites that have been extensively studied to date. Owing to their diverse biological activities, these compounds have attracted considerable attention and have been widely marketed either as therapeutic agents or as dietary supplements. However, one of the major limitations of 18 $\beta$ -GA is its poor solubility in water, which restricts its bioavailability. Therefore, its combination with other compounds and novel formulation strategies have been explored to increase its pharmacological effectiveness.<sup>2</sup>

The most significant product of liquorice root, 18-glycyrrhetic acid (18-GA), exists as two distinct epimers: 18 $\alpha$ -glycyrrhetic acid (18 $\alpha$ -GA) and 18 $\beta$ -glycyrrhetic acid (18 $\beta$ -GA). The content of 18 $\beta$ -GA in liquorice roots ranges from 0.1 % to 1.6 %, whereas 18 $\alpha$ -GA is present at less than 0.7 %.<sup>1,3</sup> In the intestine, 18 $\beta$ -GA is metabolically released following the removal of the two sugar moieties from glycyrrhizic acid<sup>3</sup>. GA, which has been reported to exhibit numerous beneficial activities in organisms, including antioxidant, anti-inflammatory, and antiapoptotic effects, is suggested to exert these effects through the hydroxyl (-OH) group at its C-3 position. Moreover, it has been reported that the oxidation of this -OH group may further enhance its beneficial effects. Additionally, the carbonyl group at the C-11 position has also been reported to play a significant role in its biological activities.<sup>4</sup>

### ABSORPTION AND METABOLISM

Following oral administration, glycosidic GA is metabolized by the intestinal flora and is subsequently absorbed entirely as GA. After absorption, the glycosidic form of GA exerts all of its systemic effects through GA. While numerous studies have provided detailed information on the absorption and metabolism of glycosidic GA, corresponding data for GA remain limited. However, studies investigating both compounds together have reported that their systemic effects are largely similar. Therefore, the known systemic properties of glycosidic GA are often considered a reference for GA.<sup>5</sup>

Experimental studies on glycosidic GA and GA indicate that the levels of these compounds can be analyzed not only in plasma but also in bile and urine. Since GA metabolites excreted via the bile are reportedly hydrolyzed by commensal bacteria, it is believed that GA undergoes enterohepatic circulation in rats. GA metabolites are secreted into the duodenum via the bile and subsequently reabsorbed, leading to the appearance of multiple secondary peaks rather than a single maximum in the plasma concentration–time curve. This phenomenon is considered a characteristic indicator of enterohepatic circulation. Because GA is excreted into the bile and reabsorbed from the intestine in both humans and rats, its elimination from the body is delayed, resulting in a prolonged plasma half-life.<sup>5</sup>

### ANTIOXIDANT PROPERTIES

Although oxygen is essential for the survival of living organisms, it can also entail potential costs. During biochemical reactions that are critical for the maintenance of life and cellular functions, oxygen is partially reduced, leading to the formation of reactive oxygen species (ROS). The generation of ROS and their metabolites

can cause oxidative damage. In this context, ROS are recognized as oxidants or free radicals because of the oxidative damage resulting from the metabolism of molecular oxygen in living organisms.<sup>6</sup>

Studies have shown that *Glycyrrhiza inflata* extract inhibits spinocerebellar ataxia type 3 (SCA3) by enhancing the activation of nuclear factor erythroid 2–related factor 2–antioxidant response elements (NFE2L2-ARE), peroxisome proliferator-activated receptor gamma coactivator 1 $\alpha$  (PPARGC1A), and peroxisome proliferator-activated receptor  $\gamma$  (PPAR $\gamma$ ). Both glycyrrhizin and *Glycyrrhiza inflata* extracts have been reported to prevent increases in ROS, cellular damage, and GSH inhibition induced by 1-methyl-4-phenylpyridinium. The antioxidant properties of the Glycyrrhiza extract are thought to be due to the presence of isoliquiritigenin in liquorice roots. Glycyrrhiza reduces oxidative stress, particularly by alleviating brain tissue damage and enhancing neuronal function and memory (Batiha et al., 2020). Furthermore, 18 $\beta$ -GA has been reported to prevent DNA damage by activating the nuclear factor erythroid 2–related factor 2 (Nrf2) and SKN-1 pathways. The same study indicated that 18 $\beta$ -GA has both protective and preventive effects against DNA damage and oxidative stress.

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### ANTI-INFLAMMATORY PROPERTIES

Infections and tissue damage caused by various pathogens trigger the inflammatory response, which is one of the fundamental defense mechanisms of the organism. Immune cells such as macrophages, dendritic cells, and mast cells are activated by pathogen-associated molecular patterns (PAMPs), such as lipopolysaccharides (LPS), or damage-associated molecular patterns (DAMPs) resulting from tissue injury. These stimuli are recognized by pattern recognition receptors (PRRs), particularly Toll-like receptors (TLRs), on the cell surface. Following this recognition, immune cells release inflammatory cytokines to restore homeostasis, a process that is physiologically beneficial, aiding in infection control and tissue repair. However, prolonged or chronic inflammation can lead to pathological reactions, resulting in tissue damage and contributing to the development of various diseases, including obesity, diabetes, asthma, rheumatoid arthritis, inflammatory bowel diseases, neurodegenerative disorders, and cancer.<sup>8</sup>

Although currently used drugs for the treatment of inflammation are effective, they have been reported to cause significant adverse effects. Therefore, existing therapeutic strategies are often insufficient for the effective and safe management of chronic inflammation, highlighting the need for safer and more targeted alternative anti-inflammatory agents.<sup>8</sup>

Studies have reported that 18 $\beta$ -GA has various pharmacological activities, including anti-inflammatory, hepatoprotective, anticancer, and antiviral effects.<sup>7,9–13</sup> A previous study reported that 18 $\beta$ -GA has neuroprotective effects by regulating the PI3K/Akt signaling pathway. The same study further indicated that GA suppresses cytokine production via the intracellular PI3K/Akt/GSK3 $\beta$  pathway, whereas 18 $\beta$ -GA enhances anti-inflammatory signaling by promoting the dissociation of the complex between the glucocorticoid receptor (GR) and heat shock protein 90 (HSP90) within the cell, thereby inhibiting inflammation.<sup>12</sup> Another study demonstrated that GA and 18 $\beta$ -GA can reduce the expression of proinflammatory genes by limiting excessive NO, PGE<sub>2</sub>, and ROS production, as well as by suppressing NF- $\kappa$ B and PI3K activities. Therefore, these compounds may be considered potential anti-inflammatory agents for the treatment of inflammatory diseases.<sup>13</sup>

### CONCLUSION

In light of the information presented and considering the side effects and costs associated with currently used drugs, the need for herbal, natural, and—more importantly—agents with minimal or no side effects has



markedly increased. The presence and quantity of molecules with essential properties for the organism, such as antioxidant and anti-inflammatory activities, are particularly important in these agents. Among such compounds, 18 $\beta$ -GA has been reported to exhibit significant antioxidant and anti-inflammatory effects by preserving mitochondrial functions, regulating neuroinflammation, and modulating key signaling pathways. These properties of 18 $\beta$ -GA have been validated through *in vivo* animal experiments and *in vitro* cell culture studies, thereby supporting its therapeutic potential at the preclinical level. On the basis of these findings, 18 $\beta$ -GA may serve as a promising agent not only for slowing the progression of neurodegenerative diseases but also for preventing and treating other inflammation-mediated disorders owing to its capacity to reduce oxidative stress, suppress inflammatory responses, and regulate signaling pathways.

### Conflict of interest and funding

At the end of the manuscript, the authors declare any potential conflicts of interest under the heading “Conflict of interest. Any financial support or project funding should be acknowledged under the heading “Funding.”

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## SILYMARIN: MECHANISMS OF ANTIOXIDANT AND ANTI-INFLAMMATORY ACTION

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### Abstract

Silymarin is a prominent medicinal flavonoid containing 25 carbon atoms and is derived from *Silybum marianum* (milk thistle) of the *Asteraceae* family. Extracted from seeds and fruits, it is a polyphenolic flavonolignan complex (65–80 %) with fatty acids (20–35 %) and is well recognized for its antioxidant and anti-inflammatory properties, particularly in the liver and other tissues. Silymarin, with a half-life of approximately 6 h, is metabolized primarily via conjugation, and its main component, silibinin, has low oral bioavailability and rapid metabolism, resulting in mostly nanomolar plasma concentrations. Silymarin acts as a direct free radical scavenger, preventing lipid peroxidation, while also protecting the organism from oxidative stress by increasing the levels of antioxidant enzymes and reducing the activity of enzymes that generate reactive oxygen species. Under stress conditions, silymarin plays a critical role in maintaining mitochondrial integrity and preserving cellular functions. Additionally, it provides further protection by activating vital components and influencing the gut microenvironment, particularly through interactions with the microbiota, suggesting new avenues for future research. Silymarin modulates the release of inflammatory mediators primarily by inhibiting signaling pathways such as the NF- $\kappa$ B, TLR4, MAPK, and JAK-STAT3 pathways. It improves the inflammatory response by reducing the secretion of proinflammatory cytokines and enhancing the activity of anti-inflammatory agents. Furthermore, by regulating the MAPK and Nrf2 signaling pathways, silymarin has both antioxidant and anti-inflammatory properties. In animal nutrition and disease prevention strategies, silymarin, either alone or in combination with compounds such as carnitine, betaine, and vitamin B12, can elicit effects comparable to the hepatoprotective outcomes observed in humans. Owing to these properties, silymarin provides a robust foundation for further research evaluating its antioxidant and anti-inflammatory effects, particularly its ability to protect the liver and preserve other organs, demonstrating its therapeutic potential in cellular, animal, and clinical studies.

**Keywords:** Inflammation, Oxidative stress, Polyphenols, Silymarin, Therapeutic potential

### INTRODUCTION

Throughout human history, various local plants have been utilized as primary sources of medicine for the prevention and treatment of acute and chronic diseases. Herbal medicines, health products, and pharmaceuticals have become the focus of numerous studies worldwide because of their safety, effectiveness, cultural acceptability, and fewer side effects. <sup>1</sup> *Silybum marianum* (L.) Gaertn., also called milk thistle or St. Mary's thistle, is a spiny plant belonging to the *Asteraceae* family. This annual or biennial species grows in dry, rocky habitats across southern and western Europe, as well as parts of the United States. The plant develops a thick, branched stem that can reach 30–90 cm in height. Its dark green leaves are glossy, edged with spines and teeth,



and display prominent white veins. The plant produces solitary, reddish–purple flower heads, with bracts ending in sharp spines. <sup>2</sup>

Under natural conditions, *Silybum marianum* spends its initial growing season in the vegetative stage, leading to its classification as a biennial species. <sup>3</sup> This plant, which grows widely in the Mediterranean and Black Seas, can reach a height of 30–100 cm; it is recognized by its light green leaves and purple or, rarely, white fruits. The extract of the plant consists of approximately 70–80 % silymarin, and the remaining 20–30 % of the fractions have incompletely characterized chemical structures. Silymarin is a mixture containing polymeric and oxidized polyphenolic compounds. <sup>4</sup>

Silymarin, a complex of flavonolignans, including silybin, isosilybin, silydianin, and silychristin derivatives, has been historically applied in the treatment of liver diseases such as cirrhosis, jaundice, and hepatitis. In addition to its hepatoprotective (liver-protective) effects, silymarin is an important Mediterranean plant distinguished by its antioxidant, anti-inflammatory, and detoxifying properties. <sup>5</sup>

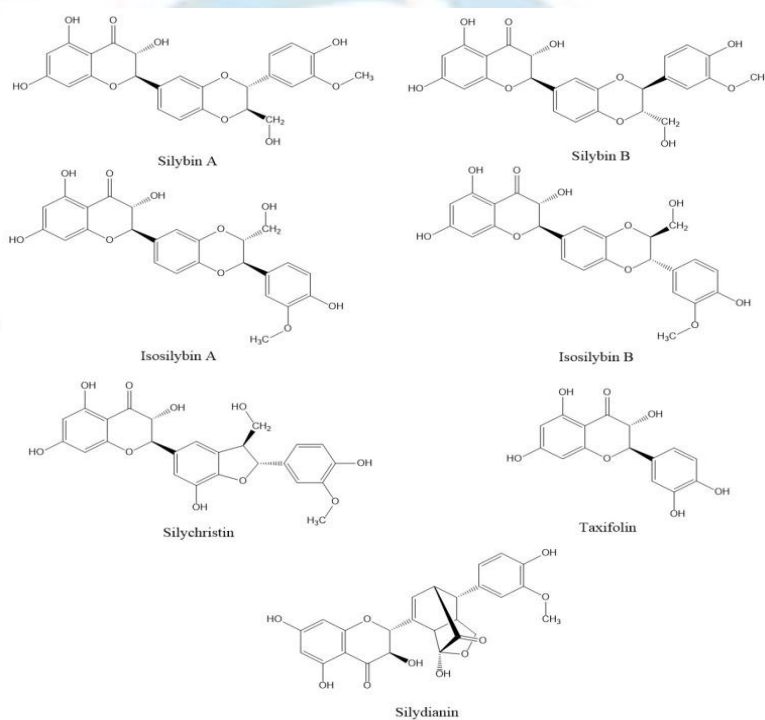


**Figure 1.** Chemical structures of the silymarin components. <sup>6</sup>

## ABSORPTION AND METABOLISM

The oral absorption of silibinin, the main active component of silymarin, is limited because of its poor water solubility. Studies conducted on animals and humans have reported that silibinin reaches its maximum plasma concentration approximately 6 h after oral administration. <sup>7</sup> The main biotransformation pathway of silibinin and its derivatives is conjugation. Like other flavonoids, silibinin is recognized by organisms as a foreign substance (xenobiotic) and is rapidly metabolized through phase II enzymes. Indeed, the oral administration of silibinin has been associated with significant dose- and time-dependent increases in the activities of glutathione S-transferase (GST) and quinone reductase (QR) enzymes in the liver, lungs, stomach, skin, and small intestine. It has been reported that silibinin present in systemic circulation exists predominantly in conjugated form. Studies conducted on humans and rats have shown that both free and conjugated forms of silibinin are rapidly eliminated, with an average elimination half-life of approximately 6.32 h. Therefore, similar to other flavonoids, silibinin—

the main component of silymarin—is characterized by relatively low bioavailability after oral administration, rapid metabolism, and plasma concentrations that generally reach nanomolar and, in some cases, micromolar levels.<sup>8,9</sup>

### ANTIOXIDANT PROPERTIES

Although oxygen is an indispensable element for the survival of living organisms, it can also lead to certain adverse consequences during metabolic processes. The partial reduction of oxygen during biochemical reactions essential for sustaining life results in the formation of reactive oxygen species (ROS). The excessive production of these species and their metabolites causes oxidative damage by harming the lipid, protein, and DNA components of cellular structures. Therefore, ROS are defined as oxidants or free radicals that are generated as a result of the metabolism of molecular oxygen in living organisms and can cause oxidative damage.<sup>10</sup> To prevent this oxidative damage, organisms have developed antioxidant defense systems. Antioxidants are biological compounds that neutralize reactive oxygen species and reduce the damage caused by oxidative stress. Among these compounds, flavonoids are powerful polyphenolic antioxidants that are naturally found in many fruits and vegetables. Silymarin stands out as an important polyphenolic antioxidant belonging to this flavonoid group.<sup>11</sup> An imbalance between reactive oxygen species (ROS) production and the effectiveness of antioxidant defense systems leads to oxidative stress. The superoxide anion ( $O_2^-$ ), a biologically important ROS, facilitates the generation of secondary toxic oxidants, including hydrogen peroxide ( $H_2O_2$ ), peroxynitrite, and related degradation products, thereby inducing cellular injury.<sup>12,13</sup> The enzyme superoxide dismutase (SOD) plays a critical role in the neutralization of these species. SOD converts superoxide radicals into hydrogen peroxide, which is subsequently reduced to water ( $H_2O$ ) through the combined actions of glutathione peroxidase (GPx) and catalase (CAT). In this way, the cellular redox balance is maintained.<sup>10,14,15</sup> Several studies have demonstrated that the administration of exogenous SOD genes has protective effects on various pathological conditions, including cancer and cardiovascular diseases (CVDs). Furthermore, it has been reported that antioxidant enzymes enhance the interaction between nuclear factor erythroid 2-related factor 2 (Nrf2) and the antioxidant response element (ARE) while simultaneously reducing the expression of nuclear factor kappa B (NF- $\kappa$ B).<sup>11,16</sup>

The ability of silymarin to activate Nrf2 pathways, a crucial mechanism in enhancing cellular antioxidant defenses, is particularly notable. Upon activation, Nrf2 translocates into the nucleus and binds to antioxidant response elements (AREs) on DNA, promoting the expression of genes encoding key antioxidant enzymes, including SOD, CAT, GPx, and heme oxygenase-1 (HO-1). In particular, HO-1 catalyzes the breakdown of heme into biliverdin, iron, and carbon monoxide, generating a potent antioxidant response. The biliverdin produced, along with its metabolite bilirubin, neutralizes free radicals, thereby providing protection against cellular oxidative damage. Consequently, silymarin promotes Nrf2 activation, enhancing the production of cytoprotective proteins and mitigating the effects of oxidative stress.<sup>17</sup> Similarly, the regulatory effect of silymarin on nuclear factor kappa B (NF- $\kappa$ B) contributes not only to the enhancement of antioxidant defenses but also to the suppression of inflammatory processes. Under normal conditions, NF- $\kappa$ B remains inactive in the cytoplasm and is bound in a complex with I $\kappa$ B proteins. However, oxidative stress or proinflammatory stimuli induce the phosphorylation and degradation of I $\kappa$ B proteins, allowing NF- $\kappa$ B to translocate into the nucleus and initiate the transcription of inflammation-related genes. Silymarin inhibits this activation step, thereby suppressing the NF-



κB pathway; in doing so, it reduces oxidative stress-associated inflammatory responses while enhancing the effectiveness of antioxidant defense mechanisms.<sup>17</sup>

### ANTI-INFLAMMATORY PROPERTIES

In both humans and animals, inflammation represents a key component of innate immune defense, generating conditions that are detrimental to invading pathogens and confining infection to affected tissues. The principal objective of acute inflammation is to eliminate pathogens, initiate tissue repair, and reestablish physiological homeostasis upon resolution.<sup>18</sup> The initiation of inflammation generally occurs when pattern recognition receptors (PRRs) of the host's innate immune system identify pathogen-associated molecular patterns (PAMPs) or damage-associated molecular patterns (DAMPs). Among these receptors, Toll-like receptors (TLRs), which are located on the cell surface, and nucleotide-binding oligomerization domain-like receptors (NLRs) in the cytoplasm play crucial roles. Once this recognition occurs, it leads to the activation and release of various inflammatory mediators, including cytokines, chemokines, and prostaglandins.<sup>19</sup> Nevertheless, inadequate resolution of the inflammatory response or disruption of immune regulation may result in the transformation of acute inflammation into a chronic condition. The persistence of chronic inflammation is considered a significant factor in the development of a range of conditions, including atherosclerosis, cancer, obesity, asthma, lung diseases, inflammatory bowel diseases, neurodegenerative disorders such as Alzheimer's and Parkinson's diseases, and various autoimmune disorders.<sup>20,21</sup>

Although the drugs currently used for inflammation treatment are effective, long-term use can lead to various side effects. This situation complicates the safe and effective management of chronic inflammation and increases the need for safer, targeted alternative anti-inflammatory agents. In this context, silymarin, a naturally derived compound, has attracted attention not only for its potent antioxidant properties but also for its anti-inflammatory, hepatoprotective, and anticancer effects.<sup>22,23</sup> Silymarin modulates inflammatory processes primarily through the nuclear factor kappa B (NF-κB) pathway. NF-κB is a key transcription factor that, when activated, upregulates the expression of numerous proinflammatory genes. Silymarin reduces the inflammatory response by inhibiting NF-κB activation and downregulating the expression of these genes.<sup>24</sup>

### CONCLUSION

While existing anti-inflammatory and antioxidant medications provide short-term benefits, prolonged use may cause serious side effects, highlighting the need for safer therapeutic alternatives. In this context, silymarin (*Silybum marianum* extract), a naturally derived compound, has attracted attention because of its versatile biological activities. Silymarin, a polyphenolic flavonolignan mixture, stands out for its antioxidant, anti-inflammatory, hepatoprotective, and anticancer effects and is capable of modulating multiple molecular signaling pathways at the cellular level. Consequently, existing evidence indicates that silymarin preserves cellular integrity by modulating mechanisms that play a central role in the pathogenesis of oxidative stress and inflammation. Owing to these properties, silymarin holds significant potential as a safe and natural pharmacological alternative in both preventive and supportive therapeutic strategies, as confirmed by *in vivo* animal studies and *in vitro* cell culture experiments.

### Conflict of interest and funding

At the end of the manuscript, the authors declare any potential conflicts of interest under the heading “Conflict of interest. Any financial support or project funding should be acknowledged under the heading “Funding.”

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## EVALUATION OF THE ANTIMICROBIAL ACTIVITY OF *ALLIUM SATIVUM* (GARLIC) ON *E. COLI* STRAINS ISOLATED FROM DIARRHEAL CALVES

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### Abstract

The antimicrobial activity of *Allium sativum* L. (garlic) against *Escherichia coli* (*E. coli*) isolates has gained increasing attention in scientific research because of the alarming increase in the number of antibiotic-resistant bacterial strains. Numerous studies have demonstrated that garlic possesses significant antibacterial properties, particularly against various strains of *E. coli*, including multidrug-resistant isolates. This study aimed to evaluate the antibacterial activity of *Allium sativum* powder against *E. coli* isolates obtained from calf diarrhea cases. Garlic powder dilutions ranging from 6.25 to 100 mg/mL were prepared and tested against 10 *E. coli* isolates from diarrheic calves via agar well diffusion and broth dilution methods to determine the MIC and MBC values. K99 antigen–positive *E. coli* isolates from diarrheic calves showed inhibition zones >15 mm only at garlic suspension concentrations of 25 mg/mL, 50 mg/mL and 100 mg/mL. Lower concentrations (6–25 mg/mL) exhibited bacteriostatic effects, whereas concentrations ≥50 mg/mL were bactericidal. As a result, garlic powder has an antimicrobial effect on *E. coli*, and these findings show that garlic powder can be used as an alternative today, where antimicrobial resistance is increasing.

### Introduction

Calf diarrhea, commonly referred to as neonatal diarrhea, is a significant health concern in dairy farming, leading to substantial morbidity and mortality rates among calves, as well as considerable economic losses for farmers (Jessop et al. 2024). Several groups of *Escherichia coli* (*E. coli*) have been implicated in outbreaks of diarrhea in calves (Naylor 2009). *E. coli* is a primary causative agent of calf diarrhea, and its impact has been intensified by the indiscriminate and inappropriate use of antimicrobial agents, resulting in considerable drug resistance (Wang et al. 2024). There is an urgent need to discover innovative alternatives that have similar effects by preventing infections due to current antibiotic resistance (Gupta & Sharma 2022).

The antimicrobial activity of *Allium sativum* (garlic) has been extensively studied because of its rich array of bioactive compounds, particularly organosulfur compounds such as allicin. Research has consistently demonstrated that these compounds exert broad-spectrum antimicrobial effects against both gram-positive and gram-negative bacteria, making garlic a promising candidate for combating antibiotic-resistant strains (Magryś et al., 2021; Bhatwalkar et al., 2021). The aim of this study was to evaluate the antimicrobial activity of *Allium sativum* (garlic) powder against K99 antigen–positive *E. coli* strains isolated from 10 cases of calf diarrhea.

### Materials and methods

#### *Preparation of garlic powder extract*

Commercially available *Allium sativum* (garlic) powder was used as the test substance. A stock solution was prepared by suspending 10 g of garlic powder in 100 mL of sterile distilled water. The suspension was vortexed thoroughly and incubated at room temperature for 24 h with continuous shaking. The suspension was

centrifuged, and the resulting supernatant was then filtered through a 0.22 µm membrane filter to obtain a sterile aqueous garlic extract. Twofold serial dilutions of the suspension were prepared, dispensed into sterile tubes, and stored at +4 °C (Circella et al., 2022).

**Bacterial strains and culture conditions**

In this study, *E. coli* strains previously isolated from diarrheic calves and characterized as K99-positive were utilized. The isolates were subcultured on MacConkey agar and incubated at 37 °C for 18–24 h. Pure colonies were suspended in sterile saline and adjusted to a turbidity equivalent to 0.5 McFarland standard (~10<sup>8</sup> CFU/mL) (Circella et al., 2022).

**Antimicrobial susceptibility assay**

The antimicrobial activity of the garlic powder extract was determined via the agar well diffusion method. Briefly, 100 µL of Mueller–Hinton agar plates were inoculated by swabbing with the *E. coli* bacterial suspensions. Wells 5 mm in diameter were cut into agar with a biopsy punch and filled with 30 µL of different concentrations of garlic suspension (ranging from 0.625 to 100 mg/mL). Sterile distilled water served as the negative control. The plates were incubated at 37 °C for 24 h, after which the zones of inhibition (mm) were measured via a caliper (Yadav et al., 2015).

**Determination of the MIC and MBC**

Serial dilutions of the garlic suspension were prepared in Mueller-Hinton broth, with each tube containing 1 mL of the diluted garlic juice. A standard inoculum of the *E. coli* isolates (1 mL) was added to each garlic concentration, as well as to a tube of broth without any antimicrobial agent, serving as a growth control. Following 24 h of incubation, the tubes were inspected for turbidity as an indicator of microbial growth. The lowest concentration of garlic suspension that completely inhibited microbial growth was recorded as the minimum inhibitory concentration (MIC). To determine the minimum bactericidal concentration (MBC), a loopful of inoculum from each broth tube was subcultured onto nutrient agar plates, which were predivided into six sections for further observation (Yadav et al., 2015).

**Results**

The inhibition zones of 10 K99 antigen–positive *E. coli* isolates recovered from calves with diarrhea were evaluated via the agar-well diffusion method (Table 1). Antimicrobial susceptibility analysis revealed that inhibition zones exceeding 15 mm were observed only at garlic suspension concentrations of 25 mg/mL, 50 mg/mL and 100 mg/mL.

**Table 1.** The inhibition zones of the *E. coli* strains were determined via the garlic powder agar-well diffusion method.

|                | 6,25 mg/mL | 12,5 mg/mL | 25,0 mg/mL | 50,0 mg/mL | 100,0 mg/mL |
|----------------|------------|------------|------------|------------|-------------|
| <i>E. coli</i> | 0          | 3±1        | 16±3       | 20±2       | 26±2        |

One-way ANOVA revealed a highly significant difference among the groups ( $F(4,45) = 403.6$ ,  $p < 0.0001$ ). Tukey’s multiple comparison test indicated that there was no significant difference between the 6.25 mg/mL and 12.5 mg/mL groups ( $p = 0.74$ ), whereas all other pairwise comparisons revealed statistically significant differences ( $p < 0.001$ ). These findings demonstrate that the inhibitory effect of *Allium sativum* (garlic) powder on *E. coli* strains increases in a concentration-dependent manner, with pronounced antibacterial activity observed from 25 mg/mL onward.

Minimal inhibitory concentration (MIC) testing indicated that lower concentrations of garlic (6 mg/mL and 12 mg/mL) had bacteriostatic effects, whereas concentrations of 25 mg/mL and above demonstrated bactericidal



activity against the *E. coli* isolates.

### Discussion and Conclusion

Our study evaluated the antimicrobial effects of *Allium sativum* (Garlic) powder on *E. coli* strains, a significant cause of calf diarrhea. Garlic powder was found to be bacteriostatic at low doses, and bacteriostatic at doses of 25 mg/mL and above against *E. coli* strains. This finding is consistent with similar studies (Garba et al., 2013; Yadav et al., 2015; Circella et al., 2022). Compared with aqueous extracts, ethanolic extracts have been shown to have superior antibacterial activity (Eghieye et al., 2024). Similarly, Merati and Boudra reported that *Allium sativum* exhibited potent antibacterial effects against multidrug-resistant *E. coli* strains isolated from broiler chickens. These findings confirmed the efficacy of garlic extracts as a viable alternative treatment option for avian colibacillosis caused by resistant *E. coli* (Merati & Boudra, 2024). The mechanisms by which *Allium sativum* exerts its antimicrobial effects are largely attributed to its high content of sulfur compounds, such as allicin, which possesses strong antibacterial properties. Furthermore, Fayyadh (2025) investigated the antibacterial activity of nanoparticles synthesized from garlic extract and reported that they significantly enhance the antibacterial activity against *E. coli* isolates, suggesting that the bioactive compounds in garlic, when appropriately formulated, can increase their effectiveness against resistant bacterial strains (Fayyadh, 2025). Additionally, a study by Anggraini et al. specifically showed that garlic extract could inhibit the growth of both *E. coli* and *Staphylococcus aureus*, demonstrating its broad-spectrum antimicrobial capabilities (Anggraini et al., 2020). As a result, garlic powder has an antimicrobial effect on *E. coli*, and these findings show that garlic powder can be used as an alternative today, where antimicrobial resistance is increasing.

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## PROTECTIVE POTENTIAL OF *CALLIGONUM POLYGONOIDES* AGAINST 6-OHDA-INDUCED DOPAMINERGIC NEURON DAMAGE

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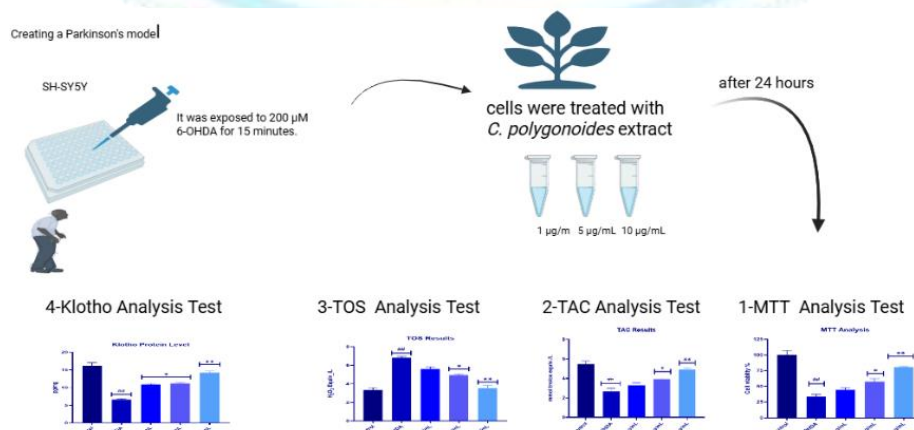
### Abstract

Parkinson's disease, a neurodegenerative disorder that is linked to behavioral abnormalities in animal models. Parkinson-like conditions are rare in pets, and the contributions of dopaminergic neuron loss and oxidative stress to the disease process involve mechanisms similar to those observed in humans. 6-Hydroxydopamine (6-OHDA), an oxidizable dopamine analog taken up via the dopamine transporter and exhibiting selective toxicity toward dopaminergic neurons in the substantia nigra pars compacta (SNpc), is widely used to induce Parkinson-like neurodegeneration.” To develop *in vitro* models of Parkinson’s disease. *Calligonum polygonoides*, an endemic perennial plant resistant to harsh environmental conditions, possesses antioxidant, antimicrobial, and cytoprotective properties. In this context, the present study investigated the neuroprotective effects of the methanol extract of *Calligonum polygonoides* (MSECP) in a 6-OHDA-induced *in vitro* Parkinson’s disease model.

To generate a Parkinson's disease model in cell culture, SH-SY5Y (neuroblastoma) cells were exposed to 200 µM 6-OHDA for 15 min and subsequently treated with *Calligonum polygonoides* extract at concentrations of 1 µg/mL, 5 µg/mL, and 10 µg/mL. After 24 h, cell viability was evaluated via the MTT test. Oxidative stress indicators were assessed by evaluating total antioxidant capacity (TAC), total oxidant status (TOS), and Klotho values. Compared with the control, treatment with 6-OHDA increased the percentage of apoptotic SH-SY5Y cells by approximately 55 %. TAC and TOS analyses indicated that this increase was associated with oxidative stress. *Calligonum polygonoides* extract was found to reduce 6-OHDA-induced apoptosis, increasing cell viability in a dose-dependent manner. Furthermore, the observed Klotho levels supported these findings. This study demonstrated the neuroprotective effects of *Calligonum polygonoides* extract in an *in vitro* 6-OHDA-induced Parkinson's disease model via MTT, TAC, TOS, and Klotho assays in SH-SY5Y cells. To gain deeper insights into the underlying mechanisms, additional investigations employing *in vivo* Parkinson’s disease models are warranted.

**Keywords:** 6-OHDA, *Calligonum polygonoides*, Klotho, Parkinson's disease, SH-SY5Y

### Graphical Summary





## INTRODUCTION

Parkinson's disease is one of the most prevalent chronic neurodegenerative disorders and is characterized by the progressive and irreversible destruction of neurons in the central nervous system (CNS). In animals, gradual cognitive decline manifests as a behavioral disorder characterized by increased brain pathology. Cats and dogs can suffer from slight age-related changes, exhibit cognitive decline, similar to mild cognitive impairment in humans, or develop a more severe cognitive dysfunction syndrome resembling dementia and Alzheimer's disease in humans. A primary characteristic of Parkinson's disease is the impairment of dopaminergic neurons, which leads to movement symptoms <sup>1</sup>.

Although the precise mechanism involved in the development of this disease is not yet known, it is understood to be caused by the misfolding of a protein called alpha-synuclein, a member of the synuclein protein family. Like other members of the synuclein family,  $\alpha$ -synuclein is an abundant protein in the nervous system. Owing to its localization and distribution within the synaptic terminal, alpha-synuclein ( $\alpha$ -synuclein) is thought to play a significant role in synaptic plasticity, vesicle dynamics, and dopamine synthesis and release. The degeneration of dopaminergic neurons in the substantia nigra results in a reduction in dopamine levels in the brain. These findings elucidate the motor symptoms of Parkinson's disease. 6-Hydroxydopamine (6-OHDA), which has selective toxicity toward dopaminergic neurons in the substantia nigra pars compacta (SNpc), is frequently used to study this disease <sup>2,3</sup>.

*Calligonum polygonoides* contains important bioactive compounds and phytochemicals that have the potential to be used to treat many diseases, including neurodegenerative and neuropsychiatric disorders. The bioactive components included dehydromevalonic lactone, furan-2,5-dimethyl, 2-methoxy-4-vinylphenol, 2,3-dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one (DDMP), deoxyspergualin, and 4-hydroxybenzenethanol <sup>4</sup>. Consequently, it possesses numerous scientifically validated bioactivities, including antibacterial, anti-inflammatory, anticancer, antidiabetic, hepatoprotective, cardioprotective, antioxidant, and antimutagenic properties <sup>5</sup>.

While numerous studies on the antioxidant activities of *Calligonum polygonoides* are available in the literature, there are no studies on its neuroprotective activity. Consequently, our research aimed to examine the neuroprotective properties of *Calligonum polygonoides* in an *in vitro* model of Parkinson's disease, emphasizing the roles of Klotho and oxidative stress pathways.

## MATERIALS AND METHODS

To create an *in vitro* model of PD, SH-SY5Y neuroblastoma cell lines were exposed to 200  $\mu$ M 6-OHDA for 15 min. The cells were subsequently administered *Calligonum polygonoides* extract at concentrations of 1, 5, and 10  $\mu$ g/mL. Following a 24-h incubation, cell viability was assessed via the MTT assay, and oxidative stress indicators, including total antioxidant capacity (TAC), total oxidant status (TOS), and Klotho levels, were analyzed.

### Cell Culture

SH-SY5Y cells were obtained from the Department of Medical Pharmacology at Bilecik Şeyh Edebali University, Faculty of Medicine (Bilecik, Türkiye). After reaching 85 % confluence, the cells were passaged and seeded into 96-well plates. The cells were maintained in a 5 % CO<sub>2</sub> incubator until they reached 80 % confluence.

To establish a PD model in cell culture, SH-SY5Y cells were subjected to 6-OHDA (1, 5, or 10 µg/mL) and incubated for 15 min. After 6-OHDA treatment, the cells were divided into groups and treated with *Calligonum polygonoides* extract (1 µg/mL, 5 µg/mL, or 10 µg/mL). Cell viability was measured 24 h post-treatment via the MTT assay. Oxidative stress was determined through total antioxidant capacity (TAC) and total oxidant status (TOS) assays, and Klotho levels were subsequently analyzed <sup>6</sup>.

#### MTT Assay

Following resuspension in fresh DMEM supplemented with 10 % FBS and 1 % antibiotics (penicillin, streptomycin, and amphotericin B), the cells were exposed to *C. polygonoides* extract at final concentrations of 1 µg/mL, 5 µg/mL, and 10 µg/mL for 24 h. The absorbance was subsequently quantified at 570 nm via a Multiskan™ GO microplate spectrophotometer (Thermo Fisher Scientific, Porto Salvo, Portugal).

#### Total Antioxidant Capacity (TAC) Assay

The total antioxidant capacity (TAC) was quantified colorimetrically via the Total Antioxidant Capacity Kit from Rel Assay Diagnostics. The experiment was conducted in accordance with the manufacturer's guidelines, and the absorbance was recorded at 660 nm. The acquired data were subjected to statistical analysis.

#### Total Oxidant Status (TOS) Assay

A Total Oxidative Stress Kit (RL0024, Rel Assay Diagnostics) was utilized for colorimetric assessment of TOS. The assay was conducted following the manufacturer's guidelines, and the absorbance was recorded at 540 nm. The acquired data were subjected to statistical analysis.

#### ELISA tests

A Klotho ELISA kit (BT Lab, LOT: 202408010) was utilized to examine alterations in ROS levels. The experiments were conducted separately on three occasions. The procedure was executed in accordance with the kit protocol, and the optical density was assessed at 450 nm.

### RESULTS

Compared with the control, exposure to 6-OHDA increased apoptosis by approximately 40 % in SH-SY5Y cells. TAC and TOS analyses indicated that this increase was caused by oxidative stress. *C. polygonoides* extract inhibited 6-OHDA-induced apoptosis and increased cell viability in a dose-dependent manner. Furthermore, elevated Klotho levels supported this improvement.

#### MTT analysis results

To establish a Parkinson's disease model, SH-SY5Y cells were exposed to 200 µM 6-OHDA for 15 min. The cells were then treated with *Calligonum polygonoides* extract (1 µg/mL, 5 µg/mL, or 10 µg/mL). After 24 h, cell viability was determined via MTT analysis. The spectrophotometric measurement of the control group was considered 100 %. The *C. polygonoides* extract and other treatment groups were compared with the control. The results showed that *C. polygonoides* extract had a dose-dependent protective effect against 6-OHDA-induced cellular damage. A slight improvement was observed in the 2 µg/mL group. At 5 µg/mL, a significant increase in cell viability ( $p < 0.05$ ) was observed, whereas at 10 µg/mL, the strongest effect ( $p < 0.01$ ) was achieved, with viability approaching normal levels.



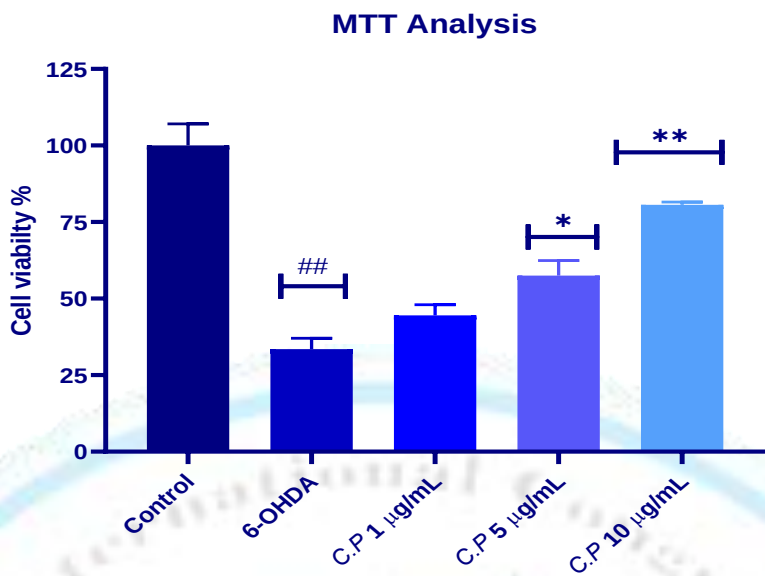


**Figure 1.** This graph shows that, compared with the control, 6-OHDA significantly reduced cell viability. Treatment with *C. polygonoides* increased cell viability in a dose-dependent manner, with doses of 5 µg/mL and 10 µg/mL providing significant protection.

### TAC and TOS analysis results

To determine oxidative damage, TAC and TOS values were examined in cell culture media collected after 24 h (Figure 2). A significant increase in TAC was observed at 10 µg/mL ( $p < 0.01$ ), which was correlated with improved cell viability. *C. polygonoides* strengthened the antioxidant defense system of the cells, and a reduction in oxidative stress was observed at higher doses. A significant decrease in TOS was detected at 5 µg/mL ( $p < 0.05$ ), and oxidant levels approached normal at 10 µg/mL ( $p < 0.01$ ). These findings indicate that *C. polygonoides* suppresses oxidative stress, supporting its neuroprotective mechanism.

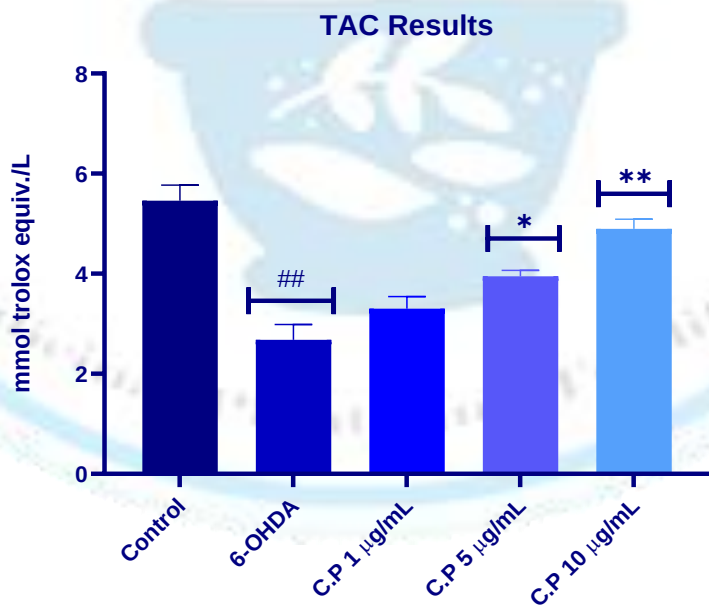


**Figure 2.** This graph shows that 6-OHDA significantly reduces total antioxidant capacity. *C. polygonoides* treatment increased the TAC in a dose-dependent manner, with significant improvements observed at 5 µg/mL and 10 µg/mL.

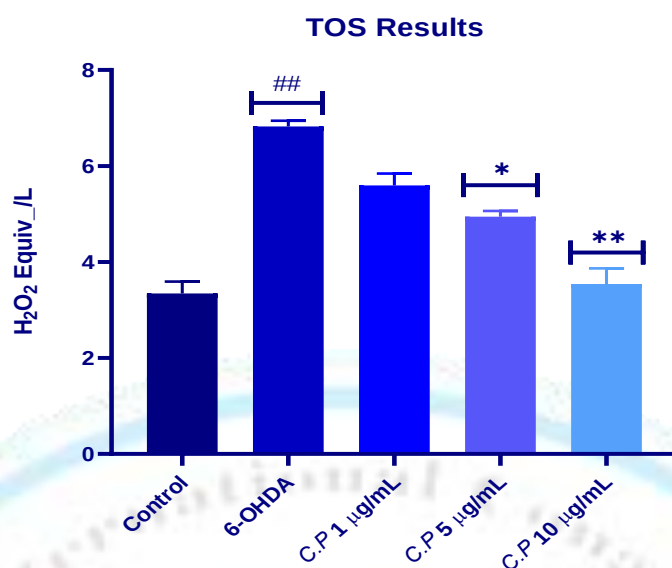


**Figure 3.** This graph shows that 6-OHDA significantly increased the total oxidant capacity. *C. polygonoides* treatment reduces TOS in a dose-dependent manner, with significant improvements observed at 2 µg/mL.

### Results of the biochemical analysis

6-OHDA significantly reduced Klotho protein levels. Treatment with *C. polygonoides* significantly increased Klotho levels at 1, 5, and 10 µg/mL in a dose-dependent manner, with the greatest increase observed at 10 µg/mL.

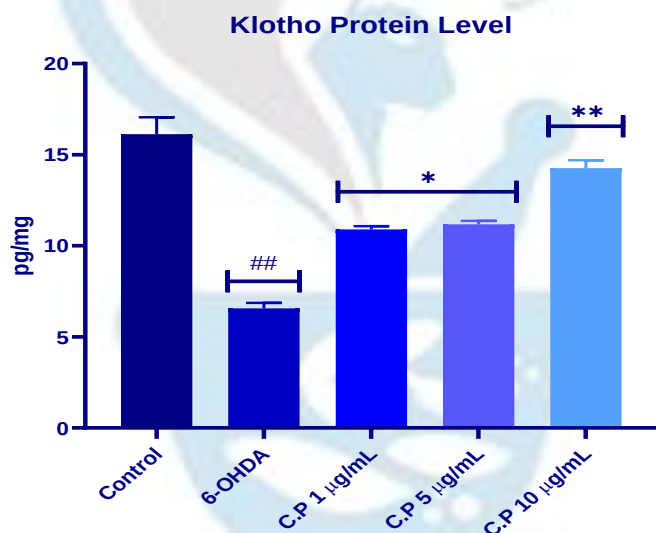


**Figure 4.** This graph shows that 6-OHDA significantly reduces Klotho protein levels. *C. polygonoides* treatment restored Klotho levels in a dose-dependent manner, with 2–5 µg/mL resulting in a significant increase and 10 µg/mL resulting in a highly significant increase.

### DISCUSSION and CONCLUSION

The neuroprotective effects of *C. polygonoides* methanol shoot extract (MSECP) were investigated in neuroblastoma (SH-SY5Y) cells via a 6-OHDA-induced *in vitro* Parkinson's disease model. Our findings revealed that the percentage of apoptotic cells, which significantly increased after 6-OHDA exposure, was reduced in a dose-dependent manner by MSECP treatment, whereas cell viability was significantly increased. These results are consistent with the antioxidant and cytoprotective properties of *C. polygonoides*. In Parkinson's disease, dopaminergic neuronal loss in the substantia nigra pars compacta is known to be the primary pathophysiological mechanism. 6-OHDA, a highly oxidizable dopamine analog, can be taken up by



dopamine transporters and selectively damages catecholaminergic neurons, including dopaminergic neurons of the SNpc, leading to the production of reactive oxygen species (ROS) such as superoxide, peroxide, and hydroxyl radicals<sup>7</sup>. The resulting oxidative stress (OS) can be exacerbated by increased cytoplasmic calcium levels (caused by glutamate excitotoxicity or loss of mitochondrial membrane potential), ultimately leading to cell death<sup>8</sup>. The changes in TAC and TOS observed in our study confirm that 6-OHDA induces oxidative stress and contributes to neurodegeneration. The increase in TAC values and decrease in TOS levels following MSECP administration suggest that the extract improves the intracellular redox balance<sup>9</sup>. The increase in Klotho levels is also noteworthy. The Klotho protein is known for its antiaging and neuroprotective properties and has been identified as a factor that suppresses oxidative stress. Therefore, the increase in Klotho expression induced by MSECP treatment can be considered a potential mechanism that enhances neuronal resilience to oxidative stress<sup>10,11</sup>. Previous studies by Liang-Wei Chen et al. reported that plant-derived phenolic compounds can reduce dopaminergic neuronal loss and exhibit protective effects against oxidative damage. Similarly, Borra K. et al. demonstrated the therapeutic effects of plant extracts on neurodegenerative disorders. Our study supports these findings and provides further evidence that *C. polygonoides* may be a potential therapeutic agent against Parkinson's disease<sup>12,13</sup>.

In conclusion, this study demonstrated the protective effects of *C. polygonoides* extract against 6-OHDA-induced neuronal damage and suggested that it may help regulate oxidative stress, which plays a critical role in the pathophysiology of Parkinson's disease. Therefore, *Calligonum polygonoides* may represent a promising therapeutic candidate for the treatment of Parkinson's disease.

**Author Contributions:** Conceptualization, S.G. and A.T.; methodology, S.G., E.N., K.K., S.B., and A.T.; formal analysis, S.G. and A.T.; investigation, S.G., E.N., K.K., S.B., and A.T.; writing—original draft preparation, S.G., E.N., K.K., S.B., and A.T.; writing—review and editing, S.G., E.N., K.K., S.B., and A.T.; supervision, A.T. All authors have read and approved the final version of the manuscript.

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## SECTION 2

### Abstract Oral Papers



## DETERMINATION OF PHENOLIC CONTENTS IN *RHODODENDRON PONTICUM* EXTRACTS

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### Abstract

Species of the genus *Rhododendron* (family *Ericaceae*) are distributed across the northern regions of Türkiye, with *Rhododendron ponticum* being the most widespread. The leaves and flowers of *Rhododendron* species have been traditionally used in many countries for the treatment of conditions such as the common cold, skin disorders, arthritis, bronchitis, asthma, and gastrointestinal diseases. The aim of the present study is to characterize the phenolic constituents of methanol extracts prepared from the leaves and flowers of *Rhododendron ponticum*. *Rhododendron ponticum* was collected Zonguldak Province, Türkiye. The species and genus were taxonomically identified at the herbarium (voucher code: ISTE-118 950). Separate methanol extracts were prepared from both the leaves and flowers using the Soxhlet extraction method. The phenolic compound profiles of the extracts were analyzed by LC–HRMS. Analyses were performed on an Exactive Plus Orbitrap instrument in both positive and negative ionization modes, and separations were carried out on a COSMOSIL 3C18-EB UHPLC column under gradient elution conditions. Samples were prepared in a methanol–water solvent system containing 0.5% acetic acid, filtered, and injected in triplicate for measurement. The use of both Full Scan and All Ion Fragmentation modes enabled structural confirmation of the compounds. In the leaf extract, the following compounds were identified: salicylic acid, 3-hydroxybenzoic acid, gallic acid, coumaric acid, caffeic acid, chlorogenic acid, quinic acid, 4-hydroxyphenylpropionic acid, catechin, epigallocatechin, rutin, quercetin, isoquercitrin, kaempferol, afzelin, nicotiflorin, myricetin, and eriodictyol. In the flower extract, salicylic acid, gallic acid, coumaric acid, caffeic acid, chlorogenic acid, quinic acid, 4-hydroxyphenylpropionic acid, catechin, rutin, quercetin, afzelin, nicotiflorin, myricetin, eriodictyol, protocatechuic acid, and daidzin were detected. Among the leaf extract constituents, quinic acid, catechin, isoquercitrin, and rutin were the most abundant, whereas in the flower extract, quinic acid, chlorogenic acid, 4-hydroxyphenylpropionic acid, and daidzin were found to be the predominant compounds, respectively. The methanol extracts of *Rhododendron ponticum* leaves and flowers contain a variety of phenolic compounds. These phenolic constituents differ between leaves and flowers in terms of both quantity and diversity.

**Keywords:** Phenolics, *Rhododendron*, *R. ponticum*

## HOMEOPATHIC REMEDIES FOR FOOT AND MOUTH DISEASE

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### Abstract

Foot-and-mouth disease (FMD) is a highly contagious, acute viral infection that affects ruminants and pigs. Although the mortality rate of the disease is around 2–5%, in young animals this rate can reach 50–60% due to acute viral myocarditis. The morbidity is high, and in susceptible cattle populations it can approach 100%. The recovery period in affected animals is prolonged, and these animals play an important role in the introduction of the disease into previously unaffected regions. Since there are many sources of transmission, prevention of the disease is not very easy. As antiviral medications are insufficient, antibiotics are often used to protect against secondary bacterial infections and the progression of complications caused by FMD lesions. Yet, in the modern context of growing antibiotic resistance, there is a strong need for safer and more effective therapeutic options. Homeopathy may serve as a supportive approach to overcoming resistance challenges in infectious diseases. Homeopathy provides a mild and comprehensive method of managing symptoms by using highly diluted remedies to target specific complaints and support the body's natural healing process. Professional veterinary consultation is vital to ensure that treatment strategies are customized to the particular needs of the affected animal. Homeopathy can act as a supportive measure to conventional practices, contributing to symptom relief and overall wellness.

**Keywords:** Complementary therapy, Foot-and-mouth disease, Homeopathy



# GERANIOL, A MONOTERPENE FRAGRANCE WITH PROMISING ANTIOXIDANT PROPERTIES: AMELIORATION OF OXIDATIVE STRESS IN THE COLON AND SMALL INTESTINE OF WISTAR RATS

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## Abstract

Oxidative stress is a common denominator of the pathogenesis of chronic diseases, and herbal extracts have shown some promise in fighting it. Natural agents, which have played a pivotal role in many drug development and research programs, will undergo continual use toward meeting the urgent need to develop effective drugs. An acyclic terpene alcohol with a flowery, rose-like odor called Geraniol (trans-3,7-dimethyl-2,6-oktadien-1-ol) is isolated from the essential oils of several aromatic plants which possesses various beneficial medicinal properties. The shifted equilibrium between the pro-oxidant (reactive oxygen species, ...) and the antioxidant (vitamin C, SOD, ...) species triggers oxidative stress which has been linked to numerous inflammatory diseases, the ageing process and carcinogenesis. This experimental study has aimed to investigate the potential antioxidant activities of Geraniol in the colon and small intestine of Wistar rats. 12 adult male Wistar rats (4–5 weeks old) were randomly divided into two groups: Control group (gavaged with physiological saline) and Geraniol group (gavaged with 100 mg/Kg of Geraniol twice weekly, 50 mg/Kg per dose). At the end of the 56-day period, all rats were anesthetized with intraperitoneal injections of Ketamine and Xylazine, and then dissected. Gastrointestinal tissue including colon and small intestine were sampled and homogenized for measurement of oxidative stress biomarkers including the total antioxidant capacity (TAC), superoxide dismutase (SOD) and malondialdehyde (MDA). As it can be seen in figure 1, oral administration of Geraniol has significantly risen TAC in small intestine in comparison with control group ( $P<0.01$ ). on the other hand, SOD activity in colon tissues of Geraniol-exposed rats has been increased meaningfully ( $P<0.05$ ). Geraniol had no significant effect on MDA formation in intestinal tissues. Hence, Geraniol efficiently elevates the power of antioxidant defense system, leading to a diminution in free radicals. The Geraniol affects antioxidant capacity by modulating the levels of oxidative stress markers in intestinal tissues. As a conclusion, the present study indicates a great potential for this monoterpene as a building block for designing and synthesizing new effective agents which, thus, offers huge importance in deriving new drugs development and progressing direction in medicinal chemistry.

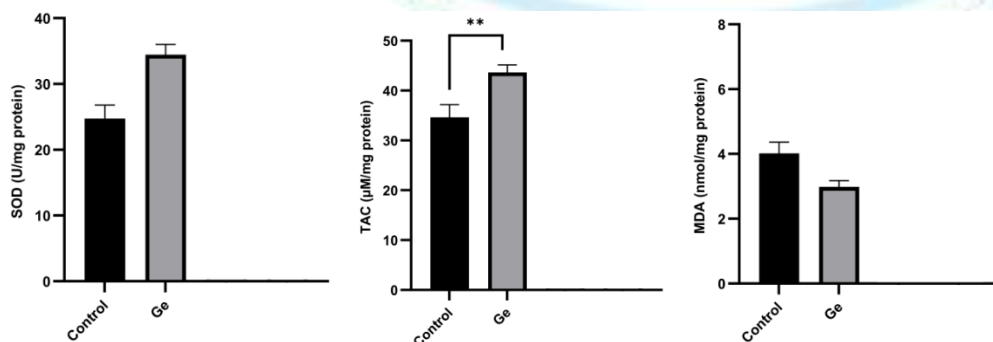


**Figure 1.** Comparison between oxidative stress markers in the small intestine. (\*\* $P<0.01$ )

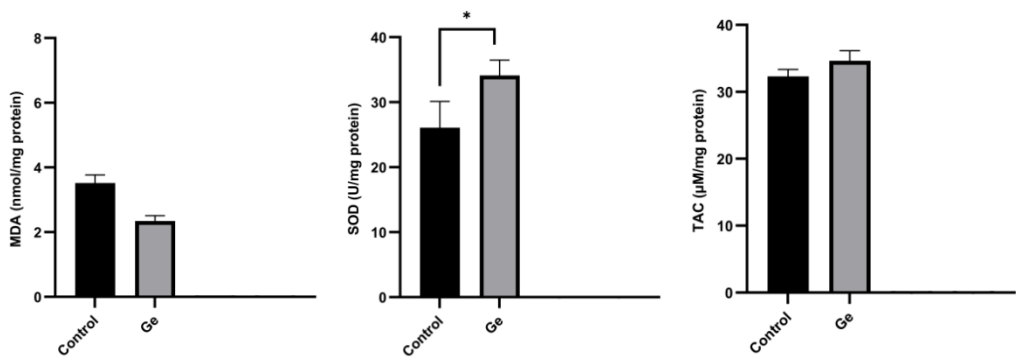


**Figure 2:** Comparison between oxidative stress markers in the colon. (\*P<0.05)

**Keywords:** Colon, geraniol, Oxidative stress, Small intestine, SOD.



## ESSENTIAL OILS IN VETERINARY MEDICINE: NATURAL SOLUTIONS FOR ANIMAL WELL-BEING

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### Abstract

Essential oils are volatile and aromatic mixtures derived from natural sources, typically composed of multiple bioactive compounds. In recent years, their antimicrobial effects, contributions to wound healing, respiratory support via inhalation, mood-regulating properties, and insect-repellent activity have increased their relevance in veterinary medicine. Başer and Franz classified essential oils for veterinary use into five categories: attractants, repellents, insecticidal/antiparasitic agents, feed additives, and therapeutic agents in disease management. These versatile biological properties highlight essential oils not only as supportive agents but also as promising alternatives for protective and therapeutic applications. For instance, the resistance of major ectoparasites to commonly used synthetic insecticides, along with the negative environmental and animal health impacts of these chemicals, has intensified the search for natural alternatives. In this context, essential oils have emerged as eco-friendly and effective agents showing acaricidal, insecticidal, repellent, and ovicidal effects against a wide range of ectoparasites such as lice, mange mites, ticks, and flies. Tea tree, lavender, thyme, clove, and cinnamon oils, in particular, have demonstrated significant mortality and ovicidal activity in both in vitro and in vivo studies. Antimicrobial investigations further support their therapeutic potential. Oils from cinnamon, clove, mint, thyme, and basil exhibited strong inhibitory activity against major bacterial pathogens including *Escherichia coli*, *Salmonella spp.*, *Pseudomonas aeruginosa*, *Campylobacter spp.*, *Staphylococcus spp.*, *Streptococcus spp.*, and *Enterococcus spp.*. Likewise, antifungal activity has been reported, with oregano, tea tree, thyme, cinnamon, and litsea oils showing notable efficacy against *Microsporum canis*, *Trichophyton mentagrophytes*, *Malassezia pachydermatis*, *Aspergillus spp.*, and *Sporothrix brasiliensis*. Another important application of essential oils is in animal nutrition. Following the 2006 ban on antibiotic growth promoters in the European Union, essential oils have gained attention as natural feed additives. Their incorporation into feed not only supports animal health but also helps mitigate the risks associated with antimicrobial resistance. In conclusion, essential oils represent versatile, sustainable, and effective agents in veterinary practice. Their broad-spectrum antimicrobial and antiparasitic properties, combined with their potential as feed additives, underline their importance as natural alternatives to conventional chemicals.

**Keywords:** Essential oils, Veterinary medicine, Ectoparasite control

## INVESTIGATION OF THE EFFICACY OF A NEWLY DEVELOPED HERBAL-BASED HLC CREAM ON NAOH-INDUCED SKIN DAMAGE IN DIABETIC RATS

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### Abstract

Wound healing in diabetic patients is defective, including impairments in neutrophil activation and responses, fibroblast migration and proliferation, and angiogenesis. Delayed wound healing may be due to high glucose-induced insulin resistance via oxidative stress, and topical application of antioxidants such as glutathione has been reported to have a healing effect in the treatment of diabetic wounds. This study aimed to investigate the effects of hLC cream on wound healing stages, free radicals, and antioxidant capacity in NaOH-induced skin damage in diabetic rats. This study consisted of Healthy, Control, Reference (Phyto), and Treatment (hLC) groups. Each group received topical cream application for 21 days. Tissue samples were taken on days 0, 7, 14, and 21, and MDA and GSH levels, as well as SOD and KAT activities, were measured. MMP-9 and NF- $\kappa$ B protein expression levels were examined by Western blotting. This study was supported by the Atatürk University Scientific Research Projects Coordination Unit under project number TDK-2023-12539. After topical application, hLC cream was found to significantly reduce inflammation by mediating the regulation of the NF- $\kappa$ B pathway. It was determined that it prevented the persistently high expression of MMP-9 in diabetic conditions and prevented irregular ECM formation. It was found to prevent lipid peroxidation by reducing free radicals and, consequently, MDA levels, a product of lipid peroxidation. It also significantly contributed to the antioxidant defense mechanism by increasing GSH levels and SOD and KAT activity, preventing oxidative stress. hLC cream was found to accelerate the wound healing process, protect the wound against inflammation, reduce oxidative stress, and support antioxidant defense. The efficacy of hLC cream has been investigated, and its safe use as an alternative solution to a common health problem has been demonstrated.

**Keywords:** Diabetes mellitus, Oxidative stress, Wound healing



## PHYTOTHERAPEUTIC STRATEGIES TO PREVENT ANTHELMINTIC RESISTANCE IN VETERINARY MEDICINE

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### Abstract

Parasitic diseases of livestock are a pervasive global problem, causing significant economic losses in both developed and developing countries. Among parasitic infections, gastrointestinal nematodes (GIN) are the primary contributors to production losses. Anthelmintics represent the most prevalent class of pharmaceuticals employed in the management of these parasites. Nevertheless, inadequate or improper application of these medications has been demonstrated to contribute to the emergence of anthelmintic resistance (AR). This issue has become increasingly prevalent in the treatment of gastrointestinal parasites in ruminants, poultry, and companion animals, resulting in reduced drug efficacy in modern pharmacological approaches. Therefore, the investigation of alternative and supportive treatment strategies in veterinary medicine has gained increasing importance. Phytotherapeutic products, particularly plant extracts, essential oils, and secondary plant metabolites, have shown promising results against anthelmintic resistance. Species such as *Azadirachta indica* A.Juss. (neem), *Artemisia annua* L., *Allium sativum* L. (garlic), *Origanum vulgare* L., *Ocimum sanctum* L., *Nigella sativa* L., *Punica granatum* L., *Thymus vulgaris* L., *Chenopodium ambrosioides* L., and *Syzygium aromaticum* (L.) Merr. & L.M.Perry have demonstrated significant efficacy against gastrointestinal nematodes and helminths in both in vitro and in vivo studies. Secondary metabolites such as terpenes, alkaloids, saponins, tannins, and flavonoids exert anthelmintic activity by affecting helminths' energy metabolism, cell membrane integrity, and neuromuscular transmission. Compounds such as azadirachtin, artemisinin, allicin, and thymol are exemplary molecules contributing to this activity. Recent reviews indicate that plant-based products possess not only antiparasitic but also antimicrobial and anti-inflammatory properties, thereby supporting animal health in a holistic manner. In this context, phytotherapy emerges as a sustainable and environmentally friendly approach to address anthelmintic resistance and is recommended for broader evaluation in veterinary clinical practice.

**Keywords:** Anthelmintics, Phytotherapy, Secondary metabolites, Parasitic diseases

## HOUSE PLANTS THAT CAN CAUSE INTOXICATIONS IN COMPANION ANIMALS

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### Abstract

Plant poisoning in companion animals represents an increasingly prevalent yet frequently underrecognized health concern, concomitant with the growing popularity of houseplants and ornamental species. Such intoxication events may result in acute toxic manifestations as well as chronic pathological conditions arising from prolonged exposure to phytotoxins. A primary contributing factor is the limited awareness among pet owners regarding the potential hazards posed by decorative plants commonly kept within households. This risk is particularly pronounced in juvenile animals, such as puppies and kittens, which are more likely to ingest plants due to curiosity, boredom, or exploratory behavior. Furthermore, the increasing amount of time pets spend indoors exacerbates the likelihood of exposure. Toxicity arises from a wide array of plant-derived secondary metabolites, including alkaloids, glycosides, saponins, and oxalates, which may exert deleterious effects following ingestion or dermal contact. For example, *Nerium oleander* contains oleandrin, a cardioactive glycoside capable of inducing fatal outcomes with the ingestion of even a single leaf. Clinical manifestations of plant poisoning range from oral mucosal irritation and gastrointestinal disturbances (vomiting, diarrhea) to respiratory distress, cardiac arrhythmias, and, in severe cases, death. Accurate diagnosis is often challenging and requires correct identification of the ingested plant species. Given that specific antidotes are rarely available for most phytotoxins, therapeutic interventions primarily involve symptomatic management and supportive care. Moreover, insufficient familiarity with toxic plant species among veterinary practitioners may further complicate case management. Accordingly, enhancing the knowledge of both pet owners and veterinarians regarding toxic plants and their associated risks is of paramount importance. Preventive strategies, including education and awareness-raising initiatives, remain the cornerstone in mitigating this avoidable yet significant threat. This resenation therefore seeks to contribute to greater awareness of this issue among pet owners.



## PROTECTIVE EFFECTS OF HESPERIDIN AGAINST TESTICULAR TOXICITY INDUCED BY DI (2-ETHYLHEXYL) PHTHALATE IN RATS

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### Abstract

In this study, we aimed to investigate the effects of hesperidin (HSP) against testicular damage induced by di(2-ethylhexyl) phthalate (DEHP). A total of 35 male Sprague Dawley rats were used in this study, and each group consisted of 7 animals: Group 1 (control, corn oil for 7 days), Group 2 (DEHP, 1 g/kg/body weight DEHP (with corn oil) given orally for 10 days), Group 3 (HSP, 200 mg/kg/body weight hesperidin given orally for 10 days), Group 4 (DEHP+HSP 100, 1 g/kg/body weight DEHP (with corn oil) and HSP 100 mg/kg/body weight orally for 10 days), and Group 5 (DEHP+HSP 200, 1 g/kg/body weight DEHP (with corn oil) and HSP 200 mg/kg/body weight orally for 10 days). 24 hours after the last administration (day 11), rats were decapitated under mild sevoflurane anesthesia, and testicular tissues were removed. Testicular samples were used for biochemical and histopathologic examination. The epididymis was used for semen extraction. As a result of the study, it was observed that DEHP had a negative effect on spermatologic values. In histopathological evaluations, testicular integrity was disrupted, and seminiferous tubules lost their normal and regular epithelial structure in the DEHP group. In biochemical analyses, GPX was significantly increased in the hesperidin-treated group compared to the control group, while the difference between the DEHP-treated group and the control group was found to be insignificant. SOD showed a significant decrease in the DEHP group. Plasma hormone analysis showed that DEHP significantly decreased the testosterone hormone level. Treatment in the presence of DEHP significantly increased the LH level. Testicular toxicity occurred in rats treated with di(2-ethylhexyl) phthalate, whereas hesperidin had a positive effect on sperm quality, oxidative stress parameters, and histopathological changes in testicular tissue.

**Keywords:** Di-(2-ethylhexyl) phthalate, Hesperidin, Sperm, Testicular toxicity

## INVESTIGATION OF THE EFFECTS OF DIFFERENT DOSES OF CURCUMIN ON AQP3 AND AQP7 PROTEINS IN RAT SPERM

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### Abstract

This study aimed to investigate the dose-dependent effects of curcumin on the expression and localization of AQP3 and AQP7 proteins, as well as on sperm quality parameters in rats. Twenty-eight adult male Sprague Dawley rats were randomly divided into four groups and administered curcumin orally for 28 days: Control (Tween 80), Low dose (100 mg/kg), Medium dose (500 mg/kg), and Toxic dose (1500 mg/kg). At the end of the experiment, testis and epididymis tissues were collected for histopathological, biochemical (Western blot), and spermatological analyses (motility, membrane damage, DNA fragmentation). Data were analyzed using SPSS v26. The toxic dose increased plasma membrane damage (21.14%) and abnormal spermatozoon rate (9.42%). DNA fragmentation was lowest in the medium dose group (8.57%), while it increased in low and toxic dose groups. Sperm motility was lower in all curcumin-treated groups compared to control. AQP3 expression increased in medium and toxic doses, while AQP7 expression increased at low dose but decreased at toxic dose. Histopathological examination revealed severe testicular damage, such as coagulative necrosis and epithelial degeneration, in the toxic dose group. The effects of curcumin on sperm are dose-dependent. While the medium dose (500 mg/kg) preserved DNA integrity, low and toxic doses negatively affected sperm quality. Changes in AQP3 and AQP7 expression indicate curcumin's complex role in osmoregulation and energy metabolism. Determining the optimal dose is critical for potential therapeutic use in male fertility.

**Keywords:** Aquaporin, Curcumin, Oxidative stress, Rat, Sperm, Cryopreservation



## THE EFFECT OF ANTIOXIDANT COMPOUNDS ON SPERM STORAGE

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### Abstract

During the stages of freezing, storage, and thawing of sperm, there may be decreases in viability and motility, DNA damage, and morphological abnormalities. These damages are caused by the plasma membrane being more sensitive to cryopreservation due to low cholesterol and high unsaturated fatty acids. To prevent this problem, antioxidants are added to the extender. These substances play an important role in reducing the stress that occurs during freezing and minimizing the damage caused by reactive oxygen species (ROS). Most of the oxygen used by spermatozoa to produce ATP in mitochondria is converted into water, while 1-2% is undesirably converted into superoxide anion ( $O_2^-$ ) and converted into hydrogen peroxide ( $H_2O_2$ ) by the superoxide dismutase (SOD) enzyme found in the cell's cytoplasm. If there are no antioxidants to reduce  $H_2O_2$  to water, it will rapidly convert to hydroxyl ( $OH^-$ ) radicals via iron ions in the environment. Although the  $H_2O_2$  metabolite is less active, it can damage the entire cell because it can pass through biological membranes and triggers apoptosis in spermatozoa. Antioxidant substances added to sperm diluents donate electrons to stabilize ROS, thereby reducing the oxidation of fatty acids in the spermatozoa membrane and causing less DNA damage. It has been reported that antioxidant compounds added to the semen of various species improve post-thaw sperm quality following cryopreservation. Hesperidin, curcumin, sulforaphane, epigallocatechin gallate, luteolin, and syringic acid have been added to sperm diluents of various types, yielding successful results. In conclusion, exogenous antioxidant supplements added to sperm diluents increase sperm quality by inhibiting oxidative stress and apoptosis at effective doses after cryopreservation.

**Keywords:** Antioxidants, Cryopreservation, Sperm

## EFFECT OF CURCUMIN SUPPLEMENTATION ON INDOXYL SULFATE AND INFLAMMATION IN CATS WITH CHRONIC KIDNEY DISEASE

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### Abstract

Chronic kidney disease (CKD) is a progressive disorder in cats in which inflammation plays a pivotal role. Increased levels of indoxyl sulfate, a uremic toxin, contribute to disease progression. Curcumin is a natural compound with anti-inflammatory properties and potential effects on indoxyl sulfate levels. This study aimed to evaluate the effects of oral curcumin supplementation in addition to standard therapy on renal function and inflammatory markers in cats with stage III CKD. A total of 24 client-owned cats were included: 16 cats with stage III CKD and 8 healthy controls. CKD cats were randomized into two groups: standard therapy only (ST; n=8) and standard therapy plus curcumin (ST + C; n=8). Curcumin was administered orally at 80 mg/cat/day, divided into two doses, for 30 days. Hematological and biochemical analyses were performed at baseline (T0), day 15 (T15), and day 30 (T30). There were no significant differences between the groups in blood urea nitrogen (BUN), creatinine, and phosphorus concentrations ( $p > 0.05$ ). Indoxyl sulfate levels showed a numerical decrease in the ST + C group, but this was not statistically significant ( $p > 0.05$ ). Inflammatory markers, including neutrophil-to-lymphocyte ratio (NLR) and systemic inflammation response index (SIRI), declined in the ST + C group beginning at T15 and remained lower than baseline values at T30 ( $p < 0.05$ ). Curcumin supplementation reduced inflammatory markers in cats with stage III CKD, supporting its potential role as an adjunct therapeutic option. However, the effect on indoxyl sulfate should be confirmed in longer-term studies with larger sample sizes.

**Keywords:** Cats, Chronic kidney disease, Curcumin, Inflammation, Indoxyl sulfate



## INVESTIGATION OF THE EFFECTS OF ZINGERONE ON APOPTOSIS AND OXIDATIVE STRESS AGAINST DIABETIC PANCREATIC DAMAGE IN RATS

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### Abstract

Diabetes is a chronic metabolic disease that develops as a result of either a deficiency of insulin, the hormone secreted from the beta cells of the pancreas, or the inability of tissues to effectively utilize this hormone. According to World Health Organization (WHO) data, approximately 420 million people had diabetes in 2014, and 1.5 million people died from diabetes-related causes in 2019. People with diabetes experience a decrease in their quality of life due to numerous complications. Prolonged hyperglycemia in diabetes mellitus leads to disruptions in carbohydrate, lipid, and protein metabolism, and causes serious damage to many tissues and organs, especially the pancreas. Hyperglycemia increases free oxygen radicals, deficient antioxidant defense systems, and, as a result, significantly increases oxidative stress. This leads to cellular structural deterioration, structural changes, and mutations in proteins and nucleic acids. In recent years, herbal therapies have been increasingly used to treat these common diseases. Extracts and active ingredients derived from plants are being investigated for their protective effects against complications caused by diabetes. Zingerone, derived from ginger and known for its potent antioxidant properties, is one such natural compound, which has been studied extensively for many years. Studies have shown that zingerone has anti-inflammatory, antimutagenic, and cytoprotective effects. This study aimed to investigate the protective effect of zingerone on the damage caused by diabetes in pancreatic tissue. For this purpose, 50 female rats were randomly assigned to five groups: control, diabetes, diabetes + metformin, diabetes + zingerone, and diabetes + metformin + zingerone. Histopathologically, atrophy in the islets of Langerhans, severe degeneration, and necrosis of islet cells were observed in the diabetes group, while these pathologies were significantly reduced in the zingerone-treated groups. Experimental studies have shown that zingerone reduces oxidative stress markers, lowers malondialdehyde (MDA) levels, and increases the activity of antioxidant enzymes (SOD, CAT, and GPx) in pancreatic tissue in diabetic animal models. Furthermore, zingerone treatment significantly reduced the expression levels of 8-OHdG, Caspase-3, and COX-2 in pancreatic tissue, which increase due to diabetes, while Nrf2 and Bcl-2 expression levels increased. Consequently, it was concluded that zingerone exerts a protective effect against diabetes-induced pancreatic damage by reducing oxidative stress and inhibiting apoptosis.

**Keywords:** Apoptosis, Diabetes mellitus, Oxidative stress, Pancreas, Zingeron

## WOUND HEALING AND ANTIOXIDANT POTENTIAL OF *HIPPOPHAE RHAMNOIDES* OIL NANOEMULSIONS: IN VITRO CHARACTERIZATION AND BIOLOGICAL EVALUATION

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### Abstract

*Hippophae rhamnoides* (HR) is a plant source notable for its high content of omega-7 fatty acids, vitamins, flavonoids, and carotenoids. Its oil exhibits antioxidant, anti-inflammatory, and regenerative activities, making it a candidate for novel therapeutic applications in both human and veterinary medicine. However, its direct use in biological systems is limited by poor solubility and dispersion. Nanoemulsion technology offers a means to enhance stability, bioavailability, and controlled delivery of lipophilic plant-derived compounds. This study investigated the physicochemical characteristics and biological effects of HR oil nanoemulsions on fibroblast proliferation, oxidative stress, and wound repair. HR fruits were collected in Erzurum (Türkiye) and Soxhlet-extracted with n-hexane. Fatty acid composition was determined by GC-FID after FAME derivatization. Ten nanoemulsions were prepared with HR oil using different surfactants (Labrasol ALF, Capmul PG-12, Span 80, Capryol 90, Lauroglycol 90, Captex 355, Myritol 318, Span 20, PEG-6 caprylic/capric glyceride, Brij 72) and Tween 20 in the aqueous phase. Ultrasonication was applied for droplet reduction. Physicochemical parameters included droplet size, zeta potential, PDI, viscosity, pH, and TEM morphology. Biological activity was assessed in L929 fibroblasts via MTT proliferation, hydrogen peroxide-induced oxidative stress, and scratch assays. GC-FID revealed palmitoleic acid (35.89%), palmitic acid (25.21%), and elaidic acid (21.2%) as dominant fatty acids. Nanoemulsions showed droplet sizes of 174–221 nm, zeta potentials of –23.5 to –35.1 mV, and skin-compatible pH. HR nanoemulsions improved fibroblast viability at 562.5–140.6 µg/mL, preserved >80% viability under oxidative stress, and promoted wound closure (25–43% at 24 h vs. 20% in controls). HR oil nanoemulsions demonstrated proliferative, antioxidant, and wound-healing effects, supporting their potential as dermal formulations for both human and veterinary medicine. Further in vivo validation is recommended to confirm therapeutic efficacy and ensure safe translation into clinical and veterinary practice.

**Keywords:** *Hippophae rhamnoides* L., Cell proliferation, Scratch test, Nanoemulsion, Fatty oil



## REGULATORY ROLE OF MORIN HYDRATE ON OXIDATIVE STRESS AND APOPTOSIS IN DIABETES-INDUCED LIVER DAMAGE

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### Abstract

Diabetes mellitus (DM) is a complex metabolic disease characterized by chronic hyperglycemia, resulting from the complete absence of insulin production, inadequate secretion, or ineffective utilization by tissues. High blood sugar levels can lead to serious complications in many organs and systems over time. While classic complications include nephropathy, retinopathy, and cardiovascular disorders, the liver's role in diabetes has become increasingly important recently. Morin hydrate, a natural flavonoid compound, is known for its anti-inflammatory, antimicrobial, neuroprotective, cardioprotective, hepatoprotective, nephroprotective, gastroprotective, anti-cancer, anti-arthritis, and potent antioxidant properties. While the protective effects of morin hydrate on various organs and systems have been described in detail in the literature, studies examining its role in diabetes-related liver damage are limited. This study aimed to investigate whether morin hydrate, which has potent antioxidant and hepatoprotective properties, has protective effects against experimentally induced diabetes and its complication, hyperglycemia. For this purpose, 60 female Sprague-Dawley rats were randomly divided into six groups. The study groups were: control, diabetes, diabetes+metformin, diabetes+metformin+MH100, diabetes+MH100, and MH100. On the 28th day of the study, all rats were sacrificed by cervical dislocation under general anesthesia. Biochemical, histopathological, and immunohistochemical analyses were performed on liver tissue samples. ALT, AST, ALP, LDH, MDA, SOD, CAT, GSH, and GPx levels were determined in liver tissue samples. Histopathologically, hepatocyte degeneration and necrosis, sinusoidal disruption, and increased Kupffer cell activation were observed in the diabetic group. Immunohistochemical analyses revealed increased expression levels of 8-OHdG and Caspase-3, while Nrf2 and Bcl-2 expression decreased. Serum samples showed an increase in toxicity markers, while antioxidant levels decreased. These findings approached normal levels in the treatment groups. In conclusion, morin hydrate has been shown to have a protective and supportive effect against hepatotoxicity caused by diabetes and its associated hyperglycemia.

**Keywords:** Apoptosis, Diabetes mellitus, Hepatotoxicity, Morin hydrate, Oxidative stress

## THE EFFECT OF THE SUPPLEMENTATION OF COMMERTIAL ESSENTIAL OIL MIXTURE APPLIED TO SIMMENTAL CALVES IN THE NEONATAL PERIOD ON GROWTH, DEVELOPMENT AND HEALTH PARAMETERS

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### Abstract

In this study, it was aimed to investigate the effects of the use of commercial essential oil mixture (CEOM), a commercial product obtained from vegetable aromatic oils on the growth, development and health parameters of calves due to diarrhea in dairy cattle enterprises as an alternative to antibiotics that leave residue. The animal material of the study consisted of 24 newborn Simmental calves raised in Atatürk University Food and Livestock Research and Application Center (GHUAM). Control and treatment groups consisted of 6 male and 6 female. After birth, 20 ml of CEOM was given orally to the calves following the morning milk feeding in the treatment group for 5 days. The 1st month weight was  $45.7 \pm 1.67$  and  $42.4 \pm 1.65$  kg in the control and treated groups, respectively, the difference in favor of the control group was insignificant. There was no difference between the control and treatment groups in terms of body measurements at birth, however, a significant ( $P < 0.05$ ) difference was observed in chest circumference in favor of the control group at the 1st month. There was no difference between the treatment and control groups in terms of hematological analyzes detected in the blood taken at the 10th day and 1 month of ages. No difference was observed between the groups in terms of immunoglobulins (IgM and IgG). It was also determined that the liquid premix had no effect on the stool score of the calves included in the trial. It was found out that CEOM does not make any difference in terms of growth, development and health of Simmental calves. In studies to be conducted on the subject, it has been suggested that higher doses of CEOM be included in the trial.

**Keywords:** Calf, Essential oil, Growth and development



## SYNERGISTIC EFFECTS OF NETTLE ESSENTIAL OIL COMBINED WITH ANTIBIOTICS AGAINST SELECTED BACTERIAL STRAINS

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### Abstract

With the increase in antibiotic-resistant bacteria and the lack of new antibiotics being brought on to market, alternative strategies need to be found to cope with infections resulting from drug-resistant bacteria. The synergistic effects between antibiotics and essential oils offer a promising approach to the current growing problem of antibiotic resistance. Antibiotic resistance is a major threat to global public health. This situation is making existing antibiotics less and less effective, and as a result, there's a growing need for new strategies to fight bacteria. Essential oils are emerging as a potential solution in this field, thanks to their broad-spectrum antimicrobial effects and diverse mechanisms of action. With the continual emergence of new bacterial strains resistant to antibiotics, the efficacy of antibiotics has dropped, and antibiotic resistance has become a global public health issue: when infections become resistant to first-line medicines, more expensive therapies must be used. This study investigated the synergistic antibacterial effects of nettle essential oil combined with three antibiotics (Erythromycin 15µg, Oxolinic acid 2µg, Netilmicin 30 µg) against bacterial strains *Bacillus cereus* BC6830, *Klebsiella pneumoniae* ATCC 70063 and *Proteus mirabilis* ATCC 12453. Disk diffusion assays measured inhibition zone diameters in millimeters for antibiotics alone and in combination with 10 µL of nettle essential oil. Results showed varying degrees of enhancement in antibacterial activity, with the highest synergy observed for Netilmicin 30 and Oxolinic acid against *K. pneumoniae*. These findings support further research on essential oil-antibiotic combinations as alternative strategies to combat antibiotic resistance. In veterinary medicine, nettle essential oil exhibits great promise as an adjuvant for antibiotics, especially against Gram-negative infections (*Klebsiella*, *Proteus*). Combining antibiotics with essential oils offers a promising strategy to reduce antibiotic doses, side effects, and resistance development, though most research remains in vitro. More in vivo and clinical studies are needed to confirm their safety and effectiveness before clinical use.

**Keywords:** Antibacterial, Essential oil, Nettle, Synergistic

## MATERNAL FLAVONOID-ENRICHED APPROACHES IMPROVE OXIDANT STATUS, ENHANCE MILK QUALITY, AND INCREASE KID BIRTH WEIGHT IN GOATS

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### Abstract

This study aimed to investigate the effects of flavonoid-enriched supplementation (FES) on combating animal stress during a transition period. Forty cross-Beetal goats were allocated into mid-pregnancy (n=20) and late pregnancy to early lactation (n=20), and each group was further sub-grouped into control (basal diet) and treated (basal diet + FES @ 120 mg/animal/day) with equal animal numbers. Flavonoid-enriched supplementation significantly increased catalase, glutathione peroxidase, and superoxide dismutase in the treated groups in both groups ( $P < 0.05$ ). The supplemented group enhanced enzymatic antioxidants in colostrum and milk compared with the control group ( $P < 0.05$ ) in late pregnancy to lactating goats. In contrast to the control group, the antioxidants significantly promoted total phenolics, lycopene, carotenoids, and total flavonoids ( $P < 0.05$ ) during mid- to late-pregnant goats. The total proteins, total antioxidant capacity, total oxidant status, and malondialdehyde levels were significantly elevated compared to the control group in mid- to late-pregnant goats as well as in colostrum and milk, respectively ( $P < 0.05$ ). Moreover, antioxidant exposure had enhanced the initial and total birth weight and survival rate of goat kids more than the control group ( $P < 0.05$ ). The results suggest that an antioxidant diet during the perinatal period may attenuate pregnancy stress, improve milk indicators, and produce healthy progeny.

**Keywords:** Biochemical profile, Flavonoid-enriched supplementation, Milk quality, Transition period



## THERAPEUTIC EFFECTS OF VARIOUS FLAVONOIDS

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### Abstract

Flavonoids are compounds naturally found in plants, often giving them their vibrant colors (such as yellow, blue, purple, and red). They belong to the polyphenolic phytochemical class, which is highly beneficial for human health. Flavonoids constitute a large portion of the most researched polyphenols in recent years. Over 5,000 flavonoids have been identified today. Flavonoids are abundant in the human diet in foods such as fruits, vegetables, tea, flowers, seeds, nuts, wine, honey, propolis, and cocoa. These compounds, which support human and animal health and help reduce the risk of disease, have been used throughout history in the treatment of various diseases. The relationship between the structural properties of flavonoids and their biological functions explains their fundamental activities. Flavonoids possess many impressive and beneficial biological activities. The basis of these benefits lies in their role as powerful antioxidants. Antioxidants combat free radicals in the body, preventing cell damage. This, in turn, helps reduce the risk of many chronic diseases. In addition, thanks to their anti-inflammatory, anticancer, cardioprotective, antibacterial, antiviral, hepatoprotective, and neuroprotective properties, they are considered as potential therapeutic agents in the prevention and treatment of many chronic diseases.

**Keywords:** Antioxidant, Flavonoid, Polyphenol

## ANTIBIOFILM ACTIVITY OF *NIGELLA SATIVA* (BLACK SEED) ESSENTIAL OIL AGAINST SELECTED PATHOGENIC BACTERIA

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### Abstract

The increase in antimicrobial resistance and tolerance over the years has become a serious public health problem, leading to the inevitable development of alternative antimicrobial agents as substitutes for industrial pharmaceutical antibiotics targeting humans and animals under the concept of one health. Essential oils (EOs) extracted from aromatic and pharmaceutical plants incorporate several bioactive compounds (phytochemicals) that positively affect human and animal health. Herein, this work aimed to examine a standardized chemical composition and screen the antimicrobial and anti-biofilm activity of essential oil—black seed (C) was tested at three concentrations (50-100- 200 µg/mL) on three bacterial strains, such as *Bacillus cereus* and *Klebsiella pneumoniae*. Biofilm was quantified by crystal violet staining in a microtiter plate assay, with optical density (OD) read at 570 nm. Black seed essential oil exhibits potent antibiofilm activity against Gram-negative bacteria at moderate doses, but its variable activity against Gram-positives and diminished efficacy at higher concentrations warrant further investigation. Data suggest that biofilm activity depends on the bacterial species and the applied concentration. Essential oils are prominent antimicrobial and anti-biofilm agents due to the presence of various active components in their composition.

**Keywords:** Antibiofilm, Essential oil, *Nigella sativa*, *Bacillus cereus*, *Klebsiella pneumoniae*



## TANNIC ACID EFFECT IN TESTICULAR INJURY: MOLECULAR PROTECTION AGAINST MSG TOXICITY

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### Abstract

Monosodium glutamate (MSG) is a controversial food additive widely used as a flavor enhancer in the food processing industry, restaurants and institutional services. Although MSG consumption is considered safe by food safety organizations, many preclinical and clinical studies, especially long-term exposure, are still questioned. Long-term MSG consumption has been shown to have negative effects on the male reproductive system. In this study, the toxic effects of MSG, a widely used food additive, on rat testicular tissue were evaluated and the potential protective role of tannic acid (TA), a natural polyphenolic compound, against this toxicity was investigated. For this purpose, 24 rats were divided into 4 groups with 6 rats in each group. The first group was the control group, the second group was MSG group (2 g/kg), the third group was TA group (50 mg/kg) and the fourth group was MSG+TA group (2 g/kg+50 mg/kg). After 21 days of treatment, testicular tissues were removed from the animals. Antioxidant oxidative stress markers (GSH, MDA) and enzyme activities (SOD, CAT, GPx) were analyzed at gene and biochemical levels. There was a decrease in GSH level and a significant increase in MDA level in the MSG group. While a decrease in SOD and CAT enzyme activities was observed, no significant change was found in GPx activity. MSG administration also suppressed the expression of antioxidant system-related genes at the gene level. The data obtained show that MSG causes impairment in antioxidant system functions in testicular tissue. It is concluded that TA treatment suppresses these harmful effects, supports cellular defense and protects testicular functions.

**Keywords:** Monosodium glutamate, Testicular toxicity, Oxidative stress, Tannic acid

## AN OVERVIEW OF QUERCETIN ANTICANCER MECHANISMS

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### Abstract

Cancer is considered a major obstacle to increasing life expectancy owing to its rapidly rising incidence and mortality rates. It involves complex biological mechanisms such as growth-promoting signals, triggering angiogenesis, and prompting invasion and metastasis, all of which make its treatment intensely complicated. Quercetin (QUE; 3,5,7,3',4'-pentahydroxyflavone), a major flavonoid found in fruits and vegetables, exhibits biological effects such as antioxidant, anti-carcinogenic, and anti-inflammatory effects. Cancer cells engage in competition with other cell types to acquire essential nutrients for development; however, due to the nutrient-limited tumor environment, they must find alternative pathways to meet energy demands for proliferation and cell growth. One such mechanism is aerobic glycolysis, which cancer cells utilize to produce energy. Although aerobic glycolysis produces only 2 ATP molecules per molecule of glucose, compared to the 36 ATP molecules generated by oxidative phosphorylation, cancer cells produce high levels of carbon-rich metabolites that can serve as precursors for de novo synthesis of nucleotides, lipids, or amino acids. The phosphoinositide 3-kinase/protein kinase B (PI3K/Akt) pathway governs the mechanism of metabolic reprogramming, which in turn triggers an increase in glucose uptake and glycolysis. The main anticancer effect of QUE is based on its ability to suppress the mitotic process by regulating pro-apoptotic, PI3K/Akt, and mitogen-activated protein kinase (MAPK) molecular pathways. Furthermore, QUE demonstrates the ability to suppress the rise in H<sub>2</sub>O<sub>2</sub> -induced reactive oxygen species (ROS) and significantly downregulate the phosphorylation of Akt, PDK1, Bcl-2-associated death promoter (BAD), and the level of tumor necrosis factor receptor 1 (TNFR1). Owing to its diverse anticancer pathways, QUE holds promise as an anti-cancer agent.

**Keywords:** Anti-carcinogenic, Aerobic glycolysis, Cancer, Quercetin, PI3K/Akt pathway



## EFFECTS OF LICORICE (*GLYCYRRHIZA GLABRA*) EXTRACT ON GAS PRODUCTION KINETICS, DEGRADABILITY, PROTOZOAL POPULATION, AND FERMENTATION PARAMETERS IN A BALANCED CATTLE DIET

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### Abstract

Medicinal plants are increasingly employed to optimize rumen fermentation and enhance feed efficiency in ruminants. Licorice (*Glycyrrhiza glabra*), renowned for its therapeutic applications, particularly in treating gastric ulcers, exhibits antibacterial, antioxidant, antifungal, anticancer, anti-inflammatory, and cytotoxic properties. This study evaluates the impact of licorice extract on rumen fermentation parameters, including gas production kinetics, methane output, protozoal populations, degradability, pH, and volatile fatty acid (VFA) profiles in an in vitro cattle rumen model. Licorice extract was incorporated into a balanced cattle diet and assessed using an in vitro rumen fluid system. Parameters measured included total gas production, methane output, protozoal counts, dry matter degradability, rumen pH, and VFA composition (acetic, propionic, butyric, valeric, and isovaleric acids). The experimental design compared control and licorice extract-treated samples, with statistical significance determined at  $p < 0.05$ . Licorice extract significantly increased total gas production ( $178.09 \pm 0.44$  mL,  $p < 0.05$ ), indicating enhanced microbial fermentation. Methane production was significantly reduced ( $p < 0.05$ ). Protozoal populations decreased markedly ( $1,245,000 \pm 6,291.53$ ,  $p < 0.05$ ), potentially improving nutrient utilization. Dry matter degradability improved significantly ( $65.30 \pm 0.17\%$ ,  $p < 0.05$ ). Rumen pH remained stable ( $6.80 \pm 0.15$ ,  $p > 0.05$ ). Licorice extract significantly altered VFA profiles, increasing valeric, isovaleric, and propionic acid concentrations ( $p < 0.05$ ), while acetic and butyric acid levels remained unchanged ( $p > 0.05$ ). Licorice extract enhances rumen fermentation by increasing gas production, reducing methane and protozoal populations, and improving degradability without affecting pH. Its selective modulation of VFAs underscores its potential to optimize feed efficiency. These findings advocate for licorice extract's integration into cattle diets to enhance productivity and sustainability. Future in vivo studies and dose optimization are recommended to validate these effects and facilitate practical application.

**Keywords:** Licorice, Gas production, Ruminants, Methane, Feed efficiency

## A NOVEL CANINE THORACIC AND PELVIC LIMB ACUPUNCTURE TRAINING MODELS

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### Abstract

The aim of this work was to develop and evaluate a realistic, reusable canine acupuncture teaching model using Elnady's plastination technique for training veterinary students at the Virginia–Maryland College of Veterinary Medicine (VMCVM). The model was designed to enhance anatomical understanding, facilitate repetitive skill practice, and reduce reliance on live animals in acupuncture training. Canine forelimb and hindlimb specimens were prepared using Elnady's plastination technique, preserving soft-tissue pliability and anatomical fidelity. Each limb was fitted with a zippered skin sleeve, allowing learners to practice needle placement at acupuncture points with the skin closed, then “unzip” to verify underlying anatomical relationships, including muscle compartments, neurovascular structures, and bony landmarks. The models were integrated into a structured canine acupuncture laboratory session consisting of pre-briefing, guided practice, and supervised repetition. After training, students and instructors completed a survey evaluating model realism, educational value, confidence in anatomical landmark identification, and overall usefulness. Survey responses indicated that the models were highly realistic and pedagogically effective. Students reported improved confidence in locating acupuncture points and understanding three-dimensional anatomical relationships relevant to safe needle insertion. Instructors emphasized the value of immediate anatomical verification, the ability to standardize teaching across multiple groups, and the durability and reusability of the specimens without the constraints of live-animal or traditional cadaver labs. Elnady-prepared canine limb specimens with zippered skin access provide a practical, high-fidelity platform for teaching canine acupuncture. This approach enables hands-on practice with direct anatomical confirmation, supports repetitive skill acquisition, and was perceived by both students and instructors as realistic and highly beneficial. It is recommended that similar plastinated models be incorporated into acupuncture and other procedural skills training in veterinary curricula to complement or reduce reliance on live-animal sessions while maintaining strong anatomical rigor.

**Keywords:** Acupuncture, Canine, Elnady, Models, Training, Veterinary



## HYPOGLYCEMIC ACTIVITY AND HEMATOLOGICAL PARAMETERS OF BLOOD IN RATS AFTER ADMINISTRATION OF *GALEGA OFFICINALIS* EXTRACT (*GALEGA OFFICINALIS* L.)

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### Abstract

Normal *Galega officinalis* L. (goat's tongue) contains the alkaloid galegine and a complex of biologically active substances that determine its hypoglycemic effect. Given the increasing prevalence of metabolic disorders, it is promising to create phytopreparations for veterinary medicine based on this plant. The aim of the study was to evaluate the effect of *Galega officinalis* extract on glucose levels and hematological parameters of rat blood as a stage in the development of a potential hypoglycemic agent for animals. The study was conducted on white outbred rats, which were administered a liquid extract of *Galega officinalis* L., prepared by the repercolation method using 40–70% ethanol. The hypoglycemic effect and the effect on hematological parameters were evaluated using standard pharmacological methods in compliance with the principles of the European Convention for the Protection of Vertebrate Animals. During the study of hematological parameters of the blood of experimental rats, it was found that in rats with metabolic syndrome (group II) the hemoglobin level exceeded the control by 23.8%, while with the administration of Arfazetin and Galega extract - by 9.7 and 6.1%, respectively ( $p < 0.05$ ). Compared with the positive control, this indicator in intact animals was lower by 23.0%, and with the use of herbal preparations - by 11.4 and 15.3%. The number of erythrocytes in group II exceeded the control by 18.2%, with treatment - by 7.8 and 5.5%, and relative to the positive control decreased by 8.8 and 10.8% ( $p < 0.05$ ). Hematocrit in animals with MS was lower than the control by 10.9%, and with the administration of herbal preparations - by 8.0 and 5.4%; relative to the positive control – increased by 3.3 and 6.0%. No significant differences were found between the groups receiving phytopreparations. Thus, the extract of *Galega officinalis* showed a pronounced hypoglycemic effect and contributed to the normalization of hematological parameters in rats with induced metabolic syndrome. The results obtained confirm the feasibility of further studies of this phytopreparation for the creation of drugs with a hypoglycemic effect and a corrective effect on the blood composition of animals.

**Keywords:** *Galega officinalis* L., Galegin, Metabolic syndrome, Hypoglycemic effect, Hematological parameters, Phytopreparation, Rats

## EXPERIMENTAL STUDY OF THE EFFECT OF *GALEGA OFFICINALIS* L. EXTRACT ON BODY WEIGHT AND BLOOD PARAMETERS OF RATS WITH METABOLIC SYNDROME

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### Abstract

Chronic consumption of excessive amounts of fructose leads to the development of characteristic components of metabolic syndrome – obesity, hyperglycemia, insulin resistance, hyperinsulinemia, carbohydrate intolerance and dyslipidemia, which is the basis for the use of herbal preparations for its correction. Purpose of the study to study the effect of *Galega officinalis* L. extract on body weight dynamics and morphological composition of blood in rats with induced metabolic syndrome in order to assess its potential as a means for the correction of metabolic disorders in veterinary practice. The experiments were performed on white outbred rats of both sexes with an average weight of 180–200 g. Metabolic syndrome was modeled by replacing drinking water with a 20% fructose solution. The animals were divided into groups: intact control, positive control and two experimental groups, which were administered *Galega officinalis* L. extract or phytopreparation Arfazetin. During the experiment, changes in body weight were recorded and hematological parameters were determined according to generally accepted methods in compliance with the principles of bioethics. It was found that after 8 weeks of use of 20% fructose solution (instead of drinking water) in rats with MS (group II), the body weight was 35.7% higher than the control, while in the case of the use of herbal preparations this indicator was slightly lower and in the III experimental group (under the conditions of use of Arfazetin at a dose of 100.0 mg/kg of body weight) was 22.3%, and in the IV experimental group (under the conditions of use of *Galega officinalis* extract at a dose of 125.0 mg/kg of body weight) – 7.7%, respectively ( $p < 0.05$ ). Relative to the positive control, the average weight of intact rats (group I) was lower by 26.3%, while in the case of the use of herbal preparations (Arfazetin and *Galega officinalis* extract) (groups III and IV) the decrease was 9.9 and 21.6%, respectively ( $p < 0.05$ ). When using hypoglycemic herbal preparations, significant changes were also found: with the administration of *Galega officinalis* extract, the weight of rats was 11.9% lower than with the administration of Arfazetin ( $p < 0.05$ ).

**Keywords:** Metabolic syndrome, Fructose, Arfazetin, *Galega officinalis*, Hypoglycemic effect, Hematological parameters, Rats



## MANAGEMENT OF BACTERIAL DERMATITIS ON THE FACE AND AROUND THE EYES IN A RABBIT WITH TOPICAL TREATMENT BASED ON HERBAL CONTENT

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### Abstract

An 11-month-old rabbit was found to have hair loss on the back, crusty and purulent discharge on half of the face, swollen eyelids, and purulent eye discharge. The crusts were gently removed, and the pus was cleaned with cotton. Microbiological swab samples were taken from the inside of the eye and the inflamed areas of the face. Microbiological culture was performed from swab samples. *Staphylococcus aureus* was isolated from facial and ocular swab samples, and *Pasteurella spp.* was additionally isolated from the facial swab. In the antibiogram analysis, the isolates were found to be sensitive to sulbactam, amoxicillin-clavulanic acid, penicillin, ceftiofur, and tetracycline, while resistant to gentamicin, trimethoprim, enrofloxacin, marbofloxacin, and ciprofloxacin. An ointment containing HLC and *Centella asiatica* was applied to the cleaned wound twice daily for three weeks. In addition, a tetracycline topical ointment was applied to the affected areas and surrounding skin twice daily for one week. After the first week, the wounds began to heal, and by the end of the third week, they were almost completely closed with noticeable hair regrowth. As a result, it was concluded that the severe superficial dermatitis caused by *Staphylococcus aureus* and *Pasteurella spp.* in this case can be successfully managed with a combination of topical antibacterial treatment—selected based on appropriate antibiotic susceptibility—and a wound healing ointment containing *Centella asiatica* and HLC.

**Keywords:** Dermatitis, Rabbit, *Staphylococcus aureus*, Treatment

## THERAPEUTIC EFFECTS OF ETHANOL EXTRACT OF PROPOLIS ON EXPERIMENTAL CUTANEOUS CANDIDIASIS IN CATTLE

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### Abstract

*Candida albicans* is a natural opportunistic flora in the skin and mucous membranes which under specific condition and excessive growth causes clinical symptoms of candidiasis in human and animals. Prophylactic and therapeutic administration of some antifungal drugs have caused drug resistance, therefore, there is a need to introduce novel therapeutic agents for treatment of candidiasis. This study evaluated the therapeutic potential of ethanolic extract of propolis in experimental induced cutaneous candidiasis in cattle, with nystatin and glycerin serving as controls. Four 4-year-old dairy cattle approximately 350 kg were included into the present study. The immune system of animals were suppressed with dexamethasone. Thorax and flanks on the right and left sides were shaved. *Candida albicans* (CPTC: 5027) suspension were inoculated intradermally at four points on each area, (5 cm equidistant from each other). The lesions on the right thorax were treated with ethanolic extract of propolis, the lesions on the left thorax treated with Nystatin ointment, and the lesions on the right flank treated with glycerin. The lesions on the left flank were left untreated. Cutaneous candidiasis was established within 5 days. Ethanol extract of propolis during 7 days and nystatin within 10 days improved the clinical signs of cutaneous candidiasis. It was concluded that ethanol extract of propolis could improve injuries of cutaneous candidiasis of cattle in a shorter time compared to nystatin. Therefore, it could be considered as a suitable alternative compared to other synthetic antifungal products. Further research is warranted to elucidate its mechanisms of action.

**Keywords:** Cattle, *Candida albicans*, Cutaneous candidiasis, Ethanol extract of propolis



## INHALATION THERAPY WITH *BRONCHO SMOKE*<sup>®</sup> IN BROILER CHICKENS WITH REPIRATORY DISORDERS CAUSED BY NEWCASTLE AND AVIAN INFLUENZA (H9N2) VIRUSES.

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### Abstract

Although inhalation therapy has been used for many years in the treatment of human respiratory diseases, it has not been widely used in veterinary medicine and is mainly used in the treatment of respiratory diseases in horses, dogs, and cats. Inhalation therapy in birds Treatment using nebulizer devices is most common in ornamental or game birds and is not common in commercial poultry. The aim of this study was to investigate the antiviral effect of a commercial product, *Broncho Smoke* (Damyar Jame co.Iran), containing: Rosemary essential oil 4% and Eucalyptus essential oil 3% in broiler chickens with respiratory disorders caused by Newcastle disease and avian influenza(H9N2) viruses, used by inhalation method under field conditions. From 6 flocks with respiratory diseases at times 0, 48, 24 and 72 hours after treatment, trachea and lungs of birds were collected and infection with NDV and AIV viruses were evaluated using the Real Time PCR method. Also, as a negative control, another housel that was not treated but had symptoms was sampled simultaneously. The results showed that using *Broncho Smoke*<sup>®</sup> in broilers suffering from Newcastle respiratory diseases and H9N2 influenza causes a reduction in mortality, decrease in the intensity of clinical symptoms (breathing sounds, sneezing and coughing, eye and nose discharge) and a faster recovery (increasing feed and water consumption). The severity of necropsy symptoms (hyperemia, hemorrhage, and secretions in the trachea, lungs, and eye conjunctiva) in chickens suffering from Newcastle disease and H9N2 influenza in chickens treated with *Broncho Smoke*<sup>®</sup> was significantly lower than in the control group, and its recovery was also It is faster. The results obtained using the Real Time(q)PCR method for the detection of NDV Fusion(F) and AIV Matrix(M) genes in broiler chickens suffering from the respiratory diseases showed that the relative expression level of this genes in the group treated with *Broncho Smoke*<sup>®</sup> compared to the control groups during the treatment period is less, which indicates the effect of this drug on replication and shedding of the Newcastle and AIV viruses.

**Keywords:** Newcastle disease, Avian influenza, H9N2, *Broncho smoke*, Broiler

# MEDICINAL PLANTS AND VETERINARY ETHNO-PHARMACOLOGY ESPECIALLY FOLK REMEDIES, BY DIFFERENT CULTURAL GROUPS IN IRAN

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## Abstract

Natural products is replenish able source of row material and serve as valuable resources for drug discovery. Bou Ali Sina (years 1 to 2 AH) described herbal and mineral medicine in the book law of the law with their effects on the human and veterinary. Iranian botanists, near 1000 medicinal and aromatic plants, speeches collected from different ecological zoon. So out of these speeches, herbalists, use more than 2000 verities traditionally. Most of them are very expert to use it as the supplements and traditional drug out of it's for treatment of their patient. In this manner, scientist interested to find out some interested compound from their practices and time of treated. Now a days the resistance of bacteria and virus, started lot of problem for human and animals life in world. In this presentation we investigated effects of few aromatic and medicinal plants, e.g *Ferula gomoza*, *Thymus vulgaris* and *Matricaria chamomilla* L. solvents extraction and essential oils of these herbs were able to reduce the fungal and bacterial.



Researcher finds out, raw plants material or extraction is my poisonous and has toxicity to humans and animals are an example of natural selection. Traditionally people in public and different villages, ecological and stats of Iran we find out one plant speeches have so many uses e.g. for different human treatment, its show plants in every stat have its uses. Therefor caution is very compulsory to use part of any plants as medical proposes. So herbal, they show both useful and harmful effects in veterinary and human beings.

**Keywords:** Veterinary, Traditional-medicine, Medicinal plants, Bacteria and virus



## THE ROLE OF MEDICINAL PLANTS IN THE MANAGEMENT OF GASTROINTESTINAL MOTILITY DISORDERS IN ANIMALS

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### **Abstract**

Gastrointestinal (GI) motility disorders in animals encompass a range of conditions that affect the movement of food and waste through the digestive tract. These disorders can lead to symptoms such as bloating, constipation, diarrhea, and abdominal pain, which not only compromise animal welfare but also result in economic losses in livestock production. Traditional pharmacological treatments often involve synthetic drugs, but concerns over side effects and antimicrobial resistance have prompted interest in alternative therapies—particularly medicinal plants. The application of medicinal plants in veterinary medicine is deeply rooted in ethnoveterinary traditions worldwide. Medical systems such as Ayurveda, Traditional Chinese Medicine, and European folk practices have long recognized the efficacy of herbs including peppermint, fennel, and ginger for managing digestive disturbances. These plants, valued for their carminative, antispasmodic, and laxative activities, have historically been employed to regulate GI motility and alleviate discomfort. What was once empirical knowledge passed down through generations is increasingly supported and refined by contemporary pharmacological studies. The therapeutic actions of medicinal plants are mediated by a diverse range of bioactive compounds, such as alkaloids, flavonoids, tannins, and essential oils. For instance, menthol—a principal constituent of peppermint—exerts smooth muscle-relaxing effects that mitigate spasms and enhance motility. Similarly, active compounds in ginger facilitate gastric emptying and stimulate intestinal transit. Because of their complex phytochemical composition, medicinal plants often function as “multi-target agents,” simultaneously addressing multiple physiological pathways involved in GI dysregulation. One of the key advantages of using medicinal plants is their lower risk of side effects and reduced potential for drug resistance. Unlike synthetic drugs that often target a single pathway, herbal remedies offer a holistic approach, modulating inflammation, enhancing immune response, and restoring microbial balance. Additionally, they are often more accessible and cost-effective, especially in rural or resource-limited settings where veterinary care may be scarce. While the therapeutic potential of medicinal plants is promising, further research is needed to standardize dosages, identify active components, and ensure safety across species. Clinical trials in veterinary medicine remain limited, and regulatory frameworks must evolve to support the integration of phytotherapy into animal healthcare. As interest grows, interdisciplinary collaboration between veterinarians, botanists, and pharmacologists will be essential for unlocking the full potential of plant-based treatments for GI motility disorders.

**Keywords:** *Tanacetum balsamita*, Bovine, Abomasum, Prokinetic

## ANTIOXIDANT AND NEUROPROTECTIVE EFFECTS OF XANTHOTHUMOL IN AN IN-VITRO MODEL OF PARKINSON'S DISEASE

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### Abstract

*Humulus lupulus L.* (hop), belonging to the Cannabaceae family, is a perennial herbaceous climbing plant. The extract of *H. lupulus* and its active compound, xanthohumol (XAN), are known to exhibit antioxidant, anti-inflammatory, and antimicrobial effects. Xanthohumol (XAN) has been demonstrated in recent in vivo studies to increase dopamine levels, reduce oxidative stress and neuroinflammation associated with Parkinson's disease, and support neuronal health by providing protective effects against neuronal excitotoxicity and mitochondrial dysfunction. Parkinson's disease is a neurodegenerative disorder that develops over time and manifests with both motor-related and non-motor symptoms. Although Parkinson-like conditions are rare in pets, the role of dopaminergic neuron loss and oxidative stress shows a mechanism similar to that in humans. In light of this information from the literature, our study aimed to determine the effects of XAN on an in vitro Parkinson's model induced by 6-OHDA. To establish a Parkinson's Disease (PD) model in cell culture, SH-SY5Y cells were exposed to 200 µM 6-OHDA for 15 minutes. After 6-OHDA application, SH-SY5Y cells were treated with XAN (50 µg/mL, 100 µg/mL, and 200 µg/mL). After 24 hours, cell viability was determined by 3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide (MTT) assays. Oxidative stress was assessed by total antioxidant capacity (TAC) and total oxidant status (TOS) assays, and PPAR-γ levels were investigated. The findings were statistically evaluated. 6-OHDA was found to increase cellular apoptosis by 50% in SH-SY5Y cells compared to the control group. TAC and TOS analyses showed that this was directly proportional to total oxidative stress. XAN prevented 6-OHDA-induced apoptosis and increased cell viability in a dose-dependent manner. The observed increase in PPAR-γ levels also supported these findings. These results suggest that specific doses of XAN may have neuroprotective effects with potential relevance for the treatment of neurodegenerative diseases. In particular, further studies are recommended to investigate the therapeutic effects of XAN in animal models of Parkinson's-like neurodegenerative processes. The results demonstrate the therapeutic effect of XAN against the in vitro Parkinson's disease model induced by 6-OHDA. To further clarify the underlying mechanisms, additional studies using in vivo Parkinson's models are warranted.

**Keywords:** 6-OHDA, Neuroblastoma, Parkinson's disease, Xanthohumol



## SECTION 3

### Full Text Poster Papers

## A REVIEW OF THE HEPATOPROTECTIVE EFFECTS OF *SILYBUM MARIANUM* EXTRACT IN SMALL ANIMAL MEDICINE

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### Abstract

Liver disorders frequently occur in both dogs and cats. There are many possible causes, including infections from viruses, bacteria, and parasites, exposure to toxins, cancerous developments, and medications such as phenobarbital, NSAIDs, and steroids. The approach to treatment and the outlook for liver disease depend on its root cause. Nonetheless, many cases can be effectively managed due to the remarkable ability of the liver to regenerate. In this regard, medicinal plants such as *Silybum marianum* may be helpful as hepatoprotective due to their bioactive compounds with antioxidant properties. *Silybum marianum* is recognized for its pharmacological benefits in treating liver disease in humans, but it is also applied in veterinary medicine. This review presents an overview of the experimental findings regarding the effects of *Silybum marianum* extract on small animals. Some studies have indicated no effects, but none have noted issues associated with the use of *Silybum marianum* extract. Nevertheless, the general consensus indicates that incorporating *Silybum marianum* extract leads to enhanced or regained health indicators or improved performance in small animals. A recommended dosage of 50-250 mg per day is advised for dogs and cats suffering from liver disorders.

**Keywords:** Cat, Dog, Liver, Milk thistle

### Introduction

The liver is responsible for several essential functions, such as the synthesis and metabolism of carbohydrates, proteins, and fats. Liver disease can disrupt the metabolism and utilization of nutrients, leading to complications with the metabolism and storage of nutrients and the breakdown of potentially harmful by-products or toxins. Additionally, the liver contributes to the production of red blood cells and the creation of factors necessary for proper blood clotting. Being crucial for various functions, liver disease can lead to significant health risks.<sup>1</sup> Liver disorders in small animals are increasingly frequent nowadays. While cats occasionally experience these issues, they are quite common in dogs. Among small animals, prevalent liver diseases include vascular anomalies, acute or chronic hepatitis, hepatic lipidosis, neoplasia, toxicosis, and cholangitis. While all breeds can be affected by these diseases, certain breeds are at a higher risk.<sup>2</sup> These at-risk breeds include Yorkshire Terriers, Cairn Terriers, Scottish Terriers, Malteses, Golden and Labrador Retrievers, Schnauzers, Poodles, Dobermans, Cocker Spaniels, and German Shepherds. In cats, no specific breed predisposition has been noted; however, overweight cats are at higher risk for a fatty liver condition known as hepatic lipidosis. Being crucial for various functions, liver disease can lead to significant health risks. Therefore, ensuring a healthy liver is vital for the overall health and well-being of small animals.<sup>3</sup>

With pet owners becoming more aware of the importance of preventive care for their pets' health in recent decades, the demand for dietary supplements has risen. Dietary supplements can not only aid in the prevention



of disease but also serve as a type of complementary or alternative medicine (CAM) that can be used either in place of or along with standard medicinal products to mitigate negative side effects of medications.<sup>4</sup> Various supplements, including curcumin, S-adenosylmethionine, and silymarin are recognized for their liver-protective properties.

*Silybum marianum*, known as milk thistle, is frequently utilized in treating both humans and animals suffering from liver failure. Silymarin is a standardized extract derived from the seeds and fruits of milk thistle, comprising at least seven flavonolignans (which include silibinin, iso-silibinin, silychristin, iso-silychristin, and silydianin) along with one flavonoid (taxifolin).<sup>5,6</sup> Research has shown that silymarin exhibits antioxidant, anti-inflammatory, and antifibrotic properties, while also encouraging choleresis, improving protein synthesis, preventing the binding of hepatotoxins, boosting GSH levels, and chelating iron. While there are many studies regarding the impact of *Silybum marianum* extract, their application in small animal medicine is still not common. This article aims to provide scientific support for the effects of *Silybum marianum* extract for small animal clinicians who require supplemental nutrition to support liver function in dogs and cats, through a review of the scientific literature.

### General Characteristics

*Silybum marianum* (Milk thistle) belongs to the *Asteraceae* (Compositae) family, which encompasses daisies, artichokes, sunflowers, thistles, and similar plants. The plant gets its name from the milky streaks found on its broad, serrated leaves. It features a prominent purple flower head. The fruits and seeds of milk thistle contain 1.3-3% flavonolignans (including silibinin and isosilibinin, silicristin, and silidianin), flavonoids (such as flavones like apigenin, chrysoeriol, eriodictyol, and flavonols like taxifolin, kaempferol, quercetin), 20-30% fatty oils (like linoleic and oleic acids, palmitic, linolenic, behenic, and others), 0.2-0.6% phytosterols (notably  $\beta$ -sitosterol), and additional compounds.<sup>1</sup> The primary active component extracted from the dried seeds and fruits of milk thistle is silymarin, which exhibits remarkable antioxidant, liver-protective, anti-inflammatory, immune-modulating, antidiabetic, anti-apoptotic, and anti-amyloidogenic effects that have been validated through extensive clinical trials. Silymarin consists of a blend of flavonolignans, including silibinin or silybin (A and B), isosilibinin or isosilybin (A and B), silychristin, silydianin, a small amount of taxifolin, and various other polyphenolic compounds.<sup>3</sup> Currently, there is increasing proof that silymarin, the active component, offers advantages for numerous liver and gallbladder conditions in humans and serves as a treatment for animals.

### Pharmacological Characteristics

Silymarin can be given either orally (PO) or through intravenous (IV) administration, and it is insoluble in water. Consequently, it is primarily eliminated via bile (80% after IV and 40%-45% after PO in the first 48 hours), with minimal amounts appearing in urine (8% after IV and 2%-5% after PO within the first 48 hours). Research by Persiani et al. shows that silybin hemisuccinate has a strong affinity for plasma proteins, and this binding capacity is consistent across various animal species, such as dogs, rats, mice, and humans.<sup>7</sup> The bioavailability of oral administration is limited, as evidenced by only 2 to 3% of the total oral dose being excreted through bile (20). Research has indicated that when silymarin is given in liposome suspensions orally, its bioavailability is enhanced. A study conducted by Yan-yu et al. on beagle dogs investigated the potential for improving the gastrointestinal absorption of silymarin that has been encapsulated using proliposome.<sup>2</sup> The

findings from the study indicated that administering silymarin in proliposome form could result in increased bioavailability compared to the administration of pure silymarin or silybin. Pharmacokinetic research by Filburn et al. involving dogs showed that the bioavailability of silybin administered alongside a phosphatidylcholine complex (phytosome) surpasses that of its conventional nonphytosome counterpart. Silybin is distributed across various tissues. Thirty minutes after administration, it predominantly accumulates in the digestive organs, including the liver, pancreas, and stomach, which are highly active glandular sites, though it is also present in the lungs. Silymarin appears to be a safe dietary supplement, with a 50% lethal dose (LD50) of 140 mg/kg via intravenous injection in dogs (which increases when the infusion rate is lower) and 10 g/kg when administered orally, with a highest tolerated dose of 300 mg/kg. When the LD50 threshold is surpassed, cardiovascular issues seem to be responsible for mortality. Additionally, silymarin appears to have no impact on embryonic development. Therefore, we can infer that the acute, subacute, and chronic toxicities associated with silymarin are quite minimal.<sup>4</sup> The most common side effects associated with silymarin include diarrhea, abdominal discomfort, itching, and nausea. The laxative effects observed with higher doses of silibinin might be associated with carrier components or heightened bile secretion. The overall percentage of adverse events documented in a meta-analysis of clinical trials was 2.36% for silibinin compared to 5.05% for the placebo. There have been no reports of fatalities or severe adverse events.

### Therapeutic Applications

Silymarin has shown various beneficial effects in pets that are helpful for treating hepatobiliary diseases, such as antioxidant, anti-inflammatory, and antifibrotic actions, making it a valuable therapeutic option for pets with liver conditions. In 1990, Paulova and her team investigated the hepatic injury caused by toxic substances, evaluating the effectiveness of silymarin (100 mg/kg BW/bid) in the treatment of liver disease induced by carbon tetrachloride in 16 beagles.<sup>8</sup> Silymarin was given for 4 days prior to exposure to carbon tetrachloride (used as the toxic agent) and continued for another 4 days afterward. Despite its use as both a preventive measure and a treatment following intoxication, it was largely ineffective in safeguarding the liver from damage. Sgorlon and colleagues observed that the levels of ALT enzymes in eight dogs with liver damage were notably decreased after being treated for 60 days with silymarin (silybin 15%, 1.5 mg/kg-BW). In conjunction with the reduction in liver enzyme activity, a lower level of the acute phase protein CuCp was noted, indicating the anti-inflammatory and antioxidant effects of silybin. The antioxidant properties of silymarin were further validated through the observed increase in paraoxonase and the significant up-regulation of superoxide dismutase.<sup>1</sup> In a separate study, administering silymarin at a dose of 28.3 mg/10 kg-BW of silybin (Hepaxan commercial product), following the manufacturer's guidelines, to 15 dogs with liver disorders resulted in significant reductions in the enzymatic liver markers AST, ALP, GLDH, and GGT. Additionally, a minor increase in albumin and globulin levels was noted, without affecting the values of LDH,  $\alpha$ -amylase, or the BUN and urea ratio. In a separate study, Soltanian and her team evaluated the therapeutic effects of silymarin versus hydrocortisone regarding clinical and hematological changes as well as organ damage (liver and heart) in dogs subjected to a low dose of LPS. They observed a notable rise in RBCs, Hb, and HCT, accompanied by a significant reduction in AST, ALP, LDH, CK-MB, and cTnI within the silymarin treated group. Similar outcomes were noted in cats administered a dose of 30 mg/kg-BW of silymarin in instances of liver injury induced by stanozolol, phenobarbital, tetracycline, and mebendazole.<sup>9</sup> In contrast to dogs, cats are particularly susceptible to the toxic effects of certain substances due to a deficiency



in their hepatic enzymes, specifically glucuronyl transferases. This relative insufficiency in the glucuronide conjugation pathway leads to a greater amount of drugs being conjugated to sulfates; however, the sulfation pathway in cats has a limited capacity, which is also lower than in other species. When given at a dose of 30 mg/kg-BW, silymarin produced effects comparable to those of N-acetylcysteine in treating liver damage resulting from acetaminophen poisoning in cats.<sup>1</sup> In cats treated with silymarin, the levels of ALT, AST, LDH, methemoglobin, and bilirubin remained unchanged, unlike those in cats that received acetaminophen alone. At doses exceeding 70 mg/bid, silymarin has demonstrated effectiveness when used alongside amiodarone, which contrasts with the antiarrhythmic properties of amiodarone and helps to prevent sustained atrial flutter by reducing or eliminating the excitable gap in dogs, likely due to the antioxidant properties of silymarin. In a study by Webb and colleagues, administering an oral dose of 10 mg/kg-BW/d of a silybin-phosphatidylcholine complex (comprising 31% silybin) over 5 days in cats resulted in increased GSH levels and improved phagocytic activity in cats infected with *E. coli*.<sup>3,1</sup>

### Conclusion

The most fascinating effects of *Silybum marianum* extract include its immunomodulatory, anti-inflammatory, regenerative, antifibrotic, antioxidant, choleric, and hepatoprotective properties. Although the use of *Silybum marianum* extract is growing in popularity and there is a substantial body of literature, the effectiveness of these extracts in individuals with liver diseases remains unclear. To validate the effectiveness suggested by earlier research, there is a need for high-quality, standardized trials that focus on dosage and the timing of treatment.

**Conflict of interest:** The authors declare no conflict of interest.

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# ACCELERATION OF SKIN WOUND HEALING USING NANO-CHITOSAN DRESSINGS INCORPORATED WITH NANO-LIPOSOMES CONTAINING HENNA EXTRACT IN RATS WITH DIABETES MELLITUS

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## Abstract

Diabetes is a systemic disease that causes neuropathy and arterial damage, impacting many tissues and organs. Diabetic foot ulcers affect approximately 15% of individuals with diabetes. Given the delayed wound healing process in patients with diabetes mellitus, major attention has recently been focused on the use of novel dressings based on medicinal plants. In this regard, the application of nanochitosan combined with nanoliposomes containing medicinal herbs has gained particular prominence. This study investigates the reparative effects of henna plant extract, encapsulated within nanoliposomes and incorporated into a nanochitosan coating, on skin wounds induced in diabetic rats. In this study, a total of 30 rats were selected and divided into two main groups: control and diabetes mellitus groups (each n=15). Each main group was subsequently subdivided into three subgroups: No dressing, Nanochitosan dressing, Nanochitosan dressing containing nanoliposomes loaded with henna extract. Diabetes mellitus was induced in the rats of the experimental group via an intraperitoneal injection of streptozotocin at a dose of 55 mg/kg of body weight. Subsequently, a full-thickness circular skin wound with an area of 1 cm<sup>2</sup> was created on the dorsal region of each rat. Based on the predefined groupings, the respective dressings were applied. The wound healing and contraction process was measured and evaluated daily. The total study duration was 35 days. The results of this study demonstrated that the wound healing process was significantly delayed in rats with diabetes mellitus compared to the control group rats. The application of the nanochitosan dressing containing nanoliposomes loaded with henna extract accelerated this reparative process and significantly enhanced the rate of wound contraction. These findings indicate that henna extract could be a potential therapeutic agent for the treatment of diabetic foot ulcers.

**Keywords:** Diabetes mellitus, Henna, Nano-chitosan, Nano-liposome, Skin wound healing

## Introduction

Diabetes mellitus, a metabolic disorder arising from a wide range of factors, disrupts the normal metabolism of carbohydrates, fats, and proteins, ultimately leading to elevated blood glucose levels. Today, diabetes mellitus is one of the most common diseases worldwide and can be considered the second most prevalent disease after cardiovascular diseases. It is characterized as a metabolic disorder with multiple causes, accompanied by hyperglycemia. This disease involves impairments in the metabolism of carbohydrates, lipids, and proteins, primarily due to defects in insulin secretion, insulin action, or both (American Diabetes Association, 2009).

The symptoms of diabetes mellitus include both long-term and short-term complications. Early symptoms include thirst, polyuria, blurred vision, and weight loss—though weight loss is more common in type I diabetes. In severe cases, depending on the type, ketoacidosis may occur, leading to symptoms such as unconsciousness, coma, and death if left untreated. Long-term effects of diabetes mellitus include the development of complications



such as retinopathy, blindness, cataracts, nephropathy, neuropathy, foot ulcers, amputations, and autonomic nervous system disorders (Balaji et al., 2019).

Various treatments for diabetic foot ulcers have been proposed. Wound debridement is among the standard treatments for such ulcers. Other therapies include electrical stimulation (light therapy and low-level laser therapy), hyperbaric oxygen, negative pressure, topical treatments, vacuum therapy, and herbal extracts as adjunctive therapies. However, it is important to note that all these treatment methods have had limited and insufficient success (Huang et al., 2023).

Henna (*Lawsonia inermis* L.), from the *Lythraceae* family, exhibits effects such as inhibiting the growth of microorganisms in wounds, anti-inflammatory activity, anti-diabetic properties, and immune system regulation (Batiha et al., 2024).

Chitosan, a natural antimicrobial, biodegradable, and biocompatible polymer, has attracted attention due to its numerous beneficial biological properties, including biocompatibility, biodegradability, wound-healing effects, and anti-tumor activity (Yadav et al., 2023). It has been widely used in various biomedical applications, such as absorbable sutures, drug delivery systems, and as a compound with anti-cancer effects and wound-healing stimulation properties (Rajinikanth et al., 2024). Diabetic wound dressing is one of the main pillars of managing this condition. Ideally, a wound dressing should alleviate injury symptoms, protect the wound, and promote healing. However, if a diabetic foot ulcer becomes infected, dressings alone cannot ensure complete healing (Kavitha et al., 2014).

A wide range of dressings for diabetic foot ulcers are available on the market. However, until now, wound dressings based on chitosan nanofibers and henna extract have not been produced or utilized (Jonidi Shariatzadeh et al., 2025). The development of such a product could represent a groundbreaking advancement in the production of next-generation dressings for diabetic wound repair. With industrial support, it could serve as a strategic product with significant export potential and economic benefits.

## **Materials & Methods**

### **Extraction of Henna Leaf Extract**

One kilogram of dried henna (*Lawsonia inermis*) and milk thistle (*Silybum marianum*) medicinal plants were separately powdered using a grinder. Then, 5 liters of 50% methanol were added to each powdered sample. Extraction was finally performed using a percolation method at 55 °C for 48 hours. To concentrate the extracts, a rotary evaporator was used at 45 °C. The obtained extracts were analyzed using a GC-MS Shimadzu QP2010 Ultra, and the software used was Turbomass ver. 5.2.

### **Preparation of Nano-liposomes Containing Plant Extract**

Initially, 60 mg of lecithin was dissolved in 15 mL of ethanol in a glass flask. The solvent was then evaporated using a rotary evaporator at 45-60 °C, forming a thin film around the flask. The formed film was hydrated with 10 mL of distilled water and glass beads. The produced liposomes, which were multilayered and micro-scale, were placed in the rotary evaporator at the highest rotation speed and above the liposomal phase transition temperature (60 °C) for 20 minutes. Finally, sonication of the liposomal samples was performed using a probe sonicator with 10 cycles of 1-minute sonication followed by 1-minute rest between each cycle, resulting in unilamellar nanoscale liposomes.

### **Loading of Henna Extracts into Nano-liposomes**

In this stage, 15 mg of each extract (henna and milk thistle) was separately dissolved in 10 mL of distilled water. To remove insoluble compounds and impurities, the solutions were filtered using Whatman filter paper. After the formation of the phospholipid layer (as described in the previous section), the extract solutions were used for hydration of the layer, thereby loading the bioactive compounds of the extracts into the liposomal vesicles. Similar to the blank liposome preparation method, the liposomal suspension was placed in a rotary evaporator at the highest rotation speed and above the liposomal phase transition temperature (60 °C) for 20 minutes. Finally, sonication of the liposomal samples was performed using a probe sonicator with 10 cycles of 1-minute sonication followed by 1-minute rest between each cycle, resulting in unilamellar nanoscale liposomes.

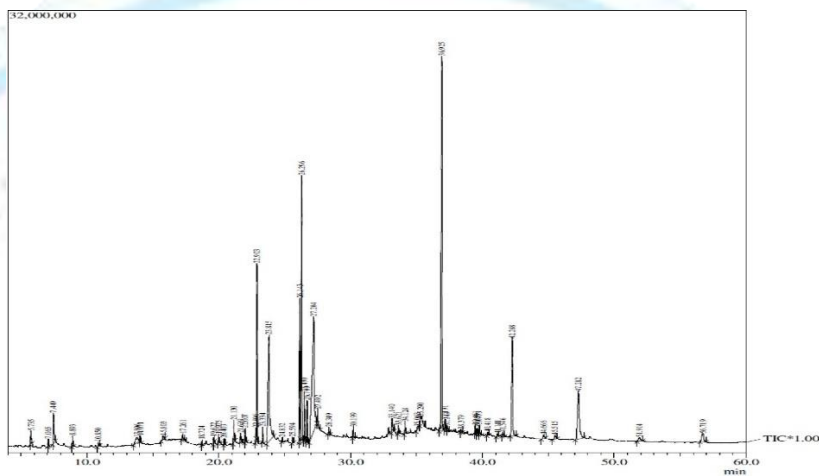


**Figure 1.** Chromatogram of the methanolic extract of *Lawsonia inermis* leaves

### Investigation of Particle Size and Zeta Potential of Nano-liposomes

Images of the produced nano-liposomes were obtained using SEM (Scanning Electron Microscopy) and TEM (Transmission Electron Microscopy). The average particle size and zeta potential of the nano-liposomes were evaluated using a DLS (Dynamic Light Scattering) device available at the Central Laboratory of Urmia University. The results indicated that the particle size of the nano-liposomes loaded with henna extract was 57.3 nanometers, and the zeta potential was -6.1 millivolts.

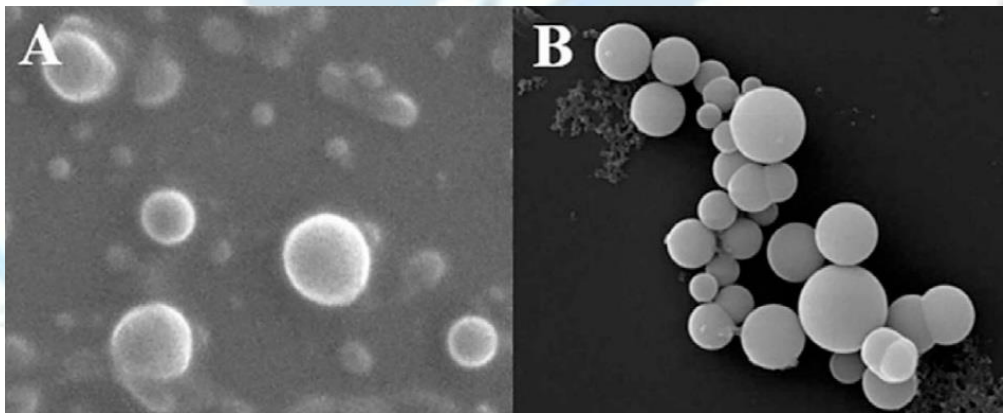


**Figure 2.** TEM (A) and SEM (B) images of nano-liposomes containing henna extract

### Fabrication of Chitosan Nanofibers and Evaluation of Physicochemical Properties (Nanoliposome and Chitosan Working Group)

To dissolve chitosan and polyvinyl alcohol (PVA), a 70% (w/w) acetic acid solution in distilled water was prepared. This solution was used to prepare the polymer solutions. Specifically, a 3% (w/w) chitosan solution and a 7% (w/w) PVA solution were prepared. The final electrospinning solution was formulated with a volumetric



ratio of 70% PVA to 30% chitosan. For each electrospinning process, 3 mL of the solution was loaded into a syringe.

Electrospinning parameters were set as follows:

- Voltage: 30 kV
- Injection speed: 1 mL/h
- Collector speed: 700 rpm
- Needle-to-collector distance: 12 cm

The electrospinning process was conducted for approximately 3 hours under the above conditions, resulting in nanofibers with dimensions of 18 × 21 cm collected on aluminum foil placed on the collector. The spinning was performed in two rounds, with 1.75 mL used per round (total 3.5 mL). A uniform white film of nanofibers was collected on the foil. The obtained nanofibers were dried in an oven at 60 °C for 24 hours.

To enhance the mechanical strength of the nanofibers, a borax solution was utilized. A 1% (w/w) borax solution in ethanol was prepared, and the nanofibers were immersed in this solution for 10 minutes to facilitate cross-linking. After this treatment, the fibers became more transparent, and a significant improvement in mechanical strength was observed.

#### Preparation of Wound Dressings Loaded with Nano-liposomes Containing Plant Extracts

The cross-linked nanofibers were used in the final stage to prepare the wound dressing. For this purpose, the fabricated nanofibers were immersed in 10 mL of nano-liposome suspensions (5 mL of nano-liposomes loaded with henna extract and 5 mL of nano-liposomes loaded with milk thistle extract, if applicable) and retained until complete absorption of the nano-liposomes onto the fibers. The fibers were then dried at 40 °C for 12 hours to achieve the final wound dressing.

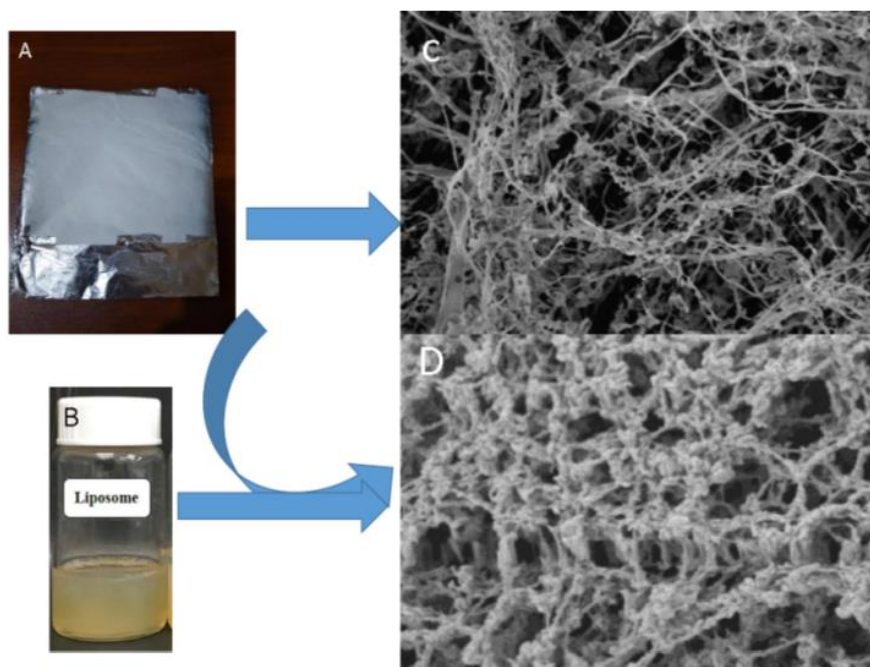


**Figure 3.** (A) Prepared chitosan nanofibers via electrospinning. (B) Nano-liposomes Containing Plant Extracts (C) Scanning electron microscopy (SEM) images of nanofibers, Pure nanofibers without liposome loading. (D) Nanofibers loaded with nano-liposomes containing henna and milk thistle extracts.

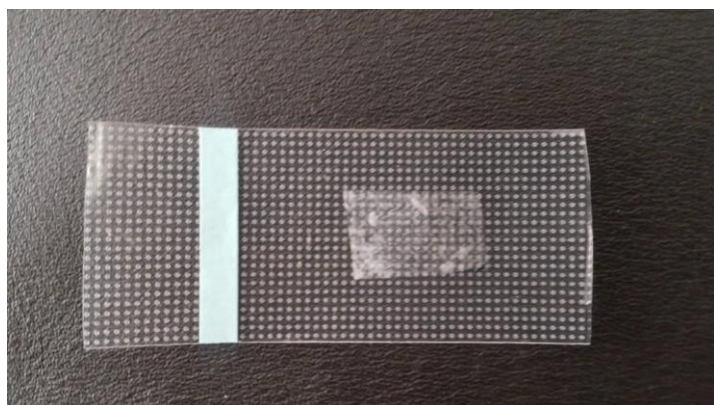


**Figure 4.** Final wound dressing composed of chitosan nanofibers and nano-liposomes encapsulating henna and milk thistle extracts.

### Preparation of Rats for Diabetes Induction and Wound Modeling

For the preclinical study of the produced nano-chitosan wound dressings, 30 male Wistar rats were purchased from the experimental animal breeding center of the Faculty of Medicine and transferred to the animal housing facility of the Veterinary Faculty specialized clinic. The animals were divided into two main groups of 15:

1. **Control group (non-diabetic)**
2. **Diabetes mellitus group (induced by streptozotocin)**

Each main group was further subdivided into three subgroups (n=5 per subgroup):

- Subgroup 1: No wound dressing applied (untreated wounds).
- Subgroup 2: Wounds dressed with plain chitosan nanofibers (without plant extracts).
- Subgroup 3: Wounds dressed with chitosan nanofiber wound dressings loaded with nano-liposomes containing henna extract.

### Induction of Diabetes Mellitus

To induce diabetes, streptozotocin (STZ) was administered intraperitoneally at a dose of 55 mg per kilogram of body weight to the rats in the diabetes group. Fasting blood glucose levels were measured 48 hours post-injection using a glucometer. Rats with blood glucose levels exceeding 200 mg per deciliter (mg/dL) were confirmed as diabetic and included in the study.

### Creation of Skin Incisions and Cutaneous Wounds

Following local anesthesia induced via subcutaneous injection of 2% lidocaine and skin disinfection, full-thickness cutaneous wounds were created in all study group rats using a specialized skin punch tool with a diameter of 10 millimeters.

### Wound Dressing and Tissue Sampling

Immediately following wound creation, dressings were applied according to the predefined group assignments (as outlined in Section 7). Dressings were replaced daily for two weeks, and wound size/closure kinetics were measured during each dressing change. Wound healing progression was monitored until day 35, after which tissue samples were collected from the wound site of all rats (Figure 5).

### Statistical Analysis

Data presented as mean  $\pm$  standard deviation (SD). Comparisons between groups used one-way ANOVA with Tukey's post-hoc test ( $p < 0.05$  considered significant).



Ethical Approval

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health (National Research Council, 2011). The research project has received the confirmation of the Institution Ethics Committee with the number of UU-AEC- Enc: 3/54



**Figure 5.** Steps of cutaneous wound creation and evaluation of the healing process by measuring wound contraction and area

Results

Wound Assessment and Healing Process Evaluation

The results of this study demonstrated that the induction of diabetes mellitus significantly impaired the wound healing process, markedly reducing wound contraction. Concurrently, the wound area in this group remained larger compared to the group of healthy rats. The application of a wound dressing containing an herbal extract significantly accelerated the healing process, facilitating rapid wound repair and reducing the wound area (Figure 6, Table 1).

Discussion

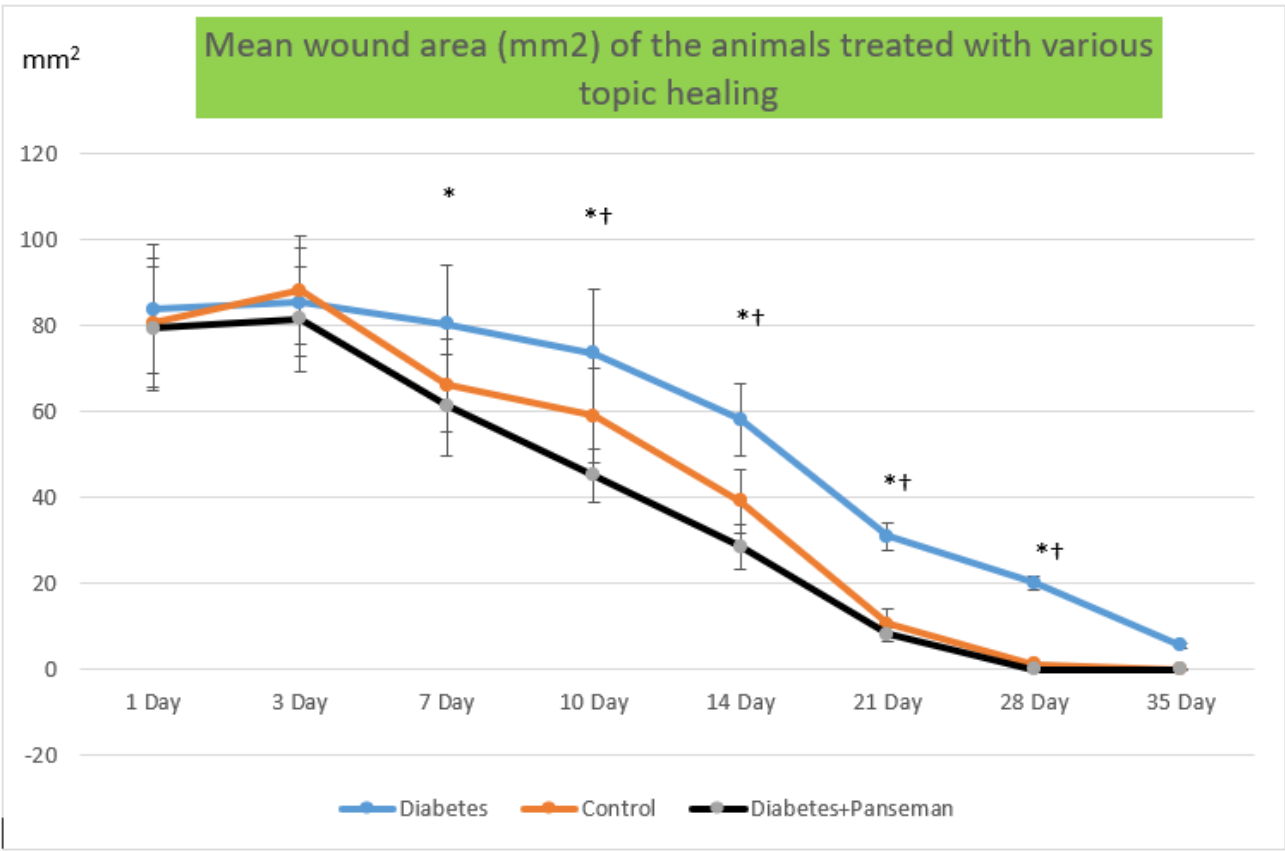
Wound management represents one of the most fundamental challenges humanity has faced since the beginning of civilization. Consequently, the search for compounds that effectively promote wound healing while possessing minimal side effects is a major goal. In this context, medicinal plants are of particular importance (Moradifar et al., 2025). Since the extract of the henna plant (*Lawsonia inermis* L.) exhibits anti-inflammatory and disinfectant properties, this research investigated the effect of this plant's extract on cutaneous wound repair. Henna extract possesses antibacterial, anti-inflammatory, and antioxidant properties, which can contribute to wound healing by accelerating the healing process, protecting the skin from infection, and reducing inflammation. The primary compounds in henna, such as lawsone, tannins, and plant acids, are effective in these properties (Batiha et al., 2024). Furthermore, they likely stimulate collagen synthesis, faster wound contraction, angiogenesis, and vasodilation (Shedoeva et al., 2019). In the investigation conducted on the group with diabetes mellitus, the use of a nanoliposome dressing containing henna extract resulted in a lower mean time to complete wound healing compared to the control group. The results indicate that the treatment group had the best effect on cutaneous wound repair in diabetic rats, compared to the other groups. It is probable that the active components of the henna plant exert anti-inflammatory and antibacterial effects, leading to a reduction in wound inflammation, bleeding, and edema.

**Table 1.** Assessment of wound area (in square millimeters, mm<sup>2</sup>) in the study groups over the 35-day study period

| Groups                    | Day 1       | Day 3       | Day 7       | Day 10      | Day 14     | Day 21     | Day 28    | Day 35  |
|---------------------------|-------------|-------------|-------------|-------------|------------|------------|-----------|---------|
| Control                   | 90.5 ± 15.1 | 88.2 ± 12.6 | 66.1 ± 10.8 | 59.0 ± 11.1 | 39.2 ± 7.4 | 10.6 ± 3.3 | 1.0 ± 0.3 | 0       |
| Nano Chitosan             | 88.3 ± 11.2 | 89.5 ± 9.8  | 65.7 ± 11.8 | 52.4 ± 8.8  | 34.8 ± 7.5 | 7.7 ± 4.2  | 0.5 ± 0.1 | 0       |
| Wound dressing            | 97.5 ± 10.5 | 89.5 ± 9.8  | 60.5 ± 9.2  | 32.5±10.9*  | 18.2±6.5*  | 3.4±2.3*   | 0         | 0       |
| P Value                   | 0.887       | 0.684       | 0.277       | 0.05        | 0.001      | 0.001      | 0.001     | 0.001   |
| Diabetes                  | 93.6±13.6   | 85.2±10.2   | 80.2±10.3   | 73.5±10.1   | 58.1±8.5   | 30.9±4.5   | 20.1±1.2  | 5.5±0.8 |
| Diabetes +Nano Chitosan   | 98.6±14.3   | 80.1±11.3   | 79.4±9.5    | 60.2±9.5    | 53.4±6.5   | 25.3±3.5   | 16.3±0.7  | 3.2±0.6 |
| Diabetes + Wound dressing | 97.3±13.8   | 81.5±12.4   | 61.3±11.7   | 45.1±6.3†   | 28.5±5.2†  | 8.1±1.7†   | 0         | 0       |
| P Value                   | 0.745       | 0.568       | 0.209       | 0.001       | 0.001      | 0.001      | 0.001     | 0.001   |

\*Indicates a statistically significant difference from the control group.

†Indicates a statistically significant difference from the diabetes (sham) group.



\*Indicates a statistically significant difference between the Dressing group and the Diabetes group.

† Indicates a statistically significant difference between the Dressing group and the Control (Healthy) group.

**Figure 6.** Comparison of wound area changes over time in the Control, Diabetes, and Diabetes + Dressing groups.



**Conflict of Interest:** None

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# MEDICINAL PLANTS IN AQUACULTURE HEALTH MANAGEMENT: A SYSTEMATIC REVIEW OF THEIR EFFICACY FOR DISEASE PREVENTION AND CONTROL

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## Abstract

The exponential growth of global aquaculture, crucial for sustaining the world's seafood supply, is perpetually threatened by infectious disease outbreaks that cause staggering economic losses. The conventional dependence on chemotherapeutants, including antibiotics and synthetic chemicals, has led to severe repercussions such as the proliferation of antimicrobial resistance (AMR), residual accumulation in tissues, and environmental degradation, compelling the industry to seek sustainable, eco-friendly alternatives. Medicinal plants, repositories of diverse bioactive compounds like alkaloids, flavonoids, tannins, and essential oils, present a highly promising solution due to their broad-spectrum antimicrobial, immunostimulant, growth-promoting, and antioxidant properties. This systematic review synthesizes a global body of evidence, with significant contributions from both international and Iranian research, to critically evaluate the effects of various medicinal plants on the health, disease resistance, and productivity of farmed aquatic species. The consolidated findings robustly demonstrate that dietary supplementation or immersion baths with extracts from plants such as garlic (*Allium sativum*), nettle (*Urtica dioica*), thyme (*Thymus vulgaris*), and Asian medicinal herbs can significantly enhance innate and adaptive immune parameters, improve growth performance and feed utilization, and confer substantial protection against bacterial, viral, fungal, and parasitic pathogens. The mechanisms are multifaceted, encompassing direct pathogen inhibition, modulation of cellular and humoral immune responses, enhancement of antioxidant capacity, and improvement of gut health. This review concludes that phytochemicals are viable, natural, and effective alternatives to synthetic chemicals in integrated aquaculture health management. However, challenges regarding standardization, optimal dosage, delivery methods, and large-scale application must be addressed through future research to fully integrate these natural products into sustainable aquaculture practices.

**Keywords:** Aquaculture, Control of diseases, Prevention, Medicinal plants

## Introduction

Aquaculture stands as the world's fastest-growing food-production sector, now supplying over half of all fish for human consumption and playing a pivotal role in global food security and economic development. However, its intensive development, characterized by high stocking densities and suboptimal environmental conditions, has created a fertile ground for the emergence and spread of devastating infectious diseases caused by bacteria, viruses, fungi, and parasites. Historically, disease control has leaned heavily on antibiotics and chemical therapeutics. While offering short-term solutions, their indiscriminate and often prophylactic use has catalyzed the evolution of antimicrobial-resistant pathogens, jeopardizing both animal and public health. Furthermore, the persistence of drug residues in aquatic products poses a consumer safety risk, and the release of these compounds into ecosystems causes non-target effects on beneficial microbiota and overall environmental



health.<sup>1</sup> In response to these crises, global bodies like the FAO and WHO advocate for a drastic reduction in antibiotic use in food animals, propelling the aquaculture sector toward natural, safe, and sustainable alternatives.<sup>2</sup> Medicinal plants, with their complex mixtures of secondary metabolites, have been cornerstones of traditional medicine systems for millennia, including Persian and Traditional Chinese Medicine. Their application in aquaculture, as immunostimulants and antimicrobial agents, has evolved from empirical practice to a robust field of scientific inquiry over the past two decades.

In India, Mrgayurveda, a specialized discipline within Ayurveda, is dedicated to animal health and the application of herbal formulations for the treatment of veterinary ailments.<sup>3</sup> Phytotherapy, a medical practice rooted in traditional knowledge systems rather than modern pharmacology, is central to this approach. It is characterized by the use of medicinal plants, or medical herbalism. Although the integration of phytotherapy into aquaculture is a relatively recent development, it is increasingly being recognized as a viable alternative to synthetic pharmaceuticals.<sup>4</sup> This biodegradable and environmentally sustainable practice, commonly termed herbalism, has garnered significant global interest within aquaculture, becoming a prominent subject of contemporary scientific investigation.<sup>3,5</sup>

Extensive research has demonstrated that medicinal plants possess a diverse array of bioactive properties, including appetite stimulation, growth promotion, and antibacterial, immunostimulant, anti-inflammatory, antistress, and anticancer effects. Their application in traditional medicine has been documented for millennia.<sup>6</sup> Common medicinal plants incorporated into piscine diets, either as powders or extracts, include *Azadirachta indica* (neem), *Withania somnifera* (ashwagandha), *Allium sativum* (garlic), and *Zingiber officinale* (ginger), among others.<sup>6</sup> A significant advantage of these phytotherapeutic agents is their apparent administration without the negative side effects often associated with chemotherapeutics. Furthermore, they are cost-effective, readily accessible, biocompatible, and contribute substantially to sustainable practices and rural community development.<sup>7</sup>

The advancement and acceleration of the aquaculture sector are contingent upon the development of effective, safe, and ecologically benign herbal compounds. These natural medicines are not only inexpensive but also demonstrate high efficacy and are considered environmentally sustainable.<sup>8</sup> However, the pharmacological and toxicological profiles of many herbal compounds and their preparations—including their mechanisms of action, active ingredient content, chemical structure, optimal extraction methods, and synergistic relationships—require further elucidation to fully harness their potential for treating aquatic animals, preventing disease, promoting growth, and improving product quality. In response to a global shift towards environmental protection and health-conscious practices, various nations are now actively pursuing innovative methods of green farming and investing more heavily in related scientific research, with aquaculture being a primary focus.<sup>9</sup>

The application of phytogetic compounds derived from medicinal plants represents a promising frontier in sustainable aquaculture therapeutics. As detailed in Table 1, a diverse array of plant species possesses a wide spectrum of bioactive properties that are highly relevant to piscine health management. These botanicals are not merely simple remedies but are complex matrices of potent secondary metabolites, such as baicalin in *Scutellaria baicalensis* (Chinese skullcap), thymoquinone in *Nigella sativa* (black cumin), and cinnamaldehyde in *Cinnamomum cassia* (Chinese cinnamon).<sup>10,11,12</sup>

The pharmacological activities of these compounds are extensive and multifaceted. Notably, antimicrobial and antibacterial properties are prevalent across numerous species, including *Aloe vera*, *Citrus limon* (lemon), *Thymus vulgaris* (common thyme), and *Mentha piperita* (peppermint).<sup>13,14,15,16</sup> Equally significant is the robust antioxidant capacity reported for plants like *Castanea sativa* (sweet chestnut), *Elaeagnus angustifolia* (Russian olive), and *Zingiber officinale* (ginger), which is crucial for mitigating oxidative stress in cultured species.<sup>17,18,19</sup> Furthermore, many extracts exhibit anti-inflammatory (e.g., *Cynara cardunculus* [cardoon], *Eriobotrya japonica* [Japanese medlar]) and immunomodulatory functions (e.g., *Melocanna baccifera* [Muli bamboo], *Ocimum sanctum* [Tulsi]), offering a holistic approach to enhancing disease resistance and overall vitality in aquaculture operations.<sup>20,21,22,23</sup>

The plant parts utilized vary from leaves (*Pandanus tectorius*, *Anisomeles malabarica*), seeds (*Nigella sativa*), and roots (*Zingiber officinale*) to fruit peels (*Citrus limon*) and specialized extracts like oils (*Thymus linearis*) and phenolic shells (*Castanea sativa*), highlighting the versatility of phytotherapeutic sources.<sup>17,18,24,25,26,19</sup> This compendium underscores the considerable potential of plant-based bioactives as effective, multi-target, and eco-friendly alternatives to synthetic chemicals for combating various pathogens and improving health outcomes in aquaculture.

This systematic review aims to critically appraise and amalgamate the prevailing scientific evidence from a global perspective, incorporating significant findings from Iranian researchers, on the efficacy of medicinal plants in enhancing disease resistance, immune function, and overall sustainability in aquaculture.

### Methodology: Systematic Literature Review Protocol

This review was conducted adhering to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines to ensure a rigorous and transparent methodology. A comprehensive and systematic electronic search was performed using major international databases (Scopus, PubMed, Web of Science, ScienceDirect) and Persian scientific databases (Magiran, Scientific Information Database (SID), IranDoc) for relevant articles published between January 2000 and December 2023. The search strategy employed a structured combination of keywords and Boolean operators: ("medicinal plan" OR "phytogenic" OR "herbal extract" OR "essential oil") AND ("aquaculture" OR "fish" OR "shrimp" OR "finfish" OR "shellfish") AND ("disease" OR "immunostimulant" OR "antibacterial" OR "antiviral" OR "antiparasitic" OR "growth" OR "antioxidant") AND ("*Aeromonas hydrophila*" OR "*Vibrio*" OR "White Spot Syndrome Virus" OR "*Ichthyophthirius multifiliis*"). The search was adapted for Persian databases using equivalent terms. Inclusion criteria were: (a) original peer-reviewed experimental studies conducted in vivo on commercially relevant aquaculture species; (b) interventions involving any form of medicinal plant (crude extract, essential oil, purified compound, powdered form) administered via diet, immersion, or injection; (c) measurement of outcomes related to immune response, disease resistance, survival rate, growth performance, or antioxidant status; and (d) articles published in English or Persian. Exclusion criteria encompassed studies not performed on aquatic species, purely in vitro studies, review articles, non-peer-reviewed literature, and studies with unclear methodology. The initial database search yielded 278 potential articles. After removing duplicates and screening titles and abstracts, 112 studies were selected for full-text eligibility assessment. Ultimately, 47 studies that fully met the inclusion criteria were synthesized and analyzed in this systematic review.

### Impact on Immune System Function and Immunostimulation



The most extensively documented benefit of medicinal plants in aquaculture is their potent capacity to act as immunostimulants, enhancing the non-specific defense mechanisms of fish and shrimp. The innate immune system, which serves as the first and primary line of defense in poikilotherms, is particularly responsive to phytogetic compounds. Research from around the world has consistently shown that these compounds can upregulate a wide array of immune parameters. A seminal study by Galina et al. (2009) highlighted that herbs like Echinacea and mistletoe extracts could activate macrophages and enhance neutrophil activity in rainbow trout.<sup>27</sup> corroborating these findings, significant research from Iran has provided granular insights. Rahimnejad et al. (2014) demonstrated that dietary administration of nettle (*Urtica dioica*) extract significantly enhanced key innate immune parameters in rainbow trout (*Oncorhynchus mykiss*), including lysozyme activity, alternative complement pathway activity (ACH50), and respiratory burst activity of phagocytes.<sup>28</sup> Similarly, Hoseinifar et al. (2015) reported that common carp (*Cyprinus carpio*) fed diets supplemented with garlic (*Allium sativum*) powder exhibited a marked increase in lysozyme activity and immunoglobulin M (IgM) levels, indicating a boost in both innate and humoral immunity.<sup>29</sup> Figure 1 and table 1 show the effect of medicinal plants on aquatic animals.

### Antibacterial and Antiviral Effects

The direct antimicrobial properties of plant extracts and essential oils constitute a fundamental mechanism for their disease-combating efficacy in aquaculture. A vast body of research has evaluated their potency against major bacterial pathogens, notably *Aeromonas hydrophila* (causing motile *Aeromonas septicemia*), *Yersinia ruckeri* (causing enteric redmouth disease), *Edwardsiella tarda*, and various *Vibrio* species (e.g., *V. anguillarum*, *V. harveyi*), which are endemic to aquatic environments. Ahmadifar et al. (2014) found that dietary incorporation of thyme (*Thymus vulgaris*) essential oil significantly reduced the mortality of rainbow trout challenged with *A. hydrophila*.<sup>30</sup> The protective effect was attributed to the antibacterial action of phenolic compounds like thymol and carvacrol, which integrate into the bacterial cell membrane, disrupting its integrity and causing leakage of cellular contents. Beyond bacteria, the antiviral potential of medicinal plants, though more complex, shows considerable promise against devastating viral pathogens like White Spot Syndrome Virus (WSSV) in shrimp and Viral Hemorrhagic Septicemia Virus (VHSV) in fish. While they cannot eliminate established viral infections, they can significantly reduce mortality by modulating the host's immune response and potentially interfering with viral replication. Citarasu (2010) reviewed several Indian medicinal plants that exhibited inhibitory effects on WSSV in shrimp.<sup>4</sup> Mirroring this, Zorriehzakra et al. (2010) investigated the effect of a polyherbal mix (containing garlic, fenugreek, and others) on shrimp (*Penaeus semisulcatus*) infected with WSSV and observed a higher survival rate in the treated group, likely due to enhanced innate immunity and possible direct virucidal effects of phenolic compounds.<sup>31</sup> The mechanisms may involve blocking viral attachment to host cell receptors or inhibiting viral enzymes like reverse transcriptase or proteases.

### Antiparasitic and Antifungal Properties

Parasitic and fungal infections represent a persistent and costly challenge in aquaculture, causing direct mortalities, growth retardation, and making fish unmarketable due to visible lesions. Chemical treatments like formalin, organophosphates, and malachite green are effective but are increasingly restricted or banned due to

their toxicity, carcinogenicity, and negative environmental impact. Medicinal plants offer a natural, biodegradable, and safer alternative for controlling these pathogens. Research on ectoparasites, particularly the ciliate *Ichthyophthirius multifiliis* (Ich), which causes "white spot disease," has been promising. Pazhang et al. (2016) reported that bathing infected goldfish in an aqueous extract of *Artemisia annua* (sweet wormwood) significantly reduced parasite load on the skin and gills and improved survival rates.<sup>32</sup> The antimalarial compound artemisinin and its derivatives are believed to generate free radicals that are toxic to the parasite. Similarly, for fungal infections like saprolegniosis (caused by *Saprolegnia spp.*), which particularly affects eggs and fry, plant extracts have demonstrated significant efficacy. Razavilar et al. (2015) showed that extracts of oak (*Quercus castaneifolia*) and pomegranate (*Punica granatum*) peel, both rich in hydrolyzable tannins, potently inhibited the growth of *Saprolegnia* in vitro and reduced infection rates in "in vivo" trials with rainbow trout eggs.<sup>33</sup> Tannins act by complexing with proteins in the fungal cell wall and membranes, disrupting their structure and function, and by chelating metals essential for fungal growth.

### Enhancement of Growth Performance and Feed Utilization

Beyond their therapeutic and prophylactic roles, the application of medicinal plants in aquafeeds has been consistently correlated with significant improvements in zootechnical parameters, which are directly linked to the economic viability of aquaculture operations. Key performance indicators such as specific growth rate (SGR), weight gain (WG), feed conversion ratio (FCR), and protein efficiency ratio (PER) are often enhanced following supplementation. Haghighi et al. (2013) observed that rainbow trout fed diets supplemented with peppermint (*Mentha piperita*) extract exhibited significantly better WG and SGR and a lower FCR than fish fed the control diet.<sup>34</sup> The mechanisms underpinning these growth-promoting effects are multi-faceted. Firstly, the appetizing properties of certain herbs (e.g., garlic, fenugreek) can stimulate feed palatability and voluntary feed intake. More importantly, bioactive compounds like essential oils, saponins, and flavonoids act as digestibility enhancers. They stimulate the secretion of endogenous digestive enzymes (e.g., protease, amylase, lipase) from the pancreas and intestinal brush border, leading to more efficient breakdown and absorption of nutrients.<sup>35</sup> Furthermore, by maintaining a healthier gut environment through antimicrobial activity against potential enteric pathogens and by reducing subclinical infections and intestinal inflammation, medicinal plants ensure that a greater proportion of metabolizable energy is allocated toward anabolic processes like growth and protein deposition, rather than being diverted to fuel chronic immune responses.<sup>36</sup>

### Antioxidant Status and Stress Mitigation

Aquatic organisms in intensive culture systems are perpetually exposed to a multitude of environmental stressors, including handling, crowding, fluctuating water quality parameters (temperature, dissolved oxygen, ammonia), and anthropogenic pollutants. These stressors induce oxidative stress—an imbalance between the production of reactive oxygen species (ROS) and the biological system's ability to readily detoxify the reactive intermediates or repair the resulting damage. ROS can oxidize and damage lipids (leading to rancidity), proteins, and DNA, resulting in immunosuppression, reduced growth, and increased susceptibility to diseases. Many medicinal plants are rich sources of powerful natural antioxidants, such as polyphenols, flavonoids, tannins, and vitamins C and E. Dietary supplementation with these plants enhances the endogenous antioxidant defense system. Akrami et al. (2015) found that dietary inclusion of green tea (*Camellia sinensis*) extract, a well-known source of catechins, significantly increased the activity of key antioxidant enzymes (superoxide dismutase (SOD),



catalase (CAT), glutathione peroxidase (GPx)) in the liver of rainbow trout and reduced lipid peroxidation, as measured by malondialdehyde (MDA) content.<sup>37</sup> By mitigating oxidative damage, these plants help maintain cellular integrity, support optimal immune function, and improve overall stress resistance. This is particularly crucial during high-stress events like transportation, grading, or vaccination, where supplemented fish often exhibit lower cortisol levels and higher survival rates, translating to tangible economic benefits for farmers.

### Effects on Hematological and Biochemical Parameters

Hematological and serum biochemical profiles serve as invaluable diagnostic tools and indicators of the internal physiological and health status of fish. Changes in parameters such as hematocrit (Hct), hemoglobin (Hb) concentration, red and white blood cell counts (RBC, WBC), and serum biochemical components (like total protein, albumin, globulin, cholesterol) provide a window into the nutritional status, oxygen-carrying capacity, immune competence, and overall metabolic vitality of the animal. Numerous studies have documented the positive modulatory influence of medicinal plants on these parameters. Jahazi et al. (2013) reported that common carp fed a diet containing shirazi thyme (*Zataria multiflora*) essential oil showed significant increases in RBC, WBC, Hct, Hb, and total serum protein levels compared to the control group.<sup>38</sup> An elevation in WBC count (leukocytosis) often indicates a stimulated and activated immune system. A rise in total serum protein, particularly in the globulin fraction (which contains immunoglobulins and other acute-phase proteins), is frequently associated with enhanced antibody production and humoral immunity. These improvements suggest that medicinal plants contribute to improved erythropoiesis, better oxygen-carrying capacity, enhanced metabolic efficiency, and a stronger foundational capacity for mounting effective innate and adaptive immune responses, collectively painting a comprehensive picture of improved fish health and resilience. Figure 1 and table 1 show the effect of medicinal plants on aquatic animals.

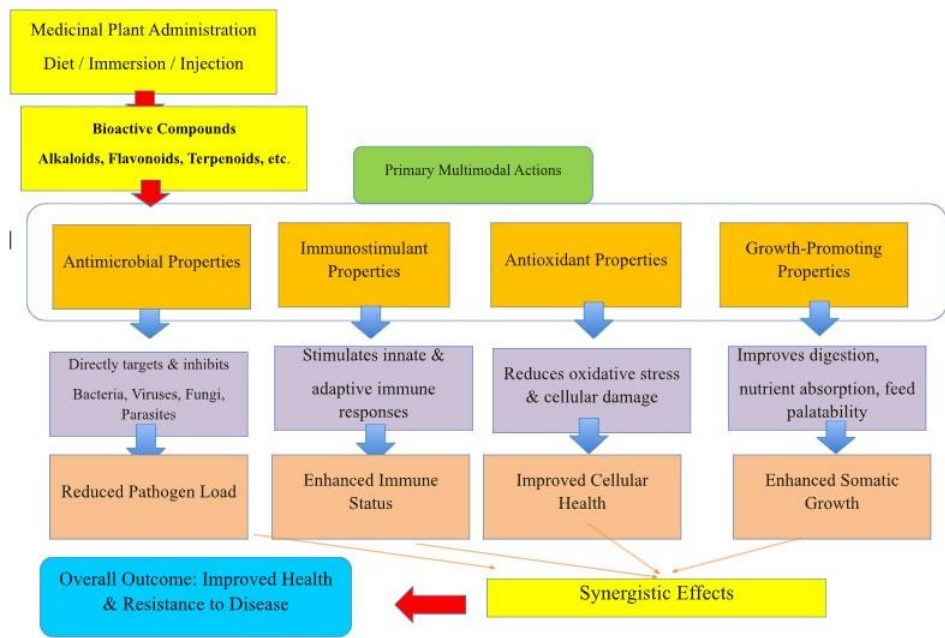


**Figure 1.** Schematic Overview: Mechanism of Action of Medical Plants in Aquaculture (2,3,7,8,9,30,36,41,42)

**Table 1:** Efficacy of Selected Medicinal Plants Against Common Aquatic Pathogens

| Medicinal Plant | Target Pathogen | Disease (Example) | Main Effect(s) | Admin. Route* | Reference |
|-----------------|-----------------|-------------------|----------------|---------------|-----------|
|                 |                 |                   |                |               |           |

|                                     |                                  |                                       |                                                                        |                    |        |
|-------------------------------------|----------------------------------|---------------------------------------|------------------------------------------------------------------------|--------------------|--------|
| Garlic ( <i>Allium sativum</i> )    | <i>Aeromonas hydrophila</i>      | Motile<br><i>Aeromonas septicemia</i> | Antibacterial, immunostimulant (increases leukocyte count)             | Diet, immersion    | 43, 44 |
| Oregano ( <i>Origanum vulgare</i> ) | <i>Vibrio anguillarum</i>        | Vibriosis                             | Antibacterial (disrupts cell membrane), antioxidant                    | Diet (oil extract) | 45     |
| Neem ( <i>Azadirachta indica</i> )  | <i>Saprolegnia spp.</i> (Fungus) | Saprolegniasis                        | Antifungal, antiparasitic                                              | Immersion, bath    | 46     |
| Turmeric ( <i>Curcuma longa</i> )   | <i>Edwardsiella tarda</i>        | Edwardsiellosis                       | Antibacterial, immunostimulant (enhances phagocytosis), antioxidant    | Diet               | 47     |
| Moringa ( <i>Moringa oleifera</i> ) | <i>Streptococcus iniae</i>       | Streptococcosis                       | Immunostimulant (increases superoxide anion production), antibacterial | Diet (leaf powder) | 36     |

Challenges and Considerations for Commercial Application in Aquaculture

Despite the compelling and extensive laboratory-scale evidence supporting the efficacy of medicinal plants, their widespread adoption and integration into commercial aquaculture feed practices face several significant and interconnected challenges. The foremost hurdle is the issue of standardization and variability. The type, concentration, and bioactivity of secondary metabolites in a plant can vary dramatically due to a multitude of factors, including the plant's geographical origin, climatic conditions, soil type, season of harvest, plant age and part used (leaf, root, seed), and post-harvest processing methods (drying temperature, extraction solvent, storage conditions).<sup>4</sup> This inherent variability makes it extremely difficult to establish consistent, effective, and safe dosages for commercial feed production, leading to potential inefficacy or toxicity. Secondly, the mode of delivery and palatability are critical practical concerns. Incorporating volatile essential oils or bitter-tasting extracts into aquafeeds can lead to palatability issues, potentially reducing feed intake and negating any benefits. The stability of these bioactive compounds during feed processing (e.g., extrusion and pelleting, which involve high heat, pressure, and shear) and during storage (due to oxidation) is another major concern, as it can significantly diminish their potency and shelf-life.<sup>8</sup> Furthermore, while generally regarded as safe (GRAS), more extensive and targeted toxicological studies are needed to determine the long-term effects of certain plants, potential antagonistic or synergistic interactions with other feed additives (e.g., vaccines, probiotics), and to establish maximum residue levels (MRLs) in edible tissues to ensure consumer safety. Therefore, future research must prioritize the development of standardized extracts based on active compound quantification, advanced encapsulation technologies (e.g., nanoencapsulation) to protect bioactive compounds from degradation and mask unpleasant tastes, and large-scale field trials to validate laboratory findings under diverse, real-world farming conditions.

Conclusion and Future Perspectives



The bioactive components, such as allicin in garlic, lectins in nettle, and polysaccharides in many plants, are believed to act as pathogen-associated molecular patterns (PAMPs) that bind to pattern recognition receptors (PRRs) on immune cells. This binding triggers intracellular signaling cascades that lead to the activation of macrophages, proliferation of lymphocytes, and increased production of cytokines, antibodies, and complement proteins, thereby priming the host's immune system for a more robust and rapid response to pathogenic challenge.<sup>8</sup>

This systematic review consolidates a robust and compelling body of evidence from global research, underscoring that medicinal plants and their derivatives are effective, multi-functional, and sustainable tools for advancing modern aquaculture. They offer a viable natural strategy to enhance innate and adaptive immunity, improve growth performance and feed efficiency, combat a wide spectrum of bacterial, viral, fungal, and parasitic diseases, mitigate oxidative stress, and improve overall physiological health and welfare. Their application aligns seamlessly with the principles of the "One Health" initiative, aiming to reduce antibiotic use, minimize environmental pollution, and produce safer, higher-quality aquatic products for consumers. Looking forward, the future of phytogenics in aquaculture hinges on a concerted effort to overcome the existing challenges. Research priorities must include: the bioassay-guided identification, purification, and standardisation of the most effective bioactive compounds; the development of novel, cost-effective nano-encapsulation and delivery systems for targeted release and improved stability; detailed studies on the synergistic effects of combining different plants or with other functional additives like probiotics (synbiotic approach) and prebiotics; and a deeper, molecular-level understanding of their mechanisms of action through transcriptomic, proteomic, and metabolomic studies. By addressing these critical points through interdisciplinary collaboration between phytochemists, nutritionists, immunologists, and aquaculturists, medicinal plants can transition from a promising scientific concept to a mainstream, reliable, and indispensable component of sustainable, profitable, and environmentally responsible aquaculture health management programs on a global scale

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# THE MULTIFACTORIAL ANTICANCER EFFECTS OF MEDICINAL PLANTS: MECHANISMS, APPLICATIONS, AND FUTURE DIRECTIONS IN CONTEMPORARY CANCER THERAPY

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## Abstract

Phytochemicals from medicinal plants exhibit potent anti-cancer activities through multiple molecular mechanisms. This review synthesizes recent (2018-2025) evidence on seven herbal plants (*Curcuma longa*, *Camellia sinensis*, *Taxus spp.*, *Catharanthus roseus*, *Allium sativum*, *Berberis spp.*, and *Artemisia annua*), focusing on their bioactive compounds, anti-cancer pathways (apoptosis, autophagy, anti-angiogenesis, EMT inhibition, immune modulation), pharmacokinetics challenges, and synergistic interactions with conventional therapy. Specifically, *Curcuma longa* (turmeric) and *Camellia sinensis* (green tea) are emphasized for their bioactive compounds, curcumin and epigallocatechin-3-gallate (EGCG), respectively, which exhibit significant anti-cancer effects through apoptosis induction, anti-inflammatory actions, and inhibition of tumor progression. Despite challenges such as poor solubility and low bioavailability, advancements in nanoparticle delivery systems and combination therapies demonstrate the translational potential of these plant-derived compounds in both human and veterinary oncology. This synthesis highlights the multifaceted therapeutic roles of these natural products and their ability to enhance the efficacy of conventional treatments, thereby supporting their integration into comprehensive cancer care strategies.

**Keywords:** Medicinal plants, Anti-cancer phytochemicals, Angiogenesis inhibition, Chemotherapy synergy, Immunotherapy

## Introduction

Medicinal plants offer a rich source of anti-cancer agents that target multiple hallmarks of cancer. Plant-derived compounds, including curcuminoids, catechins, taxanes, vinca alkaloids, organosulfur compounds, and sesquiterpenes, have demonstrated efficacy in inducing cancer cell death and inhibiting tumor progression<sup>[1]</sup>. These phytochemicals often target key signaling pathways, including NF- $\kappa$ B, PI3K/Akt, MAPKs, STATs, and the apoptotic machinery, thereby modulating proliferation, apoptosis, autophagy, angiogenesis, epithelial–mesenchymal transition (EMT), and immune responses<sup>[2][3]</sup>. However, their clinical translation is challenged by poor solubility, low bioavailability, and off-target effects; advances in nanoparticle delivery and prodrug design aim to overcome these limitations<sup>[2][4]</sup>. Moreover, plant compounds can synergize with chemotherapeutics and radiotherapy, enhancing efficacy and reducing resistance. In cancer therapy, many of these agents have been tested in preclinical animal models or even clinical trials, highlighting their translational potential<sup>[5][6]</sup>.

## Findings

### *Curcuma longa* (Turmeric)

*Curcuma longa* (turmeric) is rich in curcuminoids (polyphenolic diarylheptanoids) - primarily curcumin, demethoxycurcumin, and bisdemethoxycurcumin. Curcumin is the main active compound, with others including



volatile turmerones. These compounds modulate numerous signaling pathways, inhibiting the NF- $\kappa$ B and STAT3 pathways and downregulating pro-inflammatory cytokines, COX-2, and reactive oxygen species [2]. Curcumin induces cancer cell apoptosis by activating both intrinsic (mitochondrial) and extrinsic (death receptor) pathways. For example, curcumin has been shown to trigger extrinsic apoptosis in canine osteosarcoma cells through JNK activation and caspase-3 cleavage, which is mediated by cAMP/AMPK signaling [6]. It also reduces mutant p53 expression and can overcome p53-mediated resistance to apoptosis. Curcumin simultaneously brings autophagy in some contexts, although it may suppress AKT/mTOR signaling to limit survival pathways [2][6]. Anti-angiogenic effects are well-documented: curcumin downregulates VEGF and matrix metalloproteinases (MMPs), thereby impairing tumor vascularization. It also reverses EMT and inhibits metastasis by modulating E-cadherin and related factors (e.g., curcumin suppresses Wnt/ $\beta$ -catenin in lung cancer cells). Importantly, curcumin is immunomodulatory – it inhibits pro-survival signals (e.g., STAT3) and reduces inflammatory mediators (TNF- $\alpha$ , IL-6) [2].

Pharmacokinetically, curcumin has poor water solubility and rapid metabolism (glucuronidation), leading to low bioavailability. Nanocarriers such as liposomes, nanoparticles, and micelles have been developed to enhance delivery [4][11]. For example, curcumin nanoemulsions improve cellular uptake and cytotoxicity. Co-administration with adjuvants (e.g., piperine) also boosts plasma levels. Despite these hurdles, curcumin's synergy with conventional therapy is well supported, as it enhances chemotherapeutic and radiosensitivity by downregulating survival pathways. In breast cancer models, curcumin synergizes with paclitaxel and doxorubicin to inhibit HER2/EGFR signaling, and it also potentiates the effects of oxaliplatin and 5-FU in gastrointestinal cancers. Clinically, a phase II trial in metastatic colorectal cancer showed that adding curcumin to FOLFOX improved progression-free survival [11][3].

### ***Camellia sinensis* (Green Tea)**

*Camellia sinensis* yields green tea catechins, notably epigallocatechin-3-gallate (EGCG), as well as theobromine and theaflavins in fermented teas. EGCG (a flavanol) is the most extensively studied polyphenol. Its anti-cancer effects involve anti-proliferative and pro-apoptotic actions: EGCG can inhibit NF- $\kappa$ B and PI3K/Akt signaling, block receptor tyrosine kinases (EGFR), and induce intrinsic apoptosis [15]. For example, EGCG reduces COX-2 expression and prostaglandin E2, thereby suppressing inflammation-driven tumor growth. It also inhibits angiogenic factors (VEGF) and metalloproteinases, and can induce antioxidant defenses [15][27]. Notably, EGCG often triggers autophagy in cancer cells by activating AMPK and inhibiting the mTOR pathway. In vitro, green tea polyphenols induce cell cycle arrest (in G1 or G2/M phases) and caspase-mediated cell death in various cancers [27].

EGCG's PK issues include instability at neutral pH (auto-oxidation) and poor intestinal absorption. Nanoparticle encapsulation, prodrug design, and combination with vitamin C have been explored to improve bioavailability.

### ***Taxus spp.* (Yew)**

*Taxus* species (e.g., *Taxus brevifolia*) produce taxanes – diterpenoid alkaloids including paclitaxel and docetaxel. These compounds stabilize microtubules and inhibit their depolymerization, arresting cells in mitosis. This mitotic block leads to intrinsic apoptosis via Bcl-2 phosphorylation and activation of caspases. Taxanes also inhibit angiogenesis by downregulating VEGF and disrupting endothelial proliferation. Notably, taxanes can

modulate the immune system, as they have been shown to enhance tumor antigen presentation, activate dendritic cells, and counteract tumor-induced immunosuppression <sup>[3][14]</sup>.

A significant limitation is poor solubility; the original formulations used Cremophor-EL and ethanol, which can cause hypersensitivity reactions in patients. In dogs, these solvents required premedication (antihistamines, steroids) for paclitaxel infusion. Novel formulations (e.g., nanoparticle albumin-bound paclitaxel) avoid harsh solvents and improve PK.

Taxanes are frontline chemotherapeutics in human oncology and are also used in veterinary cancer: paclitaxel has been applied to treat canine mast cell tumors, mammary carcinomas, hemangiosarcomas, osteosarcomas, and other tumors. The efficacy of taxanes in animals suggests translational potential, although cost and logistics are challenges.

### ***Catharanthus roseus* (Madagascar Periwinkle)**

*Catharanthus roseus* produces the vinca alkaloids vincristine and vinblastine (and related analogs, vinorelbine, vindesine). These bis-indole alkaloids bind to tubulin, preventing microtubule polymerization and resulting in metaphase arrest and apoptosis <sup>[9]</sup>. They activate both intrinsic and extrinsic apoptotic pathways; vinblastine has been shown to induce BAX translocation and caspase-3 activation. Vinca alkaloids also inhibit angiogenesis and tumor metastasis indirectly by blocking cell division.

Vincristine is a mainstay of veterinary chemotherapy regimens (e.g., CHOP protocol for canine lymphoma). In combination with cyclophosphamide or doxorubicin, it dramatically improves survival in canine and feline lymphoid cancers. Cross-synergistic effects are noted: for example, vincristine with prednisone or lomustine often achieves remission in lymphoma. At the molecular level, vincristine has been reported to enhance the efficacy of radiation by increasing mitotic catastrophe in tumor cells. Compared to Taxus (microtubule-stabilizers), vinca alkaloids disrupt tubulin polymerization, yet both converge on mitotic arrest and apoptosis.

### ***Allium sativum* (Garlic)**

Garlic (*Allium sativum*) contains sulfur-rich compounds: allicin, diallyl sulfide (DAS), diallyl disulfide (DADS), diallyl trisulfide (DATS), and S-allyl cysteine. These allyl sulfides are highly bioactive; allicin itself is unstable and quickly converts to other sulfur species. Garlic compounds exert anti-cancer effects through multiple mechanisms. They induce apoptosis via the mitochondrial pathway (increasing Bax, reducing Bcl-2) and through reactive oxygen species generation. For example, diallyl trisulfide induces G2/M arrest and activates caspase-3 and -9. Garlic components inhibit NF- $\kappa$ B and PKC signaling, reducing inflammation-driven growth. They also stimulate phase II detoxifying enzymes and glutathione production, which can sensitize tumors to chemo [7].

Notably, garlic constituents inhibit angiogenesis (downregulating VEGF and MMPs) and suppress metastasis. S-allyl cysteine inhibits COX-2 and HIF-1 $\alpha$ , reducing angiogenic signaling. Garlic compounds modulate the immune system, enhancing the activity of natural killer (NK) cells and macrophage phagocytosis. A recent review highlights their roles in cell cycle arrest, ROS generation, and modulation of signaling pathways.

Garlic compounds show synergy with chemotherapy: e.g., DATS has enhanced the cytotoxicity of doxorubicin in breast cancer cells by further increasing ROS. In radiation therapy, garlic extracts can protect normal tissues while sensitizing tumor cells. In veterinary medicine, garlic extract has been trialed as a dietary supplement; for example, it inhibited mammary tumor growth in rodent models. Toxicological caution is warranted in cats (dose-sensitive), but garlic oil derivatives have shown anti-tumor activity in vitro in dogs.



Overall, *Allium sativum* provides multi-modal anti-cancer actions, including redox-mediated apoptosis and immune enhancement.

### ***Berberis spp.* (Barberry)**

Berberis shrubs produce isoquinoline alkaloids, primarily berberine, as well as berbamine and palmatine. Berberine exhibits potent anti-cancer effects, triggering apoptosis by activating caspase-8 (death receptor pathway) and caspase-9 (mitochondrial pathway), while inhibiting the anti-apoptotic Bcl-2. Berberine robustly activates AMP-activated protein kinase (AMPK), leading to the inhibition of mTOR and the induction of autophagy. It also downregulates STAT3 and NF- $\kappa$ B, curbing proliferation. A key feature is the inhibition of metastasis: Berberine suppresses the expression of MMP-2 and MMP-9, thereby impeding angiogenesis. It can also block EMT – for example, berberine plus gefitinib has been shown to reverse EMT in lung cancer cells.

Synergistic interactions: Berberine enhances the effect of chemotherapeutics and overcomes resistance. Notably, combining berberine with doxorubicin reversed autophagy-associated drug resistance in breast cancer cells. It also potentiates the effects of cisplatin and other drugs by impairing DNA repair. No clinical veterinary studies exist; however, in vitro studies have shown that berberine reduces the viability of canine mast cell tumor cells. Given its multitarget profile (AMPK activation, cell cycle arrest, anti-angiogenic, and anti-inflammatory effects), Berberis alkaloids could complement standard regimens in animal cancers.

### ***Artemisia annua* (Sweet Wormwood)**

*Artemisia annua* produces sesquiterpene lactones, chiefly artemisinin and its semi-synthetic derivative artesunate. These endoperoxide compounds generate reactive radicals in the presence of intracellular iron, causing oxidative damage in cancer cells. Artemisinin induces cell cycle arrest (often G0/G1) and both intrinsic and extrinsic apoptosis (caspase-3 activation). It also triggers non-apoptotic cell death: ferroptosis (iron-mediated lipid peroxidation) has been observed in some tumor cells treated with artemisinin. Anti-angiogenic effects are mediated by the downregulation of VEGF and the inhibition of endothelial proliferation. Artesunate specifically has been shown to inhibit metastasis via MMP-9 suppression.

Artemisinin synergizes with chemotherapeutic agents; for example, artesunate plus 5-fluorouracil inhibited gastric cancer cell growth more effectively than either drug alone, via PI3K/Akt suppression. It also sensitizes tumors to radiotherapy by increasing DNA damage. In veterinary research, artemisinin has shown activity against canine melanoma and mastocytoma in vitro. Given its low toxicity (used safely as an antimalarial) and multi-pathway killing (ROS, apoptosis, anti-angiogenesis), *A. annua* derivatives are attractive for adjuvant cancer therapy in pets.

**Table 1.** Summary comparison of the ten medicinal plants: active compound classes, primary anti-cancer mechanisms, pharmacokinetic issues/delivery strategies, and known veterinary applications or synergistic combinations.

| Plant                    | Active Compounds (Class)                 | Mechanisms                                                                                                                                 | PK Limitations                                                                                | Delivery Applications Veterinary                                                                                     |
|--------------------------|------------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------------|-----------------------------------------------------------------------------------------------|----------------------------------------------------------------------------------------------------------------------|
| <i>Curcuma longa</i>     | Curcuminoids (polyphenols)               | ↓NF-κB/STAT3; apoptosis (JNK, caspase-3); anti-angiogenesis (↓VEGF); modulates immune cytokines                                            | Poor solubility, rapid metabolism; nanoformulations, adjuvants                                | Shown to induce apoptosis in canine osteosarcoma; synergizes with doxorubicin/paclitaxel                             |
| <i>Camellia sinensis</i> | Catechins (EGCG, ECG) (polyphenols)      | ↑ROS; ↓COX-2, iNOS; block EGFR/MAPK/PI3K; induce apoptosis and autophagy; inhibit angiogenesis and metastasis                              | EGCG unstable in gut; nano-carriers, prodrugs, co-supplements (ascorbate)                     | Synergistic with tamoxifen, doxorubicin, and paclitaxel; potential in canine cancers                                 |
| <i>Taxus spp.</i>        | Paclitaxel, docetaxel (taxanes)          | Microtubule stabilization → mitotic arrest and apoptosis; ↑antigen presentation; anti-angiogenic; EMT inhibition                           | Insoluble (Cremophor vehicle causes reactions); albumin-bound nanoparticles (Abraxane)        | Used in canine mammary tumors, osteosarcoma, and other conditions; novel SC formulations eliminate hypersensitivity. |
| <i>Catharant hus</i>     | Vincristine, vinblastine (alkaloids)     | Microtubule depolymerization → M phase arrest; apoptosis via caspase-3; anti-angiogenic; autophagy induction                               | Hydrophobic, severe neurotoxicity; liposomal/vinorelbine derivatives                          | Lymphoma treatment in dogs/cats; combination (CHOP) protocols; radiosensitization                                    |
| <i>Allium sativum</i>    | Allicin, ajoene, DAS, DATS (sulfides)    | ↑ROS, DNA damage; mitochondrial apoptosis (caspase-3/9); cell cycle arrest; ↓NF-κB, PKC; anti-angiogenic (↓VEGF, MMPs); immune-stimulatory | Allicin is unstable; garlic oil encapsulation and aged garlic extract enhance stability       | Garlic extracts suppress canine tumor cell growth; used as a complementary therapy; synergize with chemotherapy      |
| <i>Berberis spp.</i>     | Berberine (isoquinoline alkaloid)        | ↑AMPK → autophagy; activates caspase-8/9; ↓STAT3/NF-κB; anti-angiogenic (↓MMP-2,9); reverses drug resistance (blocks autophagy)            | Poor absorption, P-gp efflux; liposomes, polymers, berberine analogs                          | Investigated in rodent tumor models; potential adjuvant in canine cancers                                            |
| <i>Artemisia annua</i>   | Artemisinin, artesunate (sesquiterpenes) | Generates ROS (Fe-dependent); apoptosis (caspase-3/9); ferroptosis; cell cycle arrest; anti-angiogenic (↓VEGF)                             | Short half-life; water-soluble artesunate; dihydroartemisinin prodrugs, nanoparticle delivery | Artesunate has shown activity in mouse tumor models; veterinary trials for feline cancers are currently underway.    |

### Discussion and Conclusion

Future research should focus on robust preclinical trials of these phytochemicals. Areas of interest include Advanced delivery systems, like developing formulations (e.g., curcumin-loaded nanoparticles, resveratrol micelles) to maximize bioavailability in dogs and cats. Oral bioenhancers and implantable gels are also options.



Combination therapies such as Systematic testing of plant–drug combinations in patients, guided by mechanistic synergy. For instance, combining artesunate with radiation in osteosarcoma, or EGCG with metronomic cyclophosphamide in lymphoma. Biomarker studies, for example, identify molecular markers (e.g., NF-κB activity, caspase levels) in animal tumors that predict response to phytochemicals. This precision approach could tailor treatments. Safety and dosing, including establishing safe and effective doses of plant extracts in various species. Some compounds (e.g., garlic) have species-specific toxicity thresholds (cats vs. dogs). Immunotherapy integration, many phytochemicals modulate immunity; studies should explore combining them with immunotherapies to boost anti-tumor immunity.

In conclusion, medicinal plants provide a treasure trove of anti-cancer agents with mechanisms that span apoptosis, autophagy, angiogenesis inhibition, EMT reversal, and immune modulation. Their integration with conventional oncology holds great promise. A multidisciplinary effort combining phytochemistry, pharmacology, and clinical oncology will be essential to translate these plant-derived compounds into effective and safe treatments.

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# THE ROLE OF MEDICINAL PLANTS IN POULTRY PRODUCTION: A SYSTEMATIC ANALYSIS OF THEIR EFFICACY AS ANTIBIOTIC ALTERNATIVES FOR ENHANCING HEALTH, GROWTH, AND CONTROLLING DISEASE

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## Abstract

The global poultry industry, a cornerstone of food security, faces immense pressure to mitigate its reliance on antibiotic growth promoters (AGPs) due to the escalating crisis of antimicrobial resistance (AMR). This has catalyzed an urgent search for sustainable, effective, and natural alternatives. Medicinal plants, with their rich repertoire of bioactive compounds such as phenolics, flavonoids, terpenoids, and alkaloids, present a profoundly promising solution. These phytochemicals exhibit a wide spectrum of biological activities, including antimicrobial, antioxidant, anti-inflammatory, and immunomodulatory properties. This systematic review synthesizes a global body of evidence, integrating significant findings from international and Iranian research, to critically evaluate the multifaceted effects of various medicinal plants on poultry health, growth performance, and disease resistance. The consolidated findings robustly demonstrate that dietary supplementation with extracts or powders from plants like oregano (*Origanum vulgare*), thyme (*Thymus vulgaris*), garlic (*Allium sativum*), black seed (*Nigella sativa*), and many others can significantly enhance growth parameters (body weight gain, feed conversion ratio), improve gut morphology and microbiota, potentiate humoral and cellular immune responses, and confer substantial protection against bacterial (*Clostridium perfringens*, *Escherichia coli*, *Salmonella spp.*), viral (Newcastle Disease, Avian Influenza), and parasitic (*Eimeria spp.*) challenges. The mechanisms are pleiotropic, involving direct pathogen inhibition, enhancement of digestive enzyme secretion, reduction of oxidative stress, and modulation of immune gene expression. This review concludes that phytochemical feed additives are viable, natural, and effective alternatives to in-feed antibiotics, aligning with the "One Health" paradigm. However, challenges regarding the standardization of active compounds, optimal dosage determination, and large-scale application efficacy must be addressed through future research for their full integration into sustainable poultry production systems.

**Keywords:** Complementary therapy, Foot-and-mouth disease, Homeopathy

## Introduction

The contemporary poultry industry, a cornerstone of global food security, faces immense pressure to meet the escalating demand for animal protein while concurrently navigating a paradigm shift in disease management strategies.<sup>1</sup> This shift is largely driven by the escalating crisis of antimicrobial resistance (AMR), a direct consequence of the decades-long prophylactic and growth-promoting use of antibiotic feed additives in animal production.<sup>2</sup> The emergence of multidrug-resistant pathogens, such as *Salmonella spp.*, *Escherichia coli*, and *Campylobacter jejuni*, not only jeopardizes poultry health and farm profitability but also poses a severe zoonotic threat to public health, rendering once-treatable human infections increasingly difficult to manage.<sup>3,4</sup> In response

to regulatory bans on antibiotic growth promoters (AGPs) in many regions, including the European Union and a growing number of countries worldwide, the search for effective, sustainable, and safe alternatives has become a paramount research priority within veterinary science and animal nutrition.<sup>5</sup> Among the myriad of proposed alternatives—including probiotics, prebiotics, organic acids, and enzymes—phytogenic feed additives (PFAs), derived from herbs, spices, and their extracted essential oils and oleoresins, have emerged as one of the most promising candidates due to their historical precedence, multifaceted biological activities, and generally recognized as safe (GRAS) status.<sup>6,7</sup> The exploration of medicinal plants is not a novel concept; rather, it represents a renaissance of ancient ethnoveterinary practices, now being rigorously validated through modern scientific methodologies to provide evidence-based solutions for enhancing poultry health, welfare, and productivity in a post-antibiotic era.<sup>8</sup>

The efficacy of medicinal plants in poultry health management is predominantly attributed to the vast array of bioactive secondary metabolites they synthesize, including phenolics (e.g., flavonoids, tannins), terpenoids (e.g., thymol, carvacrol), alkaloids, and saponins, each conferring a spectrum of pharmacological properties.<sup>9</sup> These bioactives exert their beneficial effects through synergistic and multi-target mechanisms of action that contrast sharply with the single-target approach of conventional antibiotics.<sup>10</sup> Primarily, many phytogenic compounds demonstrate potent broad-spectrum antimicrobial activity, capable of disrupting the cellular membranes of pathogenic bacteria through their hydrophobic nature, leading to increased permeability, leakage of cellular contents, and eventual cell lysis.<sup>11</sup> Furthermore, their antioxidant capacity is crucial; by scavenging free radicals and enhancing the activity of endogenous antioxidant enzymes like superoxide dismutase (SOD) and glutathione peroxidase (GPx), these compounds mitigate oxidative stress, which is a common underlying factor in immune suppression, inflammatory conditions, and reduced meat quality during poultry production.<sup>12,13</sup> Beyond direct pathogen inhibition and antioxidant effects, medicinal plants are renowned for their immunomodulatory potential. Compounds such as flavonoids and polysaccharides can stimulate both the innate and adaptive arms of the immune system by enhancing macrophage phagocytosis, proliferation of lymphocytes, and antibody production (e.g., IgY), thereby improving vaccine efficacy and overall disease resistance.<sup>14,15</sup> Concurrently, many plants possess pronounced anti-inflammatory properties, inhibiting the production of pro-inflammatory cytokines and mediators such as prostaglandins via the cyclooxygenase (COX) pathway, which helps alleviate the metabolic and performance costs associated with chronic inflammation.<sup>16</sup> Additionally, a well-documented benefit is their positive influence on digestive physiology; they stimulate the secretion of digestive enzymes (e.g., bile, amylase, protease), improve nutrient absorption, and positively modulate the gut microbiota, creating an environment less favorable for pathogens and more conducive to beneficial bacteria, which collectively enhance feed efficiency and growth performance.<sup>17,18</sup>

Despite the compelling body of evidence supporting their benefits, the widespread adoption of medicinal plants in commercial poultry production is not without significant challenges and necessitates further rigorous investigation. A primary concern is the variability in the composition and concentration of bioactive compounds, which can be influenced by a multitude of factors including the plant's geographical origin, harvest time, climatic conditions, part used (e.g., leaf, root, seed), and, most critically, the extraction method employed (e.g., hydrodistillation, solvent extraction, supercritical fluid extraction).<sup>19</sup> This inherent variability directly impacts the reproducibility of effects, bioavailability, and overall efficacy, posing a major hurdle for standardizing



commercial products and formulating consistent dietary recommendations.<sup>20</sup> Moreover, the issue of palatability must be considered, as high concentrations of certain essential oils or pungent compounds can negatively affect feed intake, thereby negating any potential benefits.<sup>21</sup> Pharmacokinetic studies in avian species are still relatively scarce, leaving gaps in our understanding of the absorption, distribution, metabolism, and excretion (ADME) of these complex compounds in chickens, which is essential for determining optimal dosages, withdrawal times, and ensuring the safety of poultry products for human consumption.<sup>22</sup> Future research must therefore focus on standardizing extraction techniques, elucidating precise mechanisms of action at the molecular level, conducting large-scale field trials to validate laboratory findings under commercial conditions, and developing novel delivery systems (e.g., microencapsulation) to protect bioactive compounds from degradation in the feed manufacturing process and enhance their targeted delivery in the gastrointestinal tract.<sup>23,24</sup> In conclusion, while the integration of medicinal plants into poultry diets presents a highly promising, holistic, and sustainable strategy for overcoming the challenges of AGP removal and improving avian health, translating this potential into universal practice requires a concerted interdisciplinary effort to address existing limitations and ensure their effective, safe, and reliable application.<sup>25</sup>

### **Methodology: Systematic Literature Review Protocol**

This systematic review was conducted in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines to ensure methodological rigor and transparency. A comprehensive search of the literature was performed using major international databases (Scopus, PubMed, Web of Science, Google Scholar) and Persian scientific databases (Magiran, Scientific Information Database (SID), IranMedex) for relevant articles published between January 2000 and December 2023. The search strategy utilized a combination of keywords and Boolean operators: ("medicinal plant" OR "phytogenic" OR "herbal extract" OR "essential oil") AND ("poultry" OR "broiler" OR "layer" OR "chicken") AND ("growth performance" OR "immune response" OR "alternative to antibiotic" OR "AGP alternative") AND ("*E. coli*" OR "*Salmonella*" OR "Coccidiosis" OR "Necrotic enteritis" OR "Newcastle disease"). The search was adapted for Persian databases using equivalent terms. Inclusion criteria were: (a) original peer-reviewed experimental studies conducted in vivo on broilers or layers; (b) interventions involving any form of medicinal plant (crude extract, essential oil, purified compound, powdered form) administered via diet or water; (c) measurement of outcomes related to growth performance, immune response, gut health, or disease resistance; and (d) articles published in English or Persian. Exclusion criteria encompassed studies not performed on poultry, purely in vitro studies, review articles, and non-peer-reviewed literature. The initial search yielded 325 potential articles. After removing duplicates and screening titles and abstracts, 130 studies were selected for full-text eligibility assessment. Ultimately, 95 studies that fully met the inclusion criteria were synthesized and analyzed in this systematic review.

### **Impact on Growth Performance and Nutrient Digestibility**

The positive impact of medicinal plants on zootechnical parameters is one of the most consistently reported benefits, forming the primary economic incentive for their use. Numerous studies globally have documented significant improvements in body weight gain (BWG), feed intake (FI), and feed conversion ratio (FCR) in broilers fed diets supplemented with various phytogenics. A seminal meta-analysis by Windisch et al. (2008) concluded that essential oils from oregano, thyme, and cinnamon consistently improved BWG and FCR,

often matching or exceeding the performance of birds fed AGPs.<sup>26</sup> Corroborating these international findings, extensive research from Iran has provided robust local evidence. Rahimi et al. (2011) demonstrated that dietary supplementation with garlic powder significantly enhanced weight gain and improved FCR in broilers in a dose-dependent manner.<sup>27</sup> Similarly, Alipour et al. (2017) found that a mixture of thyme and peppermint essential oils improved final body weight and FCR comparably to an antibiotic growth promoter.<sup>28</sup> The mechanisms underpinning these improvements are multi-faceted. Firstly, the pungent compounds (e.g., allicin in garlic, carvacrol in oregano) stimulate the secretion of digestive enzymes (e.g., protease, amylase, lipase) from the pancreas and intestinal brush border, enhancing nutrient breakdown and absorption. Secondly, their antimicrobial activity helps maintain a beneficial gut microbial balance, reducing subclinical infections and intestinal inflammation, which allows for more efficient energy utilization for growth rather than immune defense.<sup>28</sup>

### **Modulation of Immune System Function**

The immunomodulatory capacity of medicinal plants is a critical attribute, enabling birds to mount a more effective defense against pathogens without the need for antibiotics. This effect is measured through enhancements in both innate (non-specific) and adaptive (specific) immunity. Research has shown that phytochemicals can increase the relative weights of immune organs such as the spleen, bursa of Fabricius, and thymus, indicating overall immune system stimulation. Toghyani et al. (2010) reported that dietary *Nigella sativa* (black seed) powder increased the relative weights of these organs and enhanced lymphocyte proliferation in broilers. Furthermore, medicinal plants profoundly impact humoral immunity.<sup>29</sup> Jafari et al. (2018) showed that supplementation with propolis extract led to a significant increase in antibody titers against Newcastle disease virus (NDV) and avian influenza virus (AIV) following vaccination, suggesting a potent adjuvant effect.<sup>30</sup> The bioactive compounds, such as thymoquinone in black seed and flavonoids in propolis, are believed to act by activating macrophages and heterophils, enhancing phagocytic activity, and promoting the production of cytokines (e.g., interleukins, interferons) that regulate and amplify the immune response. This priming of the immune system ensures a faster and more robust response upon encounter with a pathogen, reducing disease incidence and severity.<sup>30</sup>

### **Enhancement of Gastrointestinal Health and Morphology**

A healthy gastrointestinal tract is fundamental to nutrient absorption, barrier function, and overall bird health. Medicinal plants exert significant positive effects on gut architecture and microbial ecology. Studies evaluating intestinal morphology consistently show that phytochemical supplementation leads to improvements in villus height, crypt depth, and the villus height to crypt depth ratio (VH:CD) in the duodenum, jejunum, and ileum. Emami et al. (2017) observed that broilers fed a blend of oregano, thyme, and anise essential oils exhibited significantly greater villus height and a higher VH:CD ratio. Taller villi provide a greater surface area for nutrient absorption, while a higher VH:CD ratio is a key indicator of improved gut health and functionality. Beyond morphology, phytochemicals significantly modulate the gut microbiota. Their antimicrobial properties are often selective, suppressing pathogenic bacteria while sparing or promoting beneficial ones.<sup>31</sup> Daneshmand et al. (2015) demonstrated that garlic extract reduced the cecal colonization of *Clostridium perfringens*, the primary pathogen responsible for necrotic enteritis. By controlling such pathogens, medicinal plants reduce the production of toxins and inflammatory responses that damage the intestinal epithelium, thereby preserving gut integrity and function, which is intrinsically linked to the improvements in growth performance discussed earlier.<sup>32</sup>



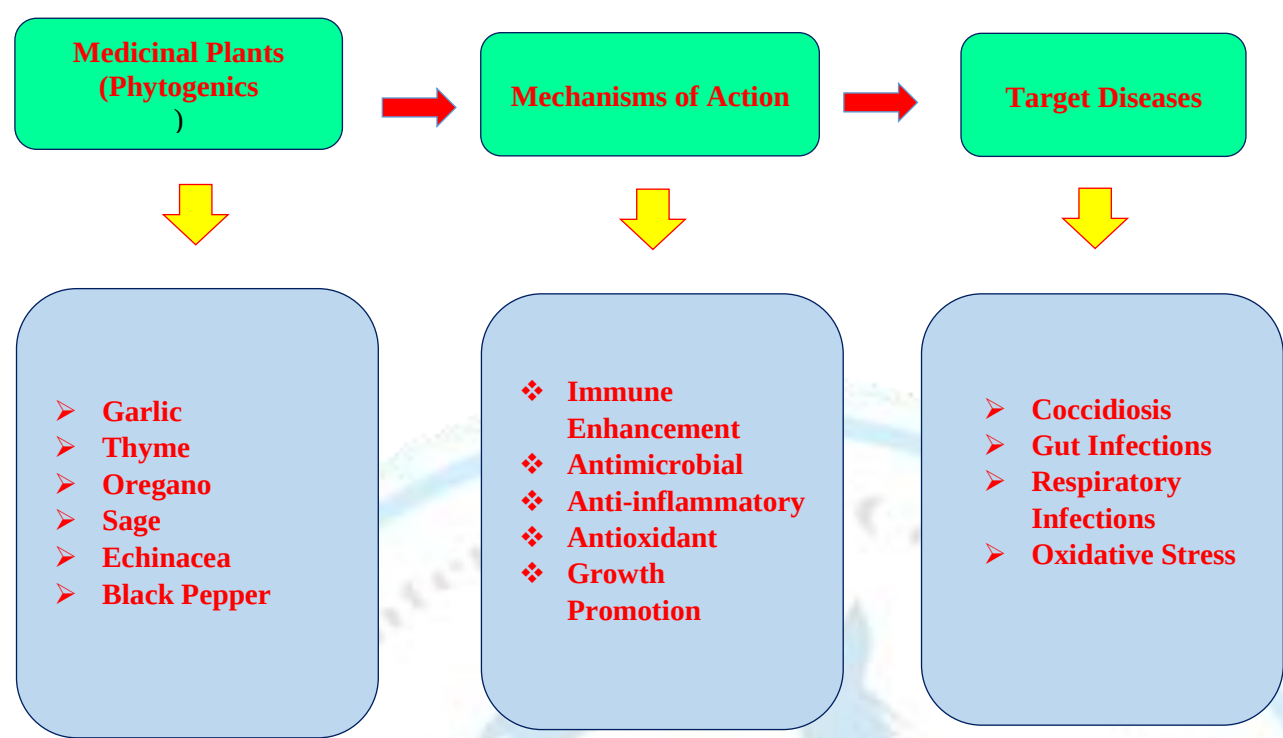


**Figure 1.** Schematic Diagram: Impact of Medicinal Plants on Poultry Diseasesn (27-39)

### Antibacterial and Antiviral Effects

The direct antimicrobial activity of plant-derived compounds is a cornerstone of their disease-preventive potential. Numerous *in vitro* and *in vivo* studies have demonstrated the efficacy of phytogenics against major bacterial pathogens in poultry, including *Salmonella Enteritidis*, *Escherichia coli*, and *Clostridium perfringens*. Salehi et al. (2016) reported that the essential oil of *Satureja khuzistanica* exhibited strong antibacterial activity against *Salmonella typhimurium*, reducing its colonization in the liver and spleen of challenged broilers. The mechanisms of action are diverse and include the disruption of the bacterial cell membrane by phenolic compounds (e.g., thymol, carvacrol), leading to leakage of cellular contents and cell death; inhibition of virulence factor production; and neutralization of bacterial toxins. Regarding viral diseases, which are not treatable with antibiotics, medicinal plants offer a supportive role by modulating the host's immune response; While they cannot cure viral infections like Newcastle Disease or Avian Influenza, they can reduce their impact. Studies on extracts of *Echinacea purpurea* and licorice root (*Glycyrrhiza glabra*) have indicated they can reduce the severity and mortality rates associated with viral challenges. Glycyrrhizic acid in licorice, for instance, has been shown to inhibit viral replication and possess direct virucidal properties, providing a layer of protection beyond immune stimulation.<sup>33</sup>

### Anticoccidial and Antiparasitic Properties

Coccidiosis, caused by protozoan parasites of the genus *Eimeria*, is one of the most prevalent and economically devastating diseases in poultry production. Widespread resistance to synthetic anticoccidial drugs has rendered many ineffective, creating a critical need for alternative control strategies. Numerous medicinal plants have demonstrated potent anticoccidial effects. Arab et al. (2006) demonstrated that dietary supplementation with garlic powder significantly reduced oocyst output in feces and intestinal lesion scores in broilers experimentally infected with *Eimeria* species.<sup>34</sup> Another study by Yakhkeshi et al. (2011) showed that a mixture of thyme and oregano essential oils was as effective as the ionophore drug monensin in controlling coccidial infection and improving the performance of infected birds. The active compounds in these plants, such

as allicin in garlic and thymol/carvacrol in thyme and oregano, are believed to work through multiple mechanisms: they may interfere with the sporulation of oocysts in the environment, damage the developmental stages (meronts, schizonts) within the intestinal mucosa, and possess anti-inflammatory properties that alleviate the tissue damage and bloody diarrhea caused by the parasite. This multifaceted action makes them effective natural agents for coccidiosis control, either alone or in rotation with chemical anticoccidials to slow and manage resistance development.<sup>35</sup>

**Antioxidant Status and Meat Quality**

Oxidative stress, resulting from an imbalance between the production of reactive oxygen species (ROS) and the body's antioxidant defenses, can impair bird health, decrease productivity, and lead to quality deterioration in meat and eggs. The inclusion of medicinal plants rich in natural antioxidants (e.g., polyphenols, flavonoids, tannins) in poultry diets enhances the endogenous antioxidant capacity of the birds. This is measured by increased activity of antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), and reduced levels of lipid oxidation markers like malondialdehyde (MDA). Hajati et al. (2014) found that adding pomegranate peel extract to broiler diets increased antioxidant enzyme activity and reduced lipid oxidation in breast meat during refrigerated storage.<sup>36</sup> Similarly, Zarei et al. (2015) reported that dietary barberry root extract improved the oxidative stability of raw and cooked chicken meat. This improvement in oxidative stability has two major benefits: it extends the shelf life of the meat, reducing spoilage and economic losses, and it provides health benefits to consumers by introducing natural antioxidants into the human diet. Furthermore, within the live bird, a better antioxidant status protects cellular integrity, supports immune function, and can mitigate the negative effects of environmental stressors.<sup>37</sup>

**Table 1- Summary Table: Effects of Specific Medicinal Plants on Poultry Diseases**

| Plant Name<br>(English) | Scientific Name         | Active Compounds  | Target Disease / Issue                                                              | Mode of Action                                                               | References |
|-------------------------|-------------------------|-------------------|-------------------------------------------------------------------------------------|------------------------------------------------------------------------------|------------|
| Thyme                   | <i>Thymus vulgaris</i>  | Thymol, Carvacrol | Respiratory infections, Bacterial infections ( <i>E. coli</i> , <i>Salmonella</i> ) | Strong antimicrobial, antispasmodic, immune booster                          | 29, 32     |
| Oregano                 | <i>Origanum vulgare</i> | Carvacrol, Thymol | Coccidiosis, Gut infections                                                         | Antiprotozoal (anti-coccidial), antibacterial, improves gut health           | 31, 32     |
| Garlic                  | <i>Allium sativum</i>   | Allicin           | Viral and bacterial infections, Intestinal worms                                    | Powerful immune booster, antiviral, antibacterial, antifungal, antiparasitic | 27, 39     |
| Turmeric                | <i>Curcuma longa</i>    | Curcumin          | Arthritis inflammation, Oxidative stress, Hepatitis                                 | Potent anti-inflammatory, antioxidant, hepatoprotective (liver protection)   | 27, 30     |



|              |                           |                            |                                            |                                                                    |        |
|--------------|---------------------------|----------------------------|--------------------------------------------|--------------------------------------------------------------------|--------|
| Sage         | <i>Salvia officinalis</i> | Phenolic compounds         | Digestive infections, Stress               | Antibacterial, anti-transpirant (reduces heat stress), antioxidant | 28, 38 |
| Echinacea    | <i>Echinacea purpurea</i> | Alkamides, Polysaccharides | Prevention of infectious diseases          | Stimulates and enhances immune system (macrophages, interferon)    | 27, 30 |
| Black Pepper | <i>Piper nigrum</i>       | Piperine                   | Digestive issues, Poor nutrient absorption | Increases digestive secretions, enhances nutrient absorption       | 39     |

Challenges and Considerations for Commercial Application

Despite the overwhelming evidence from experimental trials, the widespread adoption of medicinal plants in commercial poultry feed faces several significant challenges. The foremost issue is the lack of standardization. The concentration and profile of active compounds in a plant can vary dramatically due to factors such as geographical origin, climate, soil conditions, harvest time, and post-harvest processing methods (drying, extraction technique). This variability makes it extremely difficult to establish consistent, effective, and safe dosages for commercial feed production, leading to potential unpredictable results. Secondly, the practical aspects of incorporation pose difficulties. Some essential oils and extracts are volatile and can be lost during feed pelleting, which involves high temperatures and pressure. Their strong flavors and aromas can also affect palatability at higher concentrations, potentially reducing feed intake. Furthermore, while generally recognized as safe (GRAS), more extensive toxicological studies are needed to determine the long-term effects of certain plants, potential interactions with other feed additives (e.g., vaccines, enzymes), and to establish maximum residue levels in edible tissues. Therefore, future research must focus on standardizing extracts based on active compound content, developing protected delivery systems (e.g., microencapsulation, nanoemulsions) to enhance stability and mask odors, and conducting large-scale field trials to validate efficacy under diverse commercial conditions.

Conclusion and Future Perspectives

This systematic review consolidates a formidable body of global evidence, underscoring that medicinal plants and their derivatives are effective, multi-functional, and sustainable tools for advancing poultry production in the post-antibiotic era. They offer a viable natural strategy to enhance growth performance, improve gut health, potentiate immune competence, and combat major bacterial, viral, and parasitic diseases. Their application aligns perfectly with the "One Health" approach, aiming to reduce antibiotic use, mitigate AMR, and produce safer animal products for consumers. Looking forward, the future of phytogenics in poultry depends on a concerted effort to transcend from proof-of-concept studies to practical, reliable application. Research priorities must include: the bioassay-guided identification and standardization of active compounds; the development of advanced encapsulation technologies for targeted delivery; exploration of synergistic effects between different plants or with other additives like organic acids and prebiotics (creating synbiotic relationships); and a deeper understanding of their mechanisms of action at the molecular and genomic levels (e.g., through transcriptomics and metabolomics). By addressing the existing challenges through interdisciplinary collaboration, medicinal

plants can transition from a promising alternative to a mainstream, indispensable component of sustainable, profitable, and ethical poultry production systems worldwide.

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## EFFECTS OF HYDROALCOHOLIC EXTRACT OF *NIGELLA SATIVA* ON FERTILITY POTENTIAL IN TYPE 1 DIABETIC MICE

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### Abstract

Studies have shown that reproductive system performance is reduced under the influence of diabetes. In this research, the effect of hydroalcoholic extract of black seed (*Nigella sativa*) on sperm quality and fertility indices in male Syrian mice with type 1 diabetes was investigated. In this experimental study, 25 male Syrian mice with an average weight of  $26 \pm 2$  g were randomly divided into 5 groups of 5: Group 1: Control, receiving physiological serum. Group 2: Diabetic control, receiving physiological serum. Groups 3, 4, and 5: Diabetic mice treated with doses of 50, 100, and 200 mg/kg of hydroalcoholic black seed extract. Key sperm health indices were then evaluated. The use of hydroalcoholic black seed extract at doses of 100 and 200 mg/kg showed a significant increase in sperm count and the percentage of live sperm in the epididymal tail. Additionally, a reduction in sperm DNA damage, a decrease in sperm immaturity, a decrease in malondialdehyde levels, and an increase in testosterone levels were observed ( $P < 0.05$ ). In the examinations, the effect of hydroalcoholic black seed extract on improving sperm fertility indices and fertility rate in diabetic mice was observed.

**Keywords:** Diabetes, Black seed, Syrian mice, Fertility indices, Oxidative stress

### Introduction

Type 1 diabetes is a chronic autoimmune disease in which pancreatic beta cells are destroyed, and insulin production is halted. The lack of insulin leads to chronic hyperglycemia and disruptions in carbohydrate, fat, and protein metabolism. This condition is associated with numerous complications, including damage to reproductive organs [1]. In men with type 1 diabetes, reproductive system function can be affected. Chronic hyperglycemia, increased oxidative stress, hormonal disorders, and damage to testicular cells can reduce fertility. Type 1 diabetes can cause a decrease in sperm count, motility, and maturity, as well as an increase in the rate of sperm with damaged DNA [2, 3].

Infertility is a global issue affecting approximately 15% of couples, with male factors playing a role in half of these cases. Type 1 diabetes is recognized as one of the important and examinable factors in male infertility [4]. Black seed (*Nigella sativa*), a widely used plant in traditional medicine, contains the main active compound thymoquinone, which has antioxidant, anti-inflammatory, DNA-protective, and hormone-regulating properties [5]. This study investigates the effect of hydroalcoholic black seed extract on fertility indices, including sperm count and viability, sperm DNA damage, sperm maturity, malondialdehyde levels, and testosterone levels.

### Materials and Methods

In this experimental study, 25 male Syrian mice with an average weight of  $26 \pm 2$  g and age of 8 weeks were used. The mice were randomly divided into 5 groups of 5 [6]:

- Group 1: Control, receiving physiological serum.
- Group 2: Diabetic control, receiving physiological serum.



- Groups 3, 4, and 5: Diabetic mice treated with doses of 50, 100, and 200 mg/kg of hydroalcoholic black seed extract [7].

(The black seed extract was prepared from powder made by electric milling and 80% ethanol using a one-week maceration method. After filtering, it was concentrated using a rotary evaporator at 50 °C and 60 rpm, then dried under a hood and stored in a -60 °C freezer.)

Diabetes was induced by a single intraperitoneal injection of streptozotocin at 50 mg/kg, and the diabetic status of the mice was confirmed by measuring blood glucose. To confirm diabetes, 72 hours after STZ injection, blood glucose levels were measured via tail sampling. Mice with blood glucose greater than 250 mg/dL were considered diabetic [8]. After 30 days, the mice were anesthetized, and sperm health indices were evaluated.

Parameters examined:

- Sperm count: Using a hemocytometer slide and light microscope [9].
- Sperm viability: Using eosin-nigrosin staining [10].
- Sperm maturity: Using aniline blue staining (immature sperm, due to high histone content, appear dark blue-gray) [11].
- Sperm DNA damage: Using the acridine orange (AO) test and fluorescence microscope [12].
- Malondialdehyde (MDA) levels: Using the thiobarbituric acid reactive substances (TBARS) method by spectrophotometer [13].
- Testosterone levels: Using enzyme-linked immunosorbent assay (ELISA) kits [14].

Data analysis: Data were analyzed using SPSS software version 21, with one-way analysis of variance followed by Tukey's multiple comparison test. A P-value < 0.05 was considered the criterion for significance between groups.

## Results

Sperm count: Treatment with doses of 100 and 200 mg/kg of black seed extract led to a significant increase in total sperm count compared to the diabetic group, bringing the sperm count close to control group levels (Figure 1) (Table 1).

- Sperm viability: Administration of doses of 100 and 200 mg/kg significantly increased the percentage of live sperm compared to the diabetic group, restoring sperm viability to levels close to the control group (Figure 2) (Table 1).
- Reduction in sperm DNA damage: Treatment led to a reduction in sperm DNA damage in diabetic mice; this reduction was dose-dependent, with medium and high doses showing greater restorative effects and reducing DNA damage levels considerably close to the control group (Figure 2) (Table 2).
- Increase in immature sperm: Sperm maturity was also affected by treatment, decreasing in treated groups compared to the diabetic group. This improvement in sperm maturity was dose-dependent (Figure 2) (Table 2).
- Reduction in malondialdehyde (MDA) levels: Treatment had a remarkable effect on reducing malondialdehyde levels in diabetic mice. This reduction was clearly dose-dependent, reaching levels much lower than the untreated diabetic group and even close to the control group at medium and high doses (Figure 2) (Table 2).

• Increase in testosterone levels: While diabetes led to a decrease in this hormone, treatment with black seed extract caused a significant increase in testosterone levels in treated groups. This increase was also dose-dependent, with the highest treatment dose leading to the highest testosterone levels among all groups, including the control (Figure 2) (Table 2).

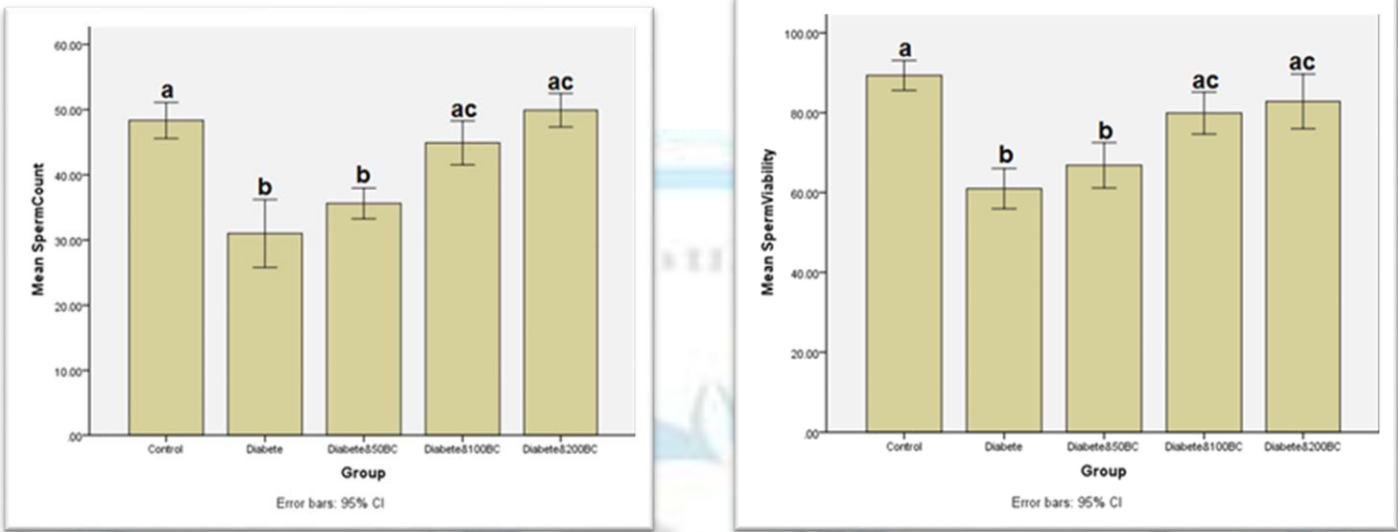


Figure 1. Figure 2.

Table 1.

| Group                 |                | Sperm Count | Sperm Viability |
|-----------------------|----------------|-------------|-----------------|
| Control               | Mean           | 48.3500     | 89.3467         |
|                       | Std. Deviation | 1.11014     | 1.51014         |
| Diabete               | Mean           | 31.0033     | 60.9833         |
|                       | Std. Deviation | 2.09682     | 2.02505         |
| Diabete&50Black Ceed  | Mean           | 35.6133     | 66.8133         |
|                       | Std. Deviation | .94500      | 2.28572         |
| Diabete&100Black Ceed | Mean           | 44.9100     | 79.9200         |
|                       | Std. Deviation | 1.35691     | 2.12113         |
| Diabete&200Black Ceed | Mean           | 49.9167     | 82.8333         |
|                       | Std. Deviation | 1.03968     | 2.75379         |
| Total                 | Mean           | 41.9587     | 75.9793         |
|                       | Std. Deviation | 7.73860     | 11.01181        |

Table 2.



| Report      |                |                 |               |         |             |
|-------------|----------------|-----------------|---------------|---------|-------------|
| Group       |                | SpermDNA Damage | SpermMaturity | MDA     | Testostrone |
| Control     | Mean           | 7.6267          | 8.2933        | 29.2033 | 1.8900      |
|             | N              | 3               | 3             | 3       | 3           |
|             | Std. Deviation | .54602          | .61493        | .71696  | .11533      |
| Diabetes    | Mean           | 20.0000         | 24.7033       | 39.6000 | 1.0200      |
|             | N              | 3               | 3             | 3       | 3           |
|             | Std. Deviation | 1.00000         | 1.12429       | 1.21655 | .05292      |
| Diabetes+LD | Mean           | 18.1833         | 20.0733       | 36.5167 | 1.2667      |
|             | N              | 3               | 3             | 3       | 3           |
|             | Std. Deviation | .74218          | .89226        | .50083  | .08505      |
| Diabetes+MD | Mean           | 7.7800          | 10.2667       | 23.0000 | 1.8533      |
|             | N              | 3               | 3             | 3       | 3           |
|             | Std. Deviation | .58924          | .64291        | 1.00000 | .16166      |
| Diabetes+HD | Mean           | 7.7500          | 8.7900        | 26.4167 | 2.5533      |
|             | N              | 3               | 3             | 3       | 3           |
|             | Std. Deviation | .66144          | .82831        | .74312  | .10504      |
| Total       | Mean           | 12.2680         | 14.4253       | 30.9473 | 1.7167      |
|             | N              | 15              | 15            | 15      | 15          |
|             | Std. Deviation | 5.83023         | 6.96751       | 6.46661 | .56257      |

Discussion

The results of this study showed that hydroalcoholic black seed extract could significantly reduce the adverse effects of type 1 diabetes on male mouse reproductive function and improve fertility indices. These findings align with the antioxidant, anti-inflammatory, and hormone-regulating properties of black seed [5].

Type 1 diabetes, by inducing hyperglycemia and oxidative stress, damages testicular cells and leads to reduced sperm count, viability, and disruptions in maturity and genetic health of sperm. Administration of black seed extract, especially at doses of 100 and 200 mg/kg, led to significant improvements in sperm count and viability. These effects are likely due to increased antioxidant enzyme activity and preservation of mitochondrial and sperm membrane health [15, 16].

Additionally, black seed extract resulted in a significant reduction in DNA damage and the percentage of immature sperm. The significant decrease in malondialdehyde levels in treated groups indicates reduced lipid peroxidation and repair of sperm cell membranes. Furthermore, administration of black seed extract caused a significant increase in testosterone levels in treated groups [17].

The 100 mg/kg dose of black seed extract was able to best modulate the negative consequences of diabetes and create notable improvements in various sperm health parameters. Although the higher dose (200 mg/kg) showed the greatest effectiveness, it did not differ markedly from the 100 mg dose.

### Conclusion

The findings of this research indicate that hydroalcoholic black seed extract, by modulating the destructive effects of diabetes, improving quantitative and qualitative sperm indices, reducing oxidative stress, enhancing hormonal function, and preserving sperm genetic health, can be considered as a complementary and natural therapeutic option in managing infertility caused by type 1 diabetes.

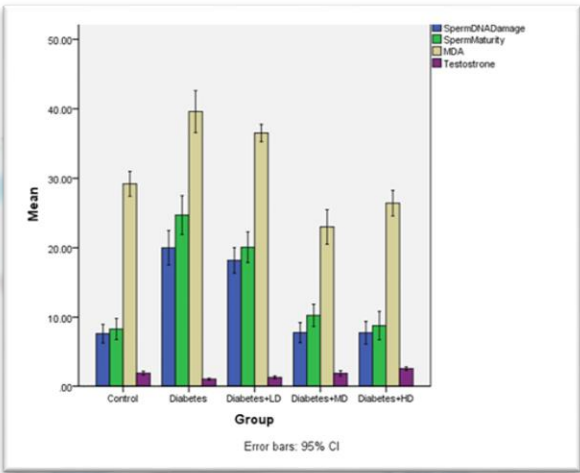


Figure 3.

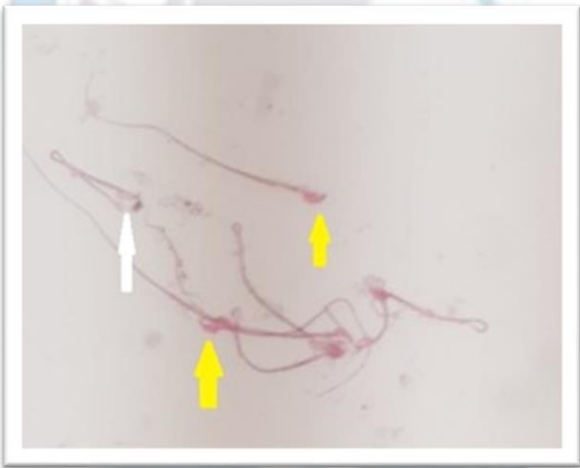


Figure 4. One live sperm with a bright head (white arrow) and several dead sperm with a red head (yellow arrow) in the diabetic group + black seed hydroalcoholic extract at a dose of 100 mg/kg.

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# A REVIEW ON IN VITRO AND IN VIVO STUDIES ON MEDICINAL PLANTS EXTRACTS EFFECTIVITY ON GASTROINTESTINAL PARASITES IN SMALL RUMINANTS

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## Abstract

Gastrointestinal helminthiasis mostly include nematodes and trematodes (flukes) of ruminant animals have increasingly become an important focus of research attention. Measures aimed at preventing clinical parasitic gastroenteritis generally attempt to avoid exposure of susceptible stock to heavily contaminated pasture during the latter part of the grazing season. This may be achieved by the strategic use of anthelmintics or grazing control, or both. Anthelmintic resistance is defined as a decrease in the efficacy of an anthelmintic against a population of parasites that is generally susceptible to that drug. The problem of anthelmintic resistance in gastrointestinal parasites of small ruminants is worldwide. Herbal medicines could probably be a good alternative to the current pharmaceutical compounds used in the market, to which drug resistance is increasing every day. To write an outline of materials and methods in this review study, online databases (Google Scholar, PubMed, Scopus, and ScienceDirect) were searched and published research articles (1992–2024) were gathered and reviewed. Overall, what we can learn from the studies results, is that herbal medicine's mechanism of action is not fully understood; if an analysis is performed to fill this hole, they would be able to suggest nontoxic and effective dosage determination methods, drug preservation, and value addition. Perhaps it is time for more research to be conducted on the use of medicinal plants and recognizing their pros and cons in the treatment of important diseases such as gastrointestinal helminth infections in small ruminants, so they can be officially used as new generation drugs in the treatment of these diseases before drug resistance to current commercial anthelmintic drugs reaches a point where they become useless.

**Keywords:** Anthelmintic, Drug resistance, Gastrointestinal parasites, Medicinal plants

## Introduction

Helminth parasites are a major cause of economic loss in small ruminants throughout the world. The major pathogenic helminth parasites of the gastrointestinal tract of ruminants are *Haemonchus spp.*, *Ostertagia spp.*, *Trichostrongylus spp.* (nematodes located in abomasum), *Nematodirus spp.*, *Trichostrongylus spp.*, *Cooperia spp.*, *Bunostomum spp.* (nematodes located in small intestine), *Oesophagostomum spp.*, *Chabertia spp.* (nematodes located in large intestine), *Fasciola hepatica* and *Dicrocoelium dendriticum* (liver flukes). Measures aimed at preventing clinical parasitic gastroenteritis generally attempt to avoid exposure of susceptible stock to heavily contaminated pasture during the latter part of the grazing season. This may be achieved by the strategic use of anthelmintics or grazing control, or both.<sup>1</sup>

Anthelmintic resistance is defined as a decrease in the efficacy of an anthelmintic against a population of parasites that is generally susceptible to that drug. This decrease in susceptibility is caused by an increase in the



frequencies of “resistance” gene alleles that result by selection through repeated use of an anthelmintic.<sup>2</sup> Reports of anthelmintic resistance in gastrointestinal nematodes have been made from South Africa, Australia, New Zealand, Malaysia, Spain, France, Denmark, UK, Brazil, and the United States.<sup>3, 4</sup> For many years, gastrointestinal nematodes (GIN) were cost-effectively controlled in small ruminants with broad-spectrum anthelmintic drugs. However, concurrently the international scientific community has raised concerns regarding the emergence of anthelmintic resistance in GIN as a global issue.<sup>5</sup>

On the other hand, infections caused by liver flukes (fascioliasis and dicrocoeliasis) are another important issue in small ruminant industry. The most important causative agent of fascioliasis is *Fasciola hepatica*, which is distributed in Europe, Africa, Asia, Oceania, and the Americas. Control of this disease depends on the strategic use of a wide range of antiparasitic drugs.<sup>6</sup> Among these drugs, triclabendazole is the only drug effective against the migrating juvenile stages in the liver parenchyma and the adult stage in the bile ducts. However, the use of triclabendazole causes drug resistance. Today, new effective treatments are used due to the emergence of drug resistance.<sup>7</sup> Dicrocoeliasis is a disease caused by *Dicrocoelium dendriticum*, a hepatic bile duct trematode that infects humans and a wide range of herbivores and is considered a major threat to livestock production in endemic areas.<sup>8</sup> The disease caused by this parasite is found throughout Europe, Asia, North Africa and North America.<sup>9</sup> There is currently no vaccine to prevent this disease, and therefore drug therapy is one of the best methods of combating. A wide range of strategies are used to control dicrocoeliasis; however, due to the emergence of resistance and the cost of treating small ruminants, alternative treatments are being sought.<sup>10</sup>

Overall, helminth infections are a major cause for reduced productivity in livestock, particularly those owned by the poor worldwide. Phytomedicine has been used for eons by farmers to treat parasitism and improve performance of livestock, and many modern commercial medicines are derived from plants. A variety of methods has been explored to validate the anthelmintic properties of such plant remedies, both in vivo and in vitro.<sup>11</sup> Herbs have nutritional and pharmaceutical properties that interact with one another polyvalently to increase the depth and breadth of the clinical effects compared with those seen in conventional pharmacological drugs.<sup>12</sup>

In the present study, the subject of discussion is a review of a number of studies conducted to conclude whether herbal compounds can be counted on as an effective alternative in the treatment of gastrointestinal parasites (nematodes and trematodes in this study) infections in small ruminants' industry.

### Materials and Methods

To conduct an in vitro study on medicinal plants extracts effectivity on Gastrointestinal nematodes, the following steps must be performed. In plant preparation formulation, extraction is the first crucial steps. The powdered plant materials should be prepared and kept in dried place where they are subjected to 99.9% methanol as a solvent using maceration technique.<sup>13</sup> Then, the mixture should be kept at room temperature for 48 hours. Then, the extract must be filtered using rotary evaporator to evaporate methanol at 60 °C with 60 RPM and kept overnight in hot air oven at 60 °C to obtain the pure crude extracts.<sup>14</sup>

To perform the egg hatch test, Eggs used in the test should be collected from feces of a donor sheep infected with desired helminths. The eggs are repeatedly washed with distilled water before initiation of the experiment. Extracts of the plants should be used as active treatment. Albendazole (99.3% pure standard reference) dissolved in DMSO (dimethyl sulfoxide) can be used as positive control while untreated eggs in water are used as negative control. Serial concentration of each desired plant extract in total volume of 0.5 ml in distilled

water is added to make concentrations of 2, 1, 0.5, 0.25, 0.125 and 0.0625 mg/ml together with water containing the eggs.<sup>15, 16</sup> The test tubes are then covered and kept in incubator at 27 °C for 48 h. The experiment should be repeated three times. Hatched larvae (dead or alive) and unhatched eggs are then counted under dissecting microscope at 40× magnification.<sup>16</sup>

To perform larval development test, eggs should be incubated at 27 °C for 24 h to obtain first stage larvae. Briefly, an aliquot of 1 ml containing 100–120 first stage larvae of desired nematodes is mixed with 5 g of feces from a sheep free of gastrointestinal nematodes and 6 serial concentrations of the plant extracts to make total volume of 7 cm<sup>3</sup> together with water containing larvae and volume of egg free of feces. Ivermectin (1%) dissolved in distilled water at 6 serial concentrations can be used as a positive control while eggs in parasite free feces are used as negative control. All samples are then incubated for 6 days at room temperature. At the end of 6th day, the wall of each cup containing the sample should be thoroughly rinsed with 10 ml of water to collect the larvae. Then, one drop of Lugol's iodine solution is added and all L3 stage larvae are counted under dissecting microscope at 40× magnification.<sup>16, 17</sup>

Parasites collection and preparation must be done next. Adult mature parasites of desired nematodes should be collected from naturally infected freshly slaughtered sheep. Immediately after death, both ends of the each collected organs (abomasum, small intestine and large intestine) should be ligated and transported to the laboratory. Then, they must be opened and their contents emptied into separate plastic buckets containing water. The parasites are recovered by passing the content through a sieve of 100 µm (a sieve of 300 µm diameter mesh is needed for *Trichostrongylus spp.*) diameter mesh and picked with wire loop. Adult mature helminths should be identified based on their morphological characteristics following the keys.<sup>14, 18</sup> Then, the parasites should be collected, washed, and kept in phosphate buffered saline (PBS) until used for in vitro test.<sup>14</sup>

In order to perform adulticide assay, a certain number of adult parasites should be used in the study. Four concentrations are employed for each plant extract. Ten actively moving worms are placed in each petri dishes filled with different concentrations of the methanolic extracts for each of the plant's materials in distilled water, and distilled water alone with ten actively moving parasites is used as the negative control group in total volume of 4 ml. Albendazole originally dissolved in DMSO and diluted in distilled water at the concentration of 1.25 mg/ml with ten parasites is used as a positive control. All tests should be repeated three times.<sup>14, 19</sup> The inhibitions of motility of the worms are used as indication of worm mortality or paralysis. Motility of worms is observed, and motile worms are counted at regular time intervals of 0-, 2-, 4-, 6-, and 8-hour post treatment. After 8 hrs, the plant extracts and albendazole are washed away, and the parasites are resuspended in PBS for 30 minutes for possible recovery of the parasite motility. Finally, the number of motile (alive) and immotile (dead) worms are counted under dissecting microscope and recorded for each concentration.<sup>14</sup>

In order to conduct an in vivo study on medicinal plants effectivity on Gastrointestinal nematodes, a mix of desired plants (Herbmix) can be used. Herbmix is added to the basal diets of meadow hay and a commercial concentrate per animal. animals are divided into four groups: infected animals (Inf), infected animals supplemented with Herbmix (Inf + Herb), uninfected control animals (C) and uninfected animals supplemented with Herbmix (C + Herb). Inf and Inf + Herb lambs are infected orally with approximately 5000 L3 larvae of certain nematode species susceptible to anthelmintics. Herbmix supplementation for Inf + Herb and C + Herb



begins on day 0 (D0). Feces are collected and eggs per gram (EPG) of feces are quantified on D0, D20, D32, D50 and D60. The mean EPG counts on each of mentioned five days, shows the effectivity rate of examined plants.<sup>20</sup>

To conduct an in vitro study on medicinal plants extracts effectivity on liver flukes, the powdered material of each plant should be soaked in ethanol three times (three to one), kept at room temperature for three days, so their extracts are extracted. After discarding the sediments, the supernatant should be placed at 40 degrees to concentrate, and the dry extracts must be kept in the refrigerator until use.<sup>21</sup>

In order to perform egg hatch test, bile ducts from sheep infected with *F. hepatica* and *D. dendriticum* should be collected from the slaughterhouse and transported to the laboratory in sterile vials. The bile should be transferred into tubes and allowed to settle for 30 minutes. The eggs are collected at the bottom of the tube and washed several times with normal saline. The eggs should be transferred to dark-colored vials containing normal saline and stored at 40 degrees. The eggs are then exposed to the extracts in 5 dilutions at 37 degrees for 24, 48, and 72 hours. The eggs should then be incubated at 28 degrees for 14 days and then examined under a microscope for the presence of miracidium.<sup>22</sup>

In order to perform adulticide test, adult *F. hepatica* and *D. dendriticum* should be collected from the livers of freshly slaughtered sheep and after washing with streptomycin, penicillin and lukewarm water, should be transported to the laboratory in sterile glass containers.<sup>23</sup> Then, 10 live and motile trematodes should be placed in each Petri dish containing RPMI culture medium and different concentrations of extracts. Phosphate buffer can be used as control group. Then, the Petri dishes should be examined at 4-hours intervals for 24 hours, in terms of parasite motility and the number of dead parasites. All tests should be repeated 3 times.<sup>24</sup>

In the in vivo trial, the oral administration experimentally liver flukes infected animals with doses of prepared extract per day for 3 consecutive days on the 63rd day post infection should be done to evaluate the anthelmintic effect of extract. A gradual decrease in fecal egg count (FEC) should be detected from the 1st day post treatment until reaching more FEC reduction by the 7th day post treatment.<sup>25</sup>

### Findings

As it was aforementioned, in vitro and in vivo Studies have been conducted on effects of medicinal plants on gastrointestinal nematodes which showed some promising results, especially on *H. contortus*. In an in vitro study conducted by Egualé et al. (2006), anthelmintic effects of crude aqueous and hydro-alcoholic extracts of the seeds of *Croton macrostachyus*, *Ekebergia capensis*, *Acacia nilotica* and *Terminalia schimperiana* on eggs and adult *H. contortus* were compared with albendazole. Major classes of secondary metabolites found in the seeds of the four mentioned medicinal plants include polyphenols, cyanogenic glycosides, saponins, phytosteroides, phenolic glycosides, flavonoids, tannins, alkaloides, antraquinone glycosides and overall findings of the study revealed that extracts from *C. macrostachyus* and *E. capensis* have potential anthelmintic effect.<sup>26</sup>

In another in vitro study conducted by Kumarasingha et al. (2016), it was discovered that two of five extracts dilutions from *Picria fel-terrae* had a significant inhibitory effect (at concentrations of 3–5 mg/ml) on the motility and development of *H. contortus* larvae in comparison with monepantel. Although the two extracts originated from the same plant, they displayed different levels of inhibition on motility and development.<sup>27</sup> In an in vivo study conducted by Váradyová et al. (2017), the effect of dietary supplementation with a mixture of selected medicinal herbs (Herbmix) contained medicinal herbs common in central Europe was examined against experimentally infected lambs with *H. contortus*. It was found out that the mixture of the medicinal herbs slow

the dynamics of infection and improve the production indicators of the animals.<sup>20</sup> An in vitro study conducted by Mumed et al. (2022), in which anthelmintic activity of methanolic Crude Extracts of three medicinal plants (*Croton macrostachyus* leaves, *Nicotiana tabacum* leaves, and *Zingiber officinale* rhizome) against adult *H. contortus* was tested. The presence of secondary metabolites such as tannins, flavonoids, steroids, and terpenoids in all extracts was revealed. The study revealed that the extracts from three plants have potential anthelmintic effect.<sup>14</sup>

Many studies have also investigated the anthelmintic effects of medicinal plants on small ruminant liver flukes and promising results were also concluded. Alvarez-Mercado et al. (2015) investigated the effects of extracts of fifteen medicinal plants on the adult stage of *F. hepatica* in comparison with triclabendazole in vitro. It was concluded that five tropical plant extracts, which included plant extracts of *Lantana camara*, *Bocconia frutescens*, *Piper auritum*, *Artemisia mexicana* and *Cajanus cajan* had promising anthelmintic effects against adult *F. hepatica* due to their secondary metabolites such as alkaloids, terpenes, tannins or flavonoids.<sup>28</sup> In an in vitro and an in vivo study conducted by Kandil et al. (2018), it was discovered that *Moringa oleifera* methanolic extract shows inhibitory activity on the vitality and hatchability of *F. hepatica* eggs in vitro. In the in vivo trial, the oral administration of *F. hepatica* experimentally infected rabbits with prepared extract was carried out. A gradual decrease in fecal egg count (FEC) was detected from the 1st day post treatment until reaching 100% FEC reduction by the 7th day post treatment.<sup>25</sup> Also, in a study conducted by Ghaffari et al. (2021), results were obtained indicating that ginger (*z. officinale*) hydroalcoholic extract contains flavonoids, saponins, tannins, terpenoids, and phenol. The mentioned compounds had lethal effect on *F. hepatica* miracidium in comparison with triclabendazole.<sup>29</sup> In another study conducted by Allahyari et al. (2024), essential oils of lavender (*Lavandula angustifolia*) contained borneol, linalool and alpha-pinene and carob (*Ceratonia siliqua*) contained nonadecane, 1,2-benzenedicarboxylic acid, dibutyl ester, heneicosan, eicosene, 1,2-benzenedicarboxylic acid and b-cedrene were compared with triclabendazole and it was found out that they had anti-hatching and lethal effects on the egg and adult stages of *F. hepatica* at different exposure times and different concentrations.<sup>21</sup> Medicinal plants secondary metabolites which are most effective against gastrointestinal helminth infection according to studies and phytochemical screening methods to detect them are mentioned in Table 1.

**Table 1.** Medicinal plants secondary metabolites and phytochemical screening methods.<sup>14</sup>

| Secondary metabolites | Phytochemical test   | Chemicals/method                                                 | Indicator                               |
|-----------------------|----------------------|------------------------------------------------------------------|-----------------------------------------|
| Alkaloids             | Mayer's test         | Mayer's reagent                                                  | Creamy precipitate                      |
| Flavonoids            | Shinoda's test       | NaOH+HCl (dilat.)                                                | Intense yellow/colorless                |
| Glycosides            | Ferric chloride test | 20%KOH + %5FeCl <sub>3</sub>                                     | Black precipitate                       |
| Phenolic compounds    | Ferric chloride test | 1%FeCl <sub>3</sub> + 1 ml of K <sub>3</sub> Fe(CN) <sub>6</sub> | Bluish green color                      |
| Phlobatannins         | HCl test             | 2 ml of 1%HCl                                                    | Deposition of a red precipitate         |
| Saponins              | Frothing test        | Heat                                                             | Formation of persistent honeycomb froth |
| Steroids              | Salkowski's test     | CHCl <sub>3</sub> +H <sub>2</sub> SO <sub>4</sub> (conc.)        | Reddish brown color                     |
| Terpenoids            |                      |                                                                  | Formation of yellow color               |
| Tannins               | Ferric chloride test | 0.1%FeCl <sub>3</sub> (dilat.)                                   | Brownish green or blue black color      |

Discussion and Conclusion

The problem of anthelmintic resistance, toxicity, and the increasing concern over the presence of drug residues in animal products has led to a renewal of interest in the use of plant-based drugs. Plant materials



evaluated in the studies had been identified from various sources to serve as anthelmintic agents. The in vitro tests using free living stages of parasitic nematodes offer a means of evaluating the anthelmintic activity of new plant compounds. In vitro techniques are preferred to in vivo methods due to their low cost, simplicity, and rapid turnover. In vitro studies have a key role in initial screening however, sounds like these studies provide no information of bioavailability, toxicity, and in vivo efficacy of tested extract/compound. Hence, in future more in vivo studies by using suitable animal models should be carried out to understand the pharmacokinetics and pharmacodynamics of the tested extract/compound. Most of the in vivo studies provide no evidence about toxicity and mechanism of action of the medicinal plant/compound, this is the most neglected aspect and strongly suggested to researchers to evaluate toxicity levels and pharmacological action of the tested plant/compound. Overall, what we can learn from the studies results, is that herbal medicine's mechanism of action is not fully understood; if an analysis is performed to fill this hole, they would be able to suggest nontoxic and effective dosage determination methods, drug preservation, and value addition.

In conclusion, parasitic infections is one of the major hindrances not only to the development of economy but also in increasing the cost of production in small ruminant industry. Cost of chemicals to control gastrointestinal parasites, development of resistance against these chemicals and presence of residues of these chemicals in livestock are major problems facing the sheep industry. Thus, according to the results obtained from many studies, the use of herbal medicines may be a good alternative to treat parasitic infections. Although traditional medicine information is diverse, no major steps have been taken to interpret and promote its use for clinical purposes, so perhaps it is time for more research to be conducted on the use of medicinal plants and recognizing their pros and cons in the treatment of important diseases such as gastrointestinal helminth infections so that they can be officially used as new generation drugs in the treatment of these diseases before drug resistance to current commercial anthelmintic drugs reaches a point where they become useless.

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# THE EFFECT OF CHITOSAN EDIBLE COATING WITH CINNAMON AND THYME ESSENTIAL ON PHYSICOCHEMICAL AND SENSORIAL PROPERTIES FRESHWATER CRAYFISH AT REFRIGERATOR TEMPERATURE

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## Abstract

The aim of this study was to investigate the effects of three chitosan variables with concentrations of (5.5, 1.0, 1.2%) and thyme essential oil (0.5-1%) and cinnamon essential oil (0.5-1%) on freshwater crabs after 7 days of storage at 4 °C in a refrigerator. Chitosan solutions were prepared in 1% acetic acid solution and mixed with 3% bovine gelatin solution at 40 °C. After homogenizing the bovine gelatin and chitosan solutions at a ratio of 30 to 70 at 45 °C, and finally thyme and cinnamon essential oil at concentrations of 0.5 and 1% were added to the prepared solution. All tests were performed in triplicate in a significant factorial experiment based on a completely randomized design. Analysis and evaluation (ANOVA) was performed using SPSS 21 statistical software at a probability level of 5% ( $p < 0.05$ ) and Duncan's multiple range test with a significant factorial at a probability level of 5% to confirm the existence of a difference between the means. The combination of 1% chitosan and 1% thyme and cinnamon essential oil mixture had a significant effect on quality retention ( $p < 0.05$ ). The combination of 1% chitosan coating and 3% gelatin with 1% thyme and cinnamon essential oil maintained the weight of freshwater crabs during shelf life and, as a result, had a significant effect ( $p < 0.05$ ) in preventing weight loss. The least weight loss was related to the treatments of 1% chitosan and 1% thyme and cinnamon mixture. Also, the highest moisture and the best sensory analysis were related to this percentage concentration of edible coating.

**Keywords:** Freshwater crab, Edible coating, Chitosan, Thyme and cinnamon essential oils, Shelf life

## Introduction

Nowadays, most researchers have focused on the use of antimicrobial compounds to prevent the growth of microbes and thus maintain the quality, freshness and safety of food. On the other hand, consumers' desire to consume natural antimicrobial products is increasing day by day, so it is thought that biodegradable coatings with natural compounds will be highly effective in the food industry to improve quality and attract consumers' attention. Despite the proven benefits of such coatings, the use of complementary methods to overcome the shortcomings of such coatings is felt.<sup>1</sup> In recent years, significant progress has been made in the field of packaging with the aim of improving the physical, chemical, microbial and organoleptic properties of food products. Active packaging uses a series of chemical and physical compounds in the coating and packaging film to improve the properties of the coating and increase the shelf life of the food. Edible coatings also have nutritional value.<sup>2</sup>

Essential oils are a class of volatile oils that are plant-based and contain heavy, volatile, and fatty chemical compounds. In essence, the pleasant smell of the organs of such plants or their taste is due to the presence of these essential oils. Various plant extracts are also used for antimicrobial packaging purposes.

The main objective of this research is to provide an impermeable layer of edible flavor on the product. Food product coatings (edible coatings) provide them with various benefits from the point of view of health, sensory and economic aspects. Some of the most important of these benefits are as follows: Due to their biodegradability, unlike synthetic films, they do not cause environmental pollution. Methods for preserving and increasing the freshness of freshwater crayfish, significant results have been obtained indicating progress in the field of the aforementioned goal. However, a point that has not been mentioned in any scientific source so far is the simultaneous use of two essential oils of thyme and cinnamon in an edible coating of chitosan and gelatin for preserving freshwater crayfish at refrigerator temperature.

### Materials and Methods

The required amount of *Zataria* thyme and cinnamon were purchased from a medicinal plant store in the spring and their scientific names were confirmed by a reputable medicinal plant laboratory. Their essential oils were prepared by hydrodistillation using a Clevenger apparatus, and the essential oils of cinnamon and thyme were extracted by water distillation. Since essential oils are sensitive to light, oxygen, and temperature, and their compounds change and evolve under such conditions, the extracted essential oils were immediately transferred to a dark glass bottle in a seal and stored in the refrigerator until use.<sup>3</sup>

In this study, freshly caught farmed freshwater crayfish samples were tested. Their average weight was  $29 \pm 5$  grams. 300 samples were taken from freshwater crayfish by random sampling method. The crayfish samples were kept at 0-4 °C (ice powder) and placed in an insulated container. After necessary examinations, healthy and uniform samples were selected, washed, skinned, and decapitated, and were divided into 35 groups including 34 treatment groups and 1 control group. Each freshwater crayfish was completely coated based on the type of treatment (A: chitosan coating with a concentration of 0% + 3% gelatin (pure coating); B: chitosan coating with concentrations of 0, 0.5, 1, 1.5, and 2% + 3% gelatin + thyme essential oil with concentrations of 0.5 and 1%; C: chitosan coating with concentrations of 0, 0.5, 1, 1.5, and 2% + 3% gelatin + cinnamon essential oil with concentrations of 0.5 and 1%; D: chitosan coating with concentrations of 0, 0.5, 1, 1.5, and 2% + 3% gelatin + thyme essential oil with concentrations of 0.5 and 1%; D: chitosan coating with concentrations of 0, 0.5, 1, 1.5, and 2% + 3% gelatin + thyme essential oil with concentrations of 0.5 and 1%, + cinnamon essential oil with concentrations of 0.5 and 1%) and then stored in polyethylene containers at refrigerator temperature. After 7 days of storage, 1 treatment was repeated every day and each treatment was repeated 3 times. In each repetition, 8 coated freshwater crab samples were tested. To form a coating on the surface of the freshwater crab samples, the samples were refrigerated for 2 hours and then packaged in polypropylene containers and stored at 4 °C and evaluated on the seventh day.<sup>4</sup>



### A: pH Measurement

A pH meter was used to determine the pH of coated long-necked crab samples according to Iranian standard No. 3195. The pH of coated freshwater long-necked crab samples was measured on the seventh day of storage.<sup>5</sup>

### B: Moisture Measurement

For the moisture test, 5 grams of coated long crab sample was taken and after placing it in an oven at 105 °C for 24 hours, it was measured according to Iranian standard number 8435 and the moisture percentage was calculated using the following formula:

$$\text{Moisture percentage} = \frac{(M0 + M1) - (M2 + M1)}{M3} \times 100$$

M0 = Sample weight before drying, M1 = Empty container weight

M2 = Sample weight after oven M3 = Sample weight before oven

### C: Sensory Analysis

In sensory evaluation, the characteristics of appearance, texture, taste, odor, then taste and overall acceptability of the samples were evaluated. Sensory analysis was performed using a 10-point method using a 10-point hedonic test and by experienced people according to the sensory parameters of odor, taste, flavor, texture and overall acceptability on the seventh day of storage and after cooking the samples by microwave (LG) for 10 minutes at a power of 700 watts. The score of each characteristic was graded as follows: (9-10) for excellent, (7-8) for good, (5-6) for fairly good to acceptable, (3-4) for poor and (1-2) for very poor. The order of using the samples was the same for all judges. It should be noted that due to the large number of treatments, the sensory test was only evaluated on the last day of storage.<sup>3</sup>

### D: Ash Measurement

To measure ash, dry ashing was used according to standard number 11143. For this purpose, 5 grams of coated freshwater crab samples were weighed and heated in an electric furnace at 550-600 degrees Celsius for 6 hours until a light gray color was obtained, and the ash was calculated according to the following formula:

$$\text{Ash percentage} = \frac{M2 - M1}{M1} \times 100$$

M1 = Weight of empty container , M2 = Weight of container and sample at the end of the work

### E: Test of Weight Loss and Weight Change of Freshwater Crayfish

Weight loss and weight change of crayfish were determined using a scale between the first and seventh days and then expressed as a percentage. The weight of the coated crayfish at the beginning of the storage period

was measured using a scale with an accuracy of 0.001 grams. Then, the weight of the coated crayfish was also measured after seven days. The amount of weight difference on the first day and the test day was calculated from the following equation:<sup>3</sup>

$$WL = \frac{W_i - W_t}{W_i} \times 100$$

Wi is the initial weight of the coated crayfish on the first day,  
Wt is the weight of the coated crayfish on the seventh day.

Results

1- pH

pH is a very important chemical factor in meat, especially marine products, including freshwater crab, which has a specific range according to the Iranian National Standard. According to the Iranian National Standard, pH should not be higher than 8, because microorganisms grow better at higher pH and have a longer shelf life. The results of measuring the pH values of the treatments on the seventh day of storage are shown in Table 1. As is clear, there is a significant difference between the pH of the different samples and the control, and the pH of all samples coated with essential oil is within the standard range. The pH of all treatments after 7 days of storage was within the standard range, i.e. less than 6.7. The highest pH measured was for sample 1, i.e. the control.

**Table 1.** Results of pH measurement of coated freshwater crab samples at refrigerator temperature

| Treatments                                 | Amount pH after 7 days of storage |                             |                         |                         |                             |
|--------------------------------------------|-----------------------------------|-----------------------------|-------------------------|-------------------------|-----------------------------|
| Chitosan concentration%                    | 0                                 | 0.5                         | 1                       | 1.5                     | 2                           |
| Gelatin+Essential oil concentration%       |                                   |                             |                         |                         |                             |
| Control (without coating and essence)      | ±0.05 <sup>AB</sup><br>7.66       | -                           | -                       | -                       | -                           |
| Pure coating (3% gelatin + chitosan)       | -                                 | 6.5±0.09 <sup>ab</sup>      | 6.45±0.18 <sup>ab</sup> | 6.31±0.1 <sup>ab</sup>  | 0.4 <sup>ab</sup><br>6.45±  |
| 3% gelatin + 0.5% thyme                    | ±0.22 <sup>ab</sup><br>6.68       | 6.4±0.19 <sup>ab</sup>      | 6.27±0.21 <sup>ab</sup> | 6.23±0.13 <sup>ab</sup> | 0.18 <sup>ab</sup><br>6.48± |
| 3% gelatin + 1% thyme                      | ±0.2 <sup>ab</sup><br>6.65        | 6.3±0.11 <sup>ab</sup>      | 6.25±0.13 <sup>ab</sup> | 6.41±0.19 <sup>ab</sup> | 0.12 <sup>ab</sup><br>6.58± |
| 3% gelatin +0.5% cinnamon                  | ±0.21 <sup>ab</sup><br>6.66       | 6.4±0.09 <sup>ab</sup>      | 6.28±0.17 <sup>ab</sup> | 6.34±0.1 <sup>ab</sup>  | 6.5±0.2 <sup>ab</sup>       |
| 3% gelatin +1% cinnamon                    | ±0.18 <sup>ab</sup><br>6.62       | 6.31±0.1 <sup>ab</sup>      | 6.25±0.03 <sup>ab</sup> | 6.4±0.11 <sup>ab</sup>  | 0.12 <sup>ab</sup><br>6.58± |
| 3% gelatin + 0.5% thyme + cinnamon mixture | ± 0.14 <sup>ab</sup><br>6.53      | 0.09 <sup>ab</sup><br>6.31± | 6.25±0.13 <sup>ab</sup> | 6.36±0.04 <sup>ab</sup> | 0.21 <sup>ab</sup><br>6.65± |
| 3% gelatin + 1% thyme + cinnamon mixture   | ±0.12 <sup>ab</sup><br>6.43       | 0.19 <sup>ab</sup><br>6.28± | 6.22±0.16 <sup>ab</sup> | 6.37±0.11 <sup>ab</sup> | 0.03 <sup>ab</sup><br>6.6±  |

Different uppercase and lowercase letters indicate significant differences (p<0.05) between treatments and the control sample.

2- Moisture



The results of measuring the moisture content of freshwater crab samples showed that immersing the samples coated with 1% chitosan and a mixture of 1% thyme and cinnamon essential oils had a greater effect on maintaining the weight of the sample. At concentrations of less than 1% chitosan, moisture loss was observed. The results of this study indicate that freshwater crab coated with 1% chitosan concentrations had more moisture than those with higher and lower chitosan percentages.

3- Sensory Analysis

The results of sensory evaluation showed that treatments containing chitosan and gelatin coating in combination with thyme and cinnamon essential oils with carvacrol and cinnamaldehyde compounds were significantly ( $p \leq 0.05$ ) more acceptable than the control sample (number one) in all sensory aspects (taste, aroma, color, texture and overall acceptability) during seven days of storage at refrigerated temperature. The lowest sensory score of the samples in terms of overall acceptability was for the control treatment (one). Overall, during seven days of storage at refrigerated temperature, the highest score was for the treatment (twenty-one) with 1% chitosan coating, 3% gelatin, and a 1% combination of thyme and cinnamon essential oils ( $p \leq 0.05$ ).

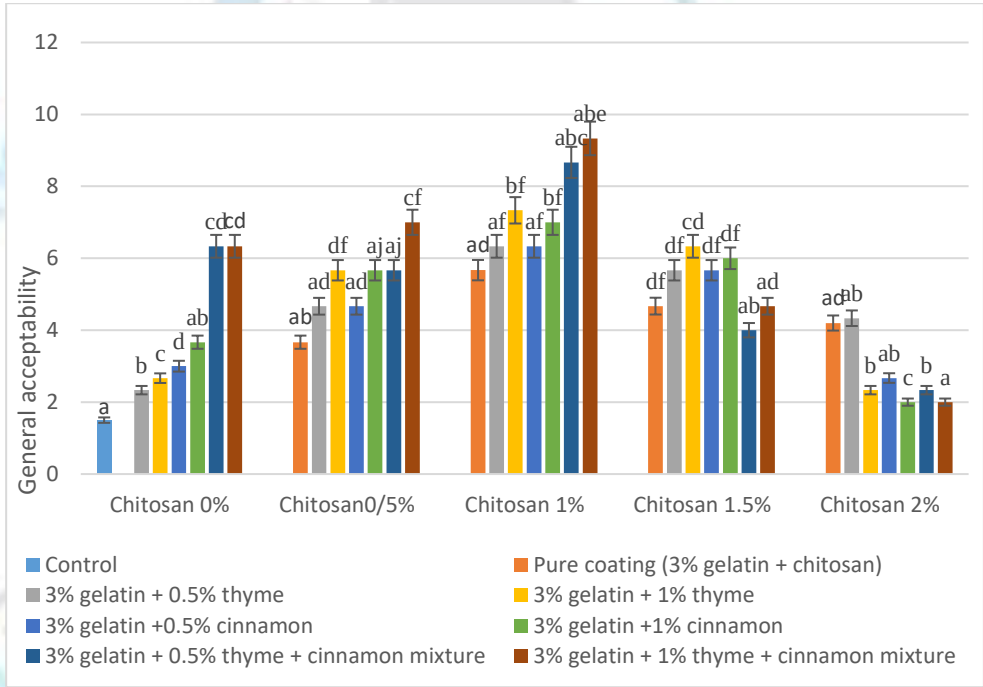


**Figure 1:** Results from the overall acceptability assessment of coated freshwater crab samples stored at refrigerated temperature for seven days.

4- Ash

Ash can be a factor for the moisture content of the product. By measuring the ash content, the amount of ash changes after 7 days was evaluated. The results of ash measurement of the samples showed that the ash of all samples was within the standard range. The lowest ash content was related to the concentration of 1% chitosan and 3% gelatin with 1% thyme and cinnamon essential oils. As the percentage of chitosan and essential oils increases, the ash also has a decreasing trend, with the lowest ash content being related to the concentration of 1% chitosan and 3% gelatin with 1% thyme and cinnamon essential oils.

5- Weight Loss and Weight Change

The graph shows the weight loss of treated freshwater crab samples after seven days of refrigeration. What can be concluded from the weight loss results is that the lowest weight loss was related to the treatment with a coating based on 1% chitosan-3% gelatin-1% thyme and cinnamon mixture. This reduction can be due to the good barrier properties of the gelatin and chitosan polymer matrix to water vapor transmission, which was able to reduce water transmission in freshwater crab muscle well. This property can be attributed to the appropriate binding strength of chitosan in aqueous solutions. The water vapor permeability properties of chitosan, like other carbohydrate polymers, depend on the relative polarity of this polymer, and more polar coatings have a lower tendency to transmit water vapor due to fewer pores.<sup>6</sup> The lowest weight loss was associated with the treatment with a coating based on 1% chitosan-3% gelatin-1% thyme and cinnamon mixture, which was in agreement with the results of Jeon *et al.* (2002), Mohan *et al.* (2012).<sup>6,7</sup>

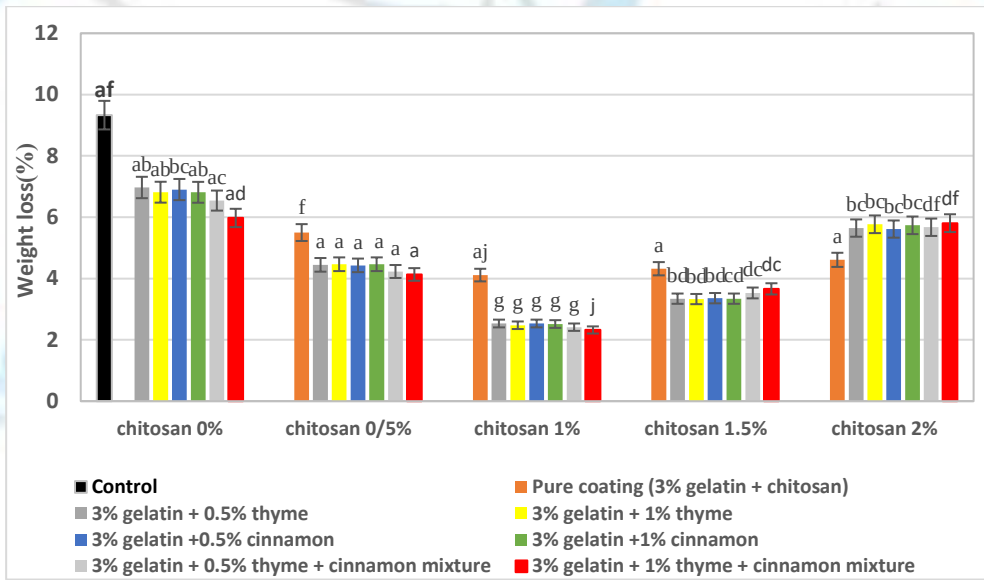


**Figure 2:** Results from measuring weight loss of coated freshwater crab samples during seven days of storage at refrigerator temperature.

Discussion and Conclusion

pH can be considered a suitable factor for chemical spoilage of freshwater crayfish, which is usually associated with the decline in the quality of freshwater crayfish, which was consistent with the results of Jeon *et al.*, (2002).<sup>6</sup> Also, several studies, including Fan *et al.* (2009),<sup>8</sup> Latou *et al.* (2014),<sup>9</sup> Yingyuad *et al.* (2006),<sup>10</sup> Vasconez *et al.* (2009),<sup>11</sup> Seabra *et al.* (2011),<sup>12</sup> have reported the property of chitosan in reducing pH at refrigerator temperature. According to the results of pH evaluation, all treatments were within the standard range, which indicated that thyme and cinnamon essential oils with carvacrol and cinnamaldehyde compounds did not have an adverse effect on the chemical properties of freshwater crayfish.

The results of this study show that freshwater crabs coated with 1% chitosan concentrations have higher moisture content than those with higher and lower chitosan concentrations. Because chitosan maintained the moisture content of the samples at this concentration, but with increasing chitosan concentration from 1%, the moisture content of freshwater crabs decreased compared to the control sample, which could be due to increased



microbial spoilage, lipolysis, and also loss of water and other nutrients. The changes in moisture content showed an increasing and then decreasing trend. This means that increasing the percentage of essential oils maintained the moisture content of the coated samples with a concentration of 1% chitosan and 3% gelatin with a mixture of thyme and cinnamon essential oils at 1%. By increasing the concentration of chitosan from 1% and essential oils from 1%, we encountered a decrease in moisture content, which could be due to changes in pH and water retention power, which removes some water-soluble nutrients from the muscles, which subsequently leads to dehydration of the meat during storage, which was in agreement with the results of Emire and Gebermariam (2010).<sup>13</sup>

Treatments with 1% chitosan coating, 3% gelatin and 1% thyme and cinnamon mixture were in a better condition than other samples in terms of smell, taste, flavor, texture and overall acceptability, and as the percentage of chitosan increased from 1%, the quality of the samples decreased and their acceptability was less. Because the firmness of the freshwater crab tissue decreased and when eaten, it had a slimy and jelly-like state, which was consistent with the results of Fan et al. (2009),<sup>8</sup> Ojagh et al. (2010).<sup>14</sup> Researchers explained the mechanism of action of chitosan to reduce fat oxidation in meat foods, its role as a scavenger of metal ions such as iron ions, which are responsible for the initiation of lipid peroxidation and the initiation of chain reactions and lead to the deterioration of the taste and smell of foods.<sup>15</sup>

According to the results of this study, it can be stated that:

1. The use of thyme and cinnamon essential oils in the coating formulation maintains the pH of the coated products within the standard range. However, in the control samples, due to the lack of coating, the pH increased.
2. Increasing the chitosan concentration to more than 0.5% had a greater effect on microorganisms than the control treatment.
3. Simultaneous use of thyme and cinnamon essential oils did not have a negative effect on the sensory properties of the product.
4. Treatments containing thyme and cinnamon essential oils had less weight loss than the control treatment.
5. By adding cinnamon and thyme essential oils to the coating formulation, an aromatic impermeable layer was created on the freshwater long-necked crab product in order to improve the coating properties and increase the shelf life of this food.
6. According to the results of chemical and sensory tests, it can be concluded that the concentrations of cinnamon and thyme essential oils are a suitable alternative to chemical preservatives because they inhibit microbial growth.
7. Considering that cinnamon and thyme essential oils are considered healthier substances than chemical preservatives, by replacing them with chemical preservatives, in addition to using their nutritional value and avoiding the harms of chemical preservatives, it is possible to produce a new functional product.

Considering the risks of chemical preservatives for consumers and also considering the high consumption of marine products including freshwater crab, replacing them with natural preservatives in part in the formulation of the coating seems necessary. Essential oil compounds extracted from cinnamon and thyme are one of these natural compounds, and in this study, its antimicrobial effect on freshwater crab was investigated. Therefore, it can be claimed that chitosan coating containing cinnamon and thyme essential oils is a completely natural and functional new product that, while eliminating the use of chemical preservatives, can replace chemical

preservatives and maintain the shelf life of the product to an acceptable level and be introduced as a new product called freshwater crab with high nutritional properties and nutritional value.

**Conflict of interest:**The authors declare no conflict of interest regarding this study.

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## IN VITRO ANTIMICROBIAL ACTIVITY OF MILKZERO<sup>®</sup> HERBAL EXTRACT AGAINST *STAPHYLOCOCCUS AUREUS* ISOLATED FROM BOVINE MASTITIS

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### Abstract

Bovine mastitis caused by *Staphylococcus aureus* (*S. aureus*) is a major challenge for dairy production. Growing antimicrobial resistance and consumer concerns about antibiotic residues highlight the need for natural alternatives. This study evaluated the in vitro antimicrobial activity of MILKZERO<sup>®</sup>, an herbal formulation of *Nigella sativa* and *Mentha piperita*, against *S. aureus* isolates from bovine mastitis. Hydro alcoholic extract of *N. sativa* and aqueous extract of *M. piperita* (10% w/v each) were combined. Seventy isolates were obtained from mastitic milk samples. MIC was determined using CLSI broth microdilution method with 0.5 McFarland suspensions. Complete inhibition was observed in 37.5% of isolates at 1:1 dilution (50 mg/ml) and 21% at 1:2 dilution (25 mg/ml). Overall, 61.5% of isolates were susceptible at tested concentrations. MILKZERO<sup>®</sup> exhibited promising antimicrobial potential against mastitis-associated *S. aureus*, supporting further in vivo evaluation for sustainable mastitis management.

**Keywords:** Bovine mastitis, *Staphylococcus aureus*, Medicinal plants, *Nigella sativa*, *Mentha piperita*, Antimicrobial activity

### Introduction

Bovine mastitis, defined as mammary gland inflammation, remains the most economically devastating disease in dairy cattle worldwide, causing substantial losses through reduced milk production, impaired milk quality, increased veterinary costs, and premature culling<sup>1</sup>. The global economic impact exceeds billions of dollars annually, affecting both individual farm profitability and the broader dairy industry<sup>2</sup>.

*Staphylococcus aureus* represents one of the most clinically significant mastitis pathogens, responsible for both acute clinical and chronic subclinical infections. This pathogen demonstrates remarkable persistence in mammary tissue, primarily due to its biofilm-forming capacity and ability to invade epithelial cells, making it particularly challenging to eliminate with conventional therapy<sup>3, 4</sup>. The prevalence of *S. aureus* in mastitis cases varies globally, with isolation rates ranging from 17-57% in clinical cases<sup>5</sup>.

Traditional antibiotic therapy faces increasing limitations, including antimicrobial resistance development, extended milk withdrawal periods, consumer safety concerns, and regulatory restrictions<sup>6, 7</sup>. Antibiotic residues in milk have been detected for periods exceeding 192 hours post-treatment, significantly longer than established withdrawal times<sup>8</sup>. These challenges have intensified the search for sustainable, natural alternatives that can effectively manage mastitis while minimizing residue concerns.

Medicinal plants have been utilized in veterinary medicine for millennia, offering antimicrobial properties through diverse bioactive compounds. The renewed scientific interest in phytotherapy stems from documented

efficacy against resistant pathogens, minimal residue formation, cost-effectiveness, and reduced likelihood of resistance development<sup>9, 10</sup>. Various plant extracts have demonstrated significant activity against mastitis pathogens, with MIC values ranging from 0.25-1.0 mg/mL<sup>11</sup>.

*Nigella sativa* (Black seed) has been extensively researched for antimicrobial properties, with thymoquinone identified as the primary bioactive compound. This quinone derivative disrupts bacterial membranes, inhibits biofilm formation, and induces oxidative stress in pathogenic bacteria<sup>12, 13</sup>. Traditional use of black seed in livestock health management has been documented across various cultures, supporting its therapeutic potential.

*Mentha piperita* (Peppermint) contains multiple antimicrobial compounds including menthol, menthone, and menthyl acetate. These terpenes demonstrate antibacterial activity through membrane disruption, enzyme inhibition, and interference with bacterial metabolism<sup>14, 15</sup>. The synergistic potential of combining multiple plant extracts may enhance therapeutic efficacy while reducing required concentrations.

The Minimum Inhibitory Concentration (MIC) assay provides standardized methodology for evaluating antimicrobial activity, allowing precise determination of effective concentrations and facilitating comparison between different compounds<sup>16</sup>. This quantitative approach is essential for establishing therapeutic protocols and clinical applications.

Despite extensive research on individual medicinal plants, limited studies have systematically evaluated combined herbal formulations against clinical mastitis isolates using standardized methodologies. The development of effective plant-based antimicrobials could revolutionize mastitis management by providing sustainable alternatives that address current therapeutic limitations while meeting consumer demands for natural approaches.

This study aimed to bridge this knowledge gap by evaluating MILKZERO<sup>®</sup>, a novel combination of *N. sativa* and *M. piperita* extracts, against *S. aureus* isolates from bovine mastitis, thereby contributing to evidence-based phytotherapy in veterinary medicine.

## Materials and Methods

### Plant Material and Extract Preparation

Fresh *Nigella sativa* seeds and *Mentha piperita* leaves were obtained from certified suppliers and authenticated by botanical experts. Plant materials were cleaned, dried at room temperature, and ground to fine powder using a mechanical grinder.

***Nigella sativa* Extract:** Hydroalcoholic extraction was performed using 50% ethanol (1:10 w/v ratio) through Soxhlet extraction method for 72 h, and concentrated using rotary evaporator at 40 °C. The final extract concentration was adjusted to 10% w/v.

***Mentha piperita* Extract:** Aqueous extraction was conducted using distilled water (1:10 w/v ratio) through maceration method at 80 °C for 4 hours with. The mixture was cooled, filtered, and concentrated to achieve 10% w/v concentration.

**MILKZERO<sup>®</sup> Formulation:** Both extracts were combined in equal proportions (10% w/v each), sterilized through 0.45 µm membrane filtration, and stored at 4 °C until use. The final formulation was subjected to phytochemical screening to confirm bioactive compound presence.



### Bacterial Identification

Primary isolation was performed on milk samples submitted to Faculty of Veterinary Medicine, Ferdowsi University of Mashhad which were inoculated on Blood Agar and Mannitol Salt Agar plates incubated at 37 °C for 24-48 hours. Suspected *S. aureus* colonies were identified through standard microbiological methods and molecular confirmation was performed using species-specific PCR targeting *nuc* gene. A total of 70 confirmed *S. aureus* isolates were selected for the study and preserved at -20 °C in Brain Heart Infusion broth containing 20% glycerol.

### Minimum Inhibitory Concentration (MIC) Determination

Antimicrobial activity evaluation followed Clinical and Laboratory Standards Institute (CLSI) guidelines for broth microdilution method. Fresh bacterial cultures were prepared by inoculating isolates on Brain Heart Infusion (BHI) agar and incubating at 37 °C for 24 hours. Bacterial suspensions were prepared in sterile saline and adjusted to 0.5 McFarland standard ( $\sim 1.5 \times 10^8$  CFU/mL).

MIC determination utilized sterile 96-well microtiter plates. Columns 1-11 received different bacterial isolates, while column 12 served as sterility control. Row A contained 100 µL undiluted MILKZERO<sup>®</sup>, followed by two-fold serial dilutions (1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128) using BHI broth. Subsequently, 100 µL of standardized bacterial suspension was added to all test wells.

Plates were incubated at 37 °C for 24 hours under aerobic conditions. MIC was defined as the lowest concentration completely inhibiting visible bacterial growth compared to positive controls.

### Data Analysis

Descriptive statistics, frequencies and percentages of susceptible isolates at each concentration were calculated. MIC distribution patterns were evaluated, and cumulative inhibition rates determined.

### Ethical Compliance

All procedures followed institutional guidelines for research involving animal-derived samples and complied with local animal welfare regulations.

## Results

### Antimicrobial Activity Assessment

The evaluation of MILKZERO<sup>®</sup> against 70 *S. aureus* isolates revealed concentration-dependent antimicrobial activity with significant variation in susceptibility patterns among clinical isolates.

### MIC Distribution Patterns

Complete growth inhibition was achieved in 26 isolates (37.5%) at the highest concentration tested (1:1 dilution, 50 mg/ml), demonstrating potent antimicrobial activity. At 1:2 dilution (25 mg/ml), 15 additional isolates (21%) showed complete inhibition, bringing the cumulative inhibition rate to 58%. Minimal activity was observed at lower concentrations, with only one isolate (1.5%) each showing inhibition at 1:4 (12.5 mg/ml) and 1:8 (6.25 mg/ml) dilutions, respectively.

Notably, 27 isolates (38.5%) exhibited visible growth even at undiluted MILKZERO<sup>®</sup> concentration, indicating intrinsic resistance or reduced susceptibility among these clinical strains. The overall susceptibility rate across all tested concentrations was 61.5% (43 out of 70 isolates) (table 1).

Concentration-Response Relationship

The study demonstrated clear concentration-dependent activity, with antimicrobial efficacy dramatically decreasing at dilutions beyond 1:2. No isolates showed inhibition at concentrations lower than 1:8, establishing the effective antimicrobial range within higher concentration spectrum.

MIC Results Summary

**Table1.** MIC distribution of *S. aureus* isolates against MILKZERO®.

| MILKZERO® Concentration | Inhibited Isolates | Percentage (%) | Cumulative (%) |
|-------------------------|--------------------|----------------|----------------|
| 50 mg/ml                | 26                 | 37.5%          | 37.5%          |
| 25 mg/ml                | 15                 | 21%            | 58.5%          |
| 12.5 mg/ml              | 1                  | 1.5%           | 60%            |
| 6.25 mg/ml              | 1                  | 1.5%           | 61.5%          |
| No inhibition           | 27                 | 38.5%          | -              |
| Total tested            | 70                 | 100%           | 61.5%          |

Discussion

This study demonstrated significant antimicrobial potential of MILKZERO® against *S. aureus* isolates from bovine mastitis, with 61.5% overall susceptibility rate. The concentration-dependent activity pattern aligned with established pharmacological principles, where higher concentrations achieved greater antimicrobial efficacy. The bioactive compounds in *N. sativa*, particularly thymoquinone, exert antimicrobial effects through multiple mechanisms including membrane disruption, biofilm inhibition, and oxidative stress induction<sup>12, 13</sup>. The hydroalcoholic extraction method employed may have optimized extraction of lipophilic compounds, enhancing overall antimicrobial potency. *M. piperita* contributes complementary antimicrobial activity through menthol, menthone, and menthyl acetate, which target bacterial membranes and essential enzymes<sup>14, 15</sup>. The aqueous extraction method preferentially extracted hydrophilic bioactive compounds, contributing to the formulation's broad-spectrum activity.

The combination of both hydrophilic and lipophilic bioactive compounds in MILKZERO® may provide synergistic antimicrobial effects through complementary mechanisms of action. This multi-target approach potentially reduces the likelihood of resistance development compared to single-compound therapies<sup>17</sup>. The 37.5% inhibition rate at undiluted concentration (50 mg/ml) and additional 21% at 1:2 dilution (25 mg/ml) suggested that MILKZERO® could be effective at clinically achievable concentrations. The dramatic reduction in activity at lower dilutions (1:4 and beyond) indicated the importance of maintaining adequate therapeutic concentrations for optimal efficacy. The observation that 38.5% of isolates showed growth even at undiluted concentration highlighted the heterogeneous nature of *S. aureus* populations. This resistance may result from various factors including biofilm formation, efflux pump activity, or intrinsic cellular resistance mechanisms<sup>18, 19</sup>. *S. aureus* isolates from chronic mastitis cases often exhibit enhanced resistance due to biofilm-associated infections and prolonged antimicrobial exposure. The biofilm matrix can significantly reduce antimicrobial penetration and efficacy, potentially explaining the resistance observed in some isolates<sup>20</sup>.



Our findings aligned with previous research demonstrating variable antimicrobial activity of plant extracts against *S. aureus*. Studies have reported MIC ranges from 0.2-50 µg/mL for various botanical antimicrobials, depending on extraction methods, strain characteristics, and phytochemical composition<sup>21</sup>. Research by Diaz et al.<sup>11</sup> reported MIC values of 0.25-1.0 mg/mL for plant extracts against bovine mastitis isolates, supporting our concentration-dependent findings. Similarly, studies on individual *N. sativa* and *M. piperita* extracts have demonstrated comparable antimicrobial activity patterns<sup>12, 14</sup>.

MILKZERO<sup>®</sup> offers several advantages over conventional antimicrobials, including reduced likelihood of resistance development, minimal residue concerns, environmental safety, and cost-effectiveness. The natural origin aligns with increasing consumer preference for organic and sustainable dairy products<sup>22</sup>. The dual-plant formulation may provide enhanced stability and broader antimicrobial spectrum compared to single-extract preparations. This approach mirrors traditional medicine practices where plant combinations often demonstrate superior therapeutic efficacy<sup>23</sup>.

The promising in vitro results support further development of MILKZERO<sup>®</sup> for mastitis management. However, comprehensive safety studies, pharmacokinetic evaluations, and controlled clinical trials are prerequisite for practical application. Integration with existing mastitis control programs may provide sustainable alternatives to conventional therapy.

The concentration-dependent activity emphasizes the importance of appropriate dosing strategies and formulation optimization. Combination approaches, where MILKZERO<sup>®</sup> could be used alongside reduced-dose conventional antimicrobials, may enhance efficacy while minimizing resistance development.

### Conclusion

This study demonstrated significant antimicrobial potential of MILKZERO<sup>®</sup>, a novel herbal formulation combining *Nigella sativa* and *Mentha piperita* extracts, against *Staphylococcus aureus* isolates from bovine mastitis. The 61.5% overall susceptibility rate, with particular effectiveness at higher concentrations (58% inhibition at 1:1 and 1:2 dilutions), indicated promising therapeutic potential.

The concentration-dependent antimicrobial activity and heterogeneous susceptibility patterns observed among clinical isolates reflect the complex nature of mastitis pathogenesis and the variable response of bacterial populations to natural antimicrobials. The combination of bioactive compounds from both medicinal plants appears to provide synergistic effects that enhance overall antimicrobial efficacy.

These findings contributed valuable evidence supporting the therapeutic application of traditional medicinal plants in modern veterinary practice. MILKZERO<sup>®</sup> represented a potential sustainable alternative or adjunct therapy for bovine mastitis management, addressing current limitations of conventional antimicrobial therapy while meeting growing demands for natural approaches in livestock health management.

The study emphasized the importance of evidence-based phytotherapy in veterinary medicine and provides a foundation for future research aimed at developing optimized herbal formulations for clinical application. Further investigation including in vivo studies, safety evaluations, and formulation optimization is warranted to establish the practical applicability of MILKZERO<sup>®</sup> in veterinary clinical practice.

**Conflict of interest:** The authors declare that they have no competing interests.

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## EFFECT OF *FERULA ASAFOETIDA* AND *ALLIUM SATIVUM* EXTRACTS ON *STRONGYLUS SPP.* IN LABORATORY CONDITIONS

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### Abstract

The high incidence of equine gastrointestinal worms and their increased resistance against anthelmintic has encouraged research into the effectiveness of rational phytotherapy. This study investigates the in vitro anti-parasitic effects of extracts of *Ferula asafoetida* and *Allium sativum*, two native plants that are widespread in Iran on *Strongylus spp.* larvae. Faecal samples were collected from horses, examined by routine parasitology methods and positive samples were used for future examination. After incubation, the third-stage larvae were harvested by the Baermann technique. A hydroalcoholic extract from the plants was used for the antiparasitic study, while tap water was used for controls. Trials for each concentration and control group were performed in three replicates. The results showed that that during the first day of exposure, the hydroalcoholic extract of *F. asafoetida* at concentration of 10, 50 and 100 mg/ml killed over the 90% of the larvae, and *A. sativum* extract at concentration of 50 and 100 mg/ml killed over the 95% of larvae ( $p < 0.05$ ). The results obtained from the bioassay show that two plant extracts have a larvicidal effect on the *Strongylus spp.* larval stages compared with the control group.

**Keywords:** *Allium sativum*, Anti-larval, Equine, *Ferula asafoetida*, In vitro

### Introduction

Normal Adult worms of *Strongylus spp.* live in the large intestine of horses and are commonly categorized as large and small strongyles [1,2]. Of the more than 100 species of internal parasites found in horses, almost half are strongyles [3]. *Strongylus spp.* is a ubiquitous and important nematode parasite of horses and related equidae [4]. They are frequently responsible not only for generally poor health, but also gastrointestinal dysfunctions, including colic and the potentially fatal condition of acute larval cyathostomosis [5]. Infective third-stage larvae (L3) ingested from contaminated pasture penetrate the mucosa of the large intestine, moult to fourth-stage larvae (L4) in the submucosa. In the case of *Strongylus vulgaris*, they then proceed to the root of the cranial mesenteric artery along the arterioles and arteries that supply the intestine, where they may cause parasitic thrombosis and arthritis [6]. Larvae of the other two species may be found in various parts of the body, including the liver, prerenal tissues, retroperitoneal tissues, and pancreas [7]. Over the past five decades, parasite control regimens involving frequent and regular anthelmintic treatment of all horses appear to have resulted in a decline in the prevalence and abundance of *S. vulgaris*, and the parasite is now considered rare in managed horse populations [8,9]; however, while the primary goal of the anthelmintic treatment regimens has been achieved, new challenges have arisen. Anthelmintic resistance in cyathostomin parasites is now being widely reported against benzimidazole-type drugs, as well as increasing levels of pyrantel resistance [6], and most recently, several publications have reported signs of ivermectin and moxidectin resistance [10]. This is further exacerbated by the world-wide. Occurrence of ivermectin and moxidectin resistance reported in *Parascaris equorum* [6]. In the face of increasing

levels of resistance against anti-parasite drugs and the damaging effects of these drugs themselves, alternative treatments are required. Anthelmintics derived from plants can be an alternative for the treatment of parasitic infections [11]. A good source of knowledge regarding the potential action of plant extracts on certain diseases and pests is constituted by research in the field of medicinal plants. Many researchers have considered the anti-cancer properties of *Allium sativum* (garlic), as well as its anti-parasite effect on *Trypanosoma spp.*, *Entamoeba histolytica*, *Giardia lamblia*, *Leishmania major*, *Cryptosporidium spp.*, *Plasmodium spp.*, *Ascaris lumbricoides*, *Heterakis gallinae*, *Ascaridia galli*, *Haemonchus contortus*, *Nippostrongylus brasiliensis*, *Ascaris suum* [12–15]. *Ferula asafoetida* has traditionally been used for its anthelmintic properties in various countries such as Iran, China and Nepal, where it has been used for treating infection with intestinal parasites [16]. Some studies have examined the anti-parasitic properties of *F. asafoetida*, including activity against *Trichomonas vaginalis* and *Schistosoma mansoni* [17], and naturally-occurring plant products such as diterpenes, phenolics and sulfurcontaining compounds are known to possess anti-Leishmania properties [18]. However, as no study has so far examined the activity of *F. asafoetida* against equine parasites, some aspects of this form of control remain unclear, and its anthelmintic activity warrants further investigation. *F. asafoetida* is an Iranian endemic medicinal plant which has been used as a spice in food. For decades, it has also been used a traditional folk phytomedicine to treat various diseases, including intestinal parasites [19]. The aim of this study is to investigate the antiparasitic effects of *F. asafoetida* and *A. sativum* plant extracts on *Strongylus spp.* larvae in vitro.

### Materials and Methods

**Preparation of parasites.** In this study faecal samples were collected from infected horses around Urmia city, West Azerbaijan, Iran during November 2014. None of the animals had been given anthelmintic treatment for at least six months before they were sampled. Faecal sample were collected directly from the rectum of horses or immediately after faecal excretion. The faecal samples were placed in plastic bags and sent to the Parasitology Laboratory, Faculty of Veterinary Medicine, Urmia University. The samples examined by routine parasitology methods and positive samples were used for future examination [20]. **Larval culture.** Culture were created for the detection of *Strongylus* larvae as follows: at least 100 grams of faeces mixed with autoclaved dry faecal samples. The sample was incubated for 3 days at 28 °C, during which time more water was added if necessary. To prevent fungal growth, the samples were mixed daily using a spatula. After incubation, the third-stage larvae were harvested after 24 hours of sedimentation by the Baermann technique. All larvae were examined and identified by microscope using morphological criteria [21]. **Preparation of hydroalcoholic extract.** The plant *F. asafoetida* was collected in March 2014 from Sabzevar, Khorasan Razavi, Iran. The plant material was shade dried for several days. The plants were powdered with electric grinder. Whole plants were dried and ground in a hammer mill, then soaked in 70% ethanol and kept at room temperature for seven days. To complete the extraction process, the material was mixed twice daily. This procedure was repeated four times and combined ethanolic extract evaporated to dryness on a rotary evaporator at temperature between 45 and 50 °C, resulting in the production of a deep dark brown semi-solid residue. The *F. asafoetida* hydroalcoholic extract (AAHE) was kept protected from light in a refrigerator at 4 °C. *A. sativum* was purchased from local market of Urmia city, Iran, and same procedure was performed as for the preparation of the *F. asafoetida* hydroalcoholic extract [22,23]. **Antiparasitic tests.** Firstly, 1ml of larval suspension containing approximately 30 larvae was incubated at 25 °C with 1 ml of hydroalcoholic extract of *F. asafoetida* at concentrations of 100, 50 and 10 mg/ml. In addition, 1 ml of larval



suspension was also incubated at 25 °C with 1ml of hydroalcoholic extract of *A. sativum* at concentrations of 100, 50, 10, 5, 2.5 and 1.25 mg/ml. These doses were selected according pilot study. The survival rate of larvae was calculated 1, 2, 3, 24 and 48 hours after treatment. The parasites were removed from the incubator and the numbers of viable parasites were counted under a microscope: death was concluded when no movement was observed in larvae after stimulation. For control group, the specimens were only incubated in tap water. Trials for each concentration and control group were performed in three replicates [24]. Statistical procedure. Data is expressed as means±standard deviation (SD). The percentage survival rate of larvae was calculated 1, 2, 3, 24 and 48 hours after treatment. The means of three replications was recorded for that time and defined as dependent variable for the statistical analysis of the data. Data obtained from the in vitro tests was analysed using ANOVA and compared by means of the Tukey's test. At every time point, the results were compared with those of the control group. Statistical analyses were performed using the SPSS ver.19.0 statistic software package (SPSS Inc., Chicago, IL). Values of ( $p < 0.05$ ) were considered significant [25].

### Results

The results obtained from the bioassay showed that the two plant extracts had a larvicidal effect on larval stages of *Strongylus* spp. The worm larvae treated with the two plants extracts demonstrated a significantly increased mortality rate compared with the control group. The results showed that on the first day of exposure, the hydroalcoholic extract of *F. asafoetida* killed over the 90% of the larvae when administered at concentration of 10, 50 and 100 mg/ml. In addition, *A. sativum* extract killed over the 95% of the tested larvae in the first 24 hours when administered at concentration of 50 and 100 mg/ml, and killed 90% after 48 hours of exposure at 10 and 5 mg/ml. The results also showed that all extract concentrations of *A. sativum* examined were effective against the larval stage of *Strongylus* spp. three hours after treatment ( $p < 0.05$ ), while the *F. asafoetida* extracts demonstrated the same results 48 hours after treatment ( $p < 0.05$ ). The results also indicate that both the 100 mg/ml *A. sativum* extract and the 50 and 100mg/ml *F. asafoetida* extracts killed all the test larvae after 48- hour incubation.

### Discussion

Both of the tested extracts demonstrated dosedependent anthelmintic activities, and the mortality rate increased significantly as the time of exposure of the larvae to the plant extracts increased. The activity of *A. sativum* ranged from a 100% mortality rate, demonstrated on day 2 of post-incubation with the 100mg/ml extract, to 84.65%, demonstrated by the lowest concentration of 1.25 mg/ml. Higher doses of *F. asafoetida* (50 and 100 mg/ml) were needed to cause 100% mortality after 48-hour exposure. The onset of an acceptable anthelmintic effect was rapid in both cases, being observed 24 hours after treatment. Ajoene, an organosulfur compound derived from *A. sativum* is known to demonstrate significant antifungal, anti-trypanosomal and antiviral activities [10]. Ajoene may cause alterations in protein and lipid transport in the parasitic larva cell membranes, leading to irreversible damage to the parasites [12]. Many pharmacological properties, including antibacterial, antioxidant, antifungal, tranquilizing, carminative, gastrointestinal, antispasmodic, laxative, pain killing, diuretic, and disinfecting activities have been confirmed for *F. asafoetida* extract [26]. *F. asafoetida* consists of three main fractions; including resin (40–64%), containing ferulic acid and its esters, coumarins, sesquiterpenecoumarins and other terpenoids, gum (25%) and essential oil (10–17%) [27]. The results of the current study indicate that hydroalcoholic extracts of *F. asafoetida* and *A. sativum* have potential anthelmintic and larvicidal activities in

vitro; however, further in vivo evaluation of the different parts and fractions is needed to make use of these plants for beneficial purposes. It is interesting to note that the extracts are combinations of many components and are not pure, so these primarily results only suggest the potency of these extracts. Nevertheless, our discovery that the plant extracts can be used as accessible source of natural anthelmintic from plants is rewarding, as it will lead to the development of a phytomedicine to act against parasite. These extracts have enormous therapeutic potential, as they can fulfil their purpose without any side effects that are often associated with synthetic compounds [28,29].

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## EFFECTS OF PLANTS OR PLANT EXTRACTS ON MILK PRODUCTION AND QUALITY IN LIVESTOCK

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### Abstract

The use of herbal medicines in the nutrition of dairy animals, especially in increasing milk production and improving milk quality, has created a significant transformation in the livestock industry. Research shows that herbs such as black cumin, turmeric and chamomile increase milk production by up to 50% by stimulating the secretion of milk-producing hormones, increasing nutrient absorption and modulating the rumen microbiome. These natural compounds also improve livestock health by reducing oxidative stress and inflammation and prevent production losses caused by environmental stresses.

On the other hand, these herbs, by changing the metabolism of fats, increase beneficial fatty acids such as CLA and omega-3 in milk and at the same time reduce cholesterol levels. These changes turn milk into a functional product with health-promoting properties. Also, the antimicrobial properties of herbs such as garlic and curcumin reduce the prevalence of mastitis and improve milk quality in terms of somatic cells.

Despite the proven benefits, challenges such as determining the exact dosage and possible interactions require further research. The use of this natural solution could simultaneously increase productivity and meet the global demand for healthy and natural milk.

**Keywords:** Functional milk, Herbal medicines, Milk production, Milk quality, Omega-3 fatty acids

### Introduction

In recent years, the use of herbal medicines as a safe and effective alternative to antibiotics and synthetic stimulants in animal husbandry has received attention<sup>1</sup>. This is especially evident in the dairy industry, where the quality and quantity of milk production are of great importance<sup>2</sup>. Several studies have shown that herbal compounds can have a positive effect on milk production and composition by improving metabolic function and strengthening the immune system of animals<sup>3,4</sup>. For example, herbs such as black cumin (*Nigella sativa*) and chamomile (*Chamomilla recutita*) have been shown to improve milk production and quality in dairy cows due to their galactagogue and anti-inflammatory properties<sup>5-7</sup>.

One of the major challenges in the dairy industry is the reduction in milk production caused by environmental stresses such as high heat and diseases such as mastitis<sup>8</sup>. Research has shown that herbal supplements such as compounds containing betaine, curcumin, and mint can increase milk production in cows under heat stress by reducing oxidative stress and improving rumen condition<sup>3,9</sup>. These compounds can also improve digestion and absorption of nutrients by modulating the rumen microbiome, which in turn leads to increased milk production and improved milk quality<sup>4,10</sup>.

Milk quality is also considered an important indicator of human health and the economic value of dairy products<sup>11</sup>. Recent studies have shown that the use of medicinal plants such as turmeric (*Curcuma longa*) and black cumin can increase the content of unsaturated fatty acids, lactose and milk protein, while reducing



cholesterol levels<sup>3,7,12</sup>. These changes not only increase the nutritional value of milk, but can also be used as a strategy for the production of functional milk<sup>13</sup>. In addition, the use of herbal medicines in the treatment of subclinical mastitis has also been investigated<sup>14</sup>. For example, herbal compounds such as curcumin and garlic, due to their anti-inflammatory and antimicrobial properties, have been able to reduce the somatic cell count in milk and improve the health of the udder of dairy cows<sup>7,15</sup>. These findings suggest that medicinal plants can be used as natural alternatives to antibiotics in the management of mastitis<sup>16</sup>. Given the increasing demand for safe and residue-free dairy products, the use of herbal medicines in the nutrition of dairy animals can be a sustainable and economical solution to improve milk production and quality<sup>17</sup>.

This article examines the effects of herbal supplements on milk production, its composition, and animal health, and analyzes the role of these compounds in the future of the dairy industry.

### **Mechanism of effect of plant extracts on milk production and quality**

Based on studies conducted in the reviewed articles, the mechanisms of effect of plant extracts on milk production and quality are as follows:

1. Modulating the rumen environment and improving digestion: Plant extracts such as black cumin and turmeric improve food digestion by changing the rumen pH and increasing the population of beneficial bacteria<sup>7</sup>. These compounds provide the necessary precursors for the synthesis of milk fat and glucose by increasing the production of volatile fatty acids (VFA), especially acetate and propionate<sup>4</sup>.
2. Antioxidant and anti-inflammatory activity: Compounds such as curcumin in turmeric and thymoquinone in black cumin improve mammary gland health by reducing oxidative stress and inhibiting inflammatory factors such as TNF- $\alpha$ <sup>3</sup>. These effects lead to a decrease in somatic cells in milk and an improvement in its quality<sup>7</sup>.
3. Galactagogue effect (milk-increasing): Some plants such as fenugreek and black cumin increase mammary gland activity by stimulating the secretion of prolactin and oxytocin hormones<sup>5</sup>. This mechanism has been clearly observed in a study on Awassi sheep.
4. Improvement of milk fatty acid profile: Plant extracts increase the ratio of unsaturated to saturated fatty acids in milk by altering lipid metabolism in the rumen and liver. This effect is particularly evident in the case of conjugated linoleic acid (CLA) and omega-3 fatty acids<sup>3</sup>.
5. Reduction of microbial load and mammary infections: Phenolic and saponin compounds present in medicinal plants act with their antimicrobial effects against mastitis-causing bacteria such as *Staphylococcus aureus*<sup>7</sup>.

These mechanisms clearly demonstrate how plant extracts can simultaneously affect milk quantity and quality. However, further research is needed to optimize the dosage and composition of these extracts.

### **Discussion**

The findings of this study and previous research clearly show that the use of herbal supplements can have positive and multifaceted effects on milk production and quality in dairy cattle. The results of various studies indicate that medicinal plants such as black cumin (*Nigella sativa*), chamomile (*Chamomilla recutita*) and turmeric (*Curcuma longa*) are able to increase milk production and improve its quality through various mechanisms, including improving rumen function, reducing oxidative stress and strengthening the immune system<sup>3,4,7</sup>. The present study is in line with the research of EL-Ghousein (2010) that the use of herbal supplements

can increase milk production by up to 50%. This increase is probably due to improved digestion and absorption of nutrients as a result of positive changes in the rumen microbial population<sup>5,10</sup>. In particular, phenolic compounds and saponins present in medicinal plants can modulate fermentation processes in the rumen, increasing the production of volatile fatty acids (VFAs), which are important precursors for milk fat synthesis<sup>18</sup>. In the context of milk quality, the findings of this study confirm the results of Butler et al. (2011) and Tudisco et al. (2010), who showed that the use of herbal supplements can improve the fatty acid profile of milk<sup>11,12</sup>. In particular, an increase in unsaturated fatty acids such as conjugated linoleic acid (CLA) and alpha-linolenic acid (ALA) has been observed in the milk of cows fed medicinal plants. These changes not only increase the nutritional value of milk, but can also have significant health benefits for consumers<sup>13</sup>.

Regarding the impact of medicinal plants on animal health, the results of this study are consistent with the findings of Nurdin et al. (2011) and Lacobellis et al. (2005) are consistent in showing that herbal compounds can be effective in the prevention and treatment of subclinical mastitis<sup>7,15</sup>. This effect is likely due to the anti-inflammatory and antimicrobial properties of compounds such as curcumin and allicin found in medicinal plants<sup>16</sup>. However, there are some challenges in the use of herbal supplements. As Benchaar et al. (2008) have pointed out, the appropriate dosage and combination of medicinal plants is very important to achieve the desired effects<sup>1</sup>. Also, breed differences and environmental conditions can affect the response of livestock to herbal supplements<sup>2</sup>.

Overall, the findings of this study and previous research indicate that the use of herbal supplements can be considered as a sustainable and economical strategy to improve milk production and quality in the livestock industry. However, further research is needed to determine optimal doses and possible interactions of these compounds with other dietary components<sup>17</sup>.

### Conclusion

The use of herbal medicines in the nutrition of dairy animals has been considered as a new and effective solution for improving milk production and quality. Numerous studies have shown that medicinal plants such as black cumin, chamomile and turmeric have a significant effect on increasing milk production and improving its quality through various mechanisms, including improving rumen function, reducing oxidative stress and strengthening the immune system. These natural compounds are not only able to increase milk production by 50%, but also improve its nutritional value by improving the fatty acid profile of milk and reducing cholesterol levels. One of the most important advantages of using medicinal plants is their ability to prevent and treat diseases such as mastitis. This feature is especially important in a situation where antibiotic resistance has become a global challenge. Herbal compounds with their anti-inflammatory and antimicrobial properties can be used as safe and effective alternatives to antibiotics while preventing drug residues in milk. Despite their numerous benefits, the use of herbal supplements in the dairy industry also comes with challenges. Determining optimal doses for different livestock breeds, investigating possible interactions with other diet components, and standardizing herbal formulations are among the issues that require further research. Also, geographical differences and environmental conditions can affect the effectiveness of these compounds.

Finally, it can be said that the integration of traditional knowledge about medicinal plants with modern animal husbandry technologies provides an exceptional opportunity to produce higher quality and healthier milk. This approach not only helps to increase the economic productivity of livestock farmers, but also responds to the



growing consumer demand for natural and chemical-free dairy products. By continuing research in this field and addressing existing challenges, the use of herbal medicines can become one of the main pillars of the milk industry in the future and play an important role in the sustainable development of this industry.

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## THE USE OF MEDICINAL PLANTS AND ESSENTIAL OILS IN THE TREATMENT OF MASTITIS IN DAIRY COWS

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### Abstract

Mastitis is one of the most common and costly diseases in dairy cows, manifesting in clinical and subclinical forms. The subclinical form, due to the absence of visible symptoms and delayed diagnosis, causes greater economic losses, including reduced milk production and lower quality of dairy products. This disease is primarily caused by bacteria such as *Staphylococcus aureus* and *Escherichia coli*, and its conventional treatment relies on antibiotics. However, the indiscriminate use of antibiotics has led to microbial resistance, drug residues in milk, and public health concerns. In recent years, medicinal plants have gained attention as alternative or complementary therapies. Active compounds such as allicin (garlic), curcumin (turmeric), and thymol (thyme) exhibit antibacterial, anti-inflammatory, and immune-boosting properties. Studies have shown that plant extracts, particularly combinations like garlic and neem, have synergistic effects in inhibiting mastitis-causing bacteria. Additionally, essential oils such as cinnamon and clove, by disrupting bacterial biofilms, are effective in controlling this disease. These findings suggest that plant-based products can help reduce antibiotic use and promote more sustainable mastitis management.

**Keywords:** Antibiotics, Essential oils, Mastitis, Medicinal plants, Microbial resistance

### Introduction

Mastitis in dairy cows manifests in both clinical and subclinical forms. The clinical form presents with obvious symptoms such as swelling, warmth, pain, and changes in milk color or consistency, while the subclinical form lacks visible signs and is only detectable through elevated somatic cell counts (SCC) in milk. Research indicates that subclinical mastitis affects 20-50% of dairy herds, typically at higher rates than clinical cases<sup>1</sup>. International reports show mastitis accounts for 40-50% of dairy cow diseases, with significant economic impacts including reduced milk yield, discarded contaminated milk, treatment costs, premature culling, and lower dairy quality<sup>2</sup>. The estimated cost per clinical mastitis case ranges from \$128 to \$444, while subclinical mastitis averages \$110 loss per lactation<sup>3</sup>.

The global milk supply primarily comes from dairy cows (81%), buffalo (15%), and goats, sheep and camels (4% combined). With world milk production at approximately 887 million tons in 2021<sup>4</sup> and projected to reach 930 million tons in 2022, udder health is critical not only for farmers but for the entire dairy supply chain<sup>2,5</sup>. Mastitis remains one of the most economically significant challenges facing the industry<sup>6</sup>.

This multifactorial inflammatory disease of the mammary gland, classified as a transition period disorder<sup>7</sup>, is primarily caused by bacteria including *Streptococcus agalactiae*, *Streptococcus dysgalactiae*, *Streptococcus uberis*, *Staphylococcus aureus*, *Corynebacterium bovis*, *Staphylococcus epidermidis*, *Escherichia coli*, *Klebsiella*, *Enterobacter aerogenes*, *Mycoplasma*, and coagulase-negative staphylococci<sup>8,9</sup>. Transition diseases



continue to pose major challenges, reducing production, profitability, and affecting both animal and public health  
7.

Conventional mastitis treatment relies heavily on antibiotics. However, their widespread and often excessive use has led to serious challenges including increased microbial resistance, drug residues in milk and dairy products, reduced therapeutic efficacy, and public health risks <sup>10</sup>. Antibiotic residues in milk may cause allergic reactions, disrupt gut microbiota, and contribute to drug resistance in humans <sup>11</sup>.

Given these issues, natural alternatives - particularly medicinal plants - have gained attention. These plants contain active compounds such as flavonoids, tannins, alkaloids, terpenoids and essential oils that exhibit antibacterial, anti-inflammatory, antioxidant and immune-stimulating properties <sup>12</sup>. Thus, medicinal plants are being explored not only for prevention but as complementary or alternative treatments for mastitis management <sup>10</sup>. Numerous studies demonstrate that medicinal plants, through active compounds like allicin, flavonoids, terpenoids and tannins, possess antimicrobial, antioxidant and immune-enhancing effects. Edward et al. (2017) found ethanolic extracts of garlic (*Allium sativum*) and neem (*Azadirachta indica*) leaves, both individually and combined, showed synergistic effects significantly inhibiting growth of *Staphylococcus aureus* and *E. coli*<sup>13</sup>.

Sharun et al. (2021) identified several effective medicinal plants including turmeric (*Curcuma longa*), holy basil (*Ocimum sanctum*), Indian gooseberry (*Phyllanthus emblica*), and immune-boosting plants like *Tinospora cordifolia* and *Withania somnifera*. These plants reduce inflammation, enhance local immune responses and inhibit Gram-positive bacteria<sup>14</sup>.

Essential oils (EOs) have also emerged as effective alternatives. Lopes et al. (2020) reported oils like thyme, cinnamon, fennel and clove, through their phenolic compounds, can inhibit pathogenic bacteria and disrupt biofilms<sup>15</sup>. Caneschi et al. (2023) demonstrated essential oils like thymol (in thyme) and cinnamaldehyde (in cinnamon) strongly inhibit *S. aureus* by penetrating cell membranes and disrupting respiratory mechanisms<sup>16</sup>.

This study aims to evaluate the efficacy of plant-derived compounds in bovine mastitis management. The findings will provide evidence-based recommendations for integrating phytotherapeutic approaches into mastitis control programs, potentially reducing antibiotic dependence while maintaining animal health and productivity.

### 1. Synergistic Effects with Conventional Antibiotics

Certain herbal combinations (e.g., garlic + neem) as well as essential oils (e.g., thyme, cinnamon, clove) enhanced the efficacy of conventional antibiotics by disrupting bacterial biofilms, increasing membrane permeability, and lowering the required minimum inhibitory concentrations (MICs). These synergistic interactions suggest a potential for reduced antibiotic dosages when combined with plant-derived compounds.

### 2. Anti-inflammatory and Immunomodulatory Properties

Several plants such as *Tinospora cordifolia*, *Withania somnifera*, and *Phyllanthus emblica* exhibited anti-inflammatory and immune-enhancing properties, contributing to reduced somatic cell counts (SCC) and improved udder tissue healing. These effects are particularly beneficial in managing subclinical mastitis and in preventing recurrence.

### 3. Biofilm Disruption and Resistance Control

Essential oils from *Thymus vulgaris*, *Origanum vulgare*, and *Cinnamomum cassia* were found to be effective in disrupting established bacterial biofilms, which are often responsible for chronic or recurrent mastitis.

Their active components—such as thymol, carvacrol, and cinnamaldehyde—target microbial respiratory chains and structural integrity, helping overcome antibiotic resistance.

4. Diverse Routes of Application

The reviewed therapies were administered via multiple routes: topically (ointments, sprays, oils), orally (extracts, juices), and intramammary (experimental formulations). Topical application was most common and showed promising localized effects, especially when combined with anti-inflammatory agents.

5. Safety and Milk Quality Improvement

Most plant-based therapies showed favorable safety profiles, with minimal risk of residue accumulation in milk. Additionally, many of the reviewed botanicals contributed to improved milk parameters such as pH, electrical conductivity, and SCC—indicators of udder health and milk quality.

6. Potential for Economic and Practical Viability

Though large-scale cost analyses are limited, the use of phytotherapeutics has potential to lower treatment costs, reduce milk discard due to antibiotic withdrawal periods, and decrease losses from culling or chronic infection. The availability of these plants in many dairy-producing regions further supports their feasibility for integration into mastitis control programs.

Table 1. Medicinal plants used in treating mastitis in dairy cows

| Category | Plant                             | Usage Method                           | Mechanism of Action                                           | Key Results and Applications                                      | Reference |
|----------|-----------------------------------|----------------------------------------|---------------------------------------------------------------|-------------------------------------------------------------------|-----------|
| Herbal   | <i>Allium sativum</i> (Garlic)    | Ethanollic extract, in vitro           | Allicin; penetrates bacteria and inhibits growth              | Inhibition zone ~12–14 mm vs. <i>S. aureus</i> and <i>E. coli</i> | 13        |
| Herbal   | <i>Azadirachta indica</i> (Neem)  | Ethanollic extract, in vitro           | Direct antibacterial effect vs. <i>S. aureus</i>              | Inhibition zone ~10–11 mm; <i>E. coli</i> resistant               | 13        |
| Herbal   | Combined Neem + Garlic            | Combined ethanollic extracts, in vitro | Synergistic effect, reduced CFU/ml for <i>S. aureus</i>       | More effective than single extracts                               | 13        |
| Herbal   | <i>Curcuma longa</i> (Turmeric)   | Topical or oral                        | NFκB inhibition, reduced <sup>16</sup> inflammatory cytokines | Lower SCC, reduced inflammation, improved milk quality            | 14        |
| Herbal   | <i>Ocimum sanctum</i> (Tulsi)     | Topical extract / spray                | Anti-inflammatory, antioxidant, immunomodulator               | Reduced udder swelling and inflammation                           | 14        |
| Herbal   | <i>Tinospora cordifolia</i>       | Oral                                   | Immune booster, anti-inflammatory                             | Enhanced local immune response                                    | 14        |
| Herbal   | <i>Morinda citrifolia</i>         | Oral (juice)                           | Lower milk pH, EC and bacterial load                          | Improved milk quality, reduced inflammation                       | 14        |
| Herbal   | <i>Minthostachys verticillata</i> | Topical essential oil                  | Antibacterial and biofilm inhibition                          | Inhibits <i>S. aureus</i> growth                                  | 14        |
| Herbal   | <i>Liquidambar orientalis</i>     | Plant extract                          | Antibacterial, anti-inflammatory                              | Initial therapeutic potential                                     | 14        |
| Herbal   | <i>Cinnamon cassia</i>            | Extract or oil                         | Antibacterial vs. Gram-positive bacteria                      | Potential for microbial load reduction                            | 14        |



|        |                            |                      |                                        |                                        |    |
|--------|----------------------------|----------------------|----------------------------------------|----------------------------------------|----|
| Herbal | <i>Phyllanthus emblica</i> | Extract              | Antioxidant and antibacterial          | Protective potential                   | 14 |
| Herbal | <i>Murraya koenigii</i>    | Extract              | Flavonoid-based antibacterial activity | Demonstrated antibacterial action      | 14 |
| Herbal | <i>Glycyrrhiza glabra</i>  | Topical/oral extract | Anti-inflammatory and antibacterial    | Alleviates inflammation                | 14 |
| Herbal | <i>Withania somnifera</i>  | Extract              | Anti-inflammatory, immune booster      | Enhances body resistance               | 14 |
| Herbal | <i>Terminalia chebula</i>  | Extract              | Antibacterial, antioxidant             | Tissue protection and pathogen control | 14 |
| Herbal | <i>Prosopis juliflora</i>  | Extract              | Potential antibacterial                | Promising for combination therapy      | 14 |

**Table 2.** Essential Oils Used in Treating Mastitis in Dairy Cows

| Essential Oil                                  | Main Active Compound      | Primary Mode of Action                             | Key Results and Therapeutic Application                                        | Reference |
|------------------------------------------------|---------------------------|----------------------------------------------------|--------------------------------------------------------------------------------|-----------|
| <i>Thymus vulgaris</i> (Thyme)                 | Thymol, Carvacrol         | Antibacterial, anti-biofilm                        | Inhibits <i>S. aureus</i> & biofilm; comparable to antibiotics                 | 15,16     |
| <i>Origanum vulgare</i> (Oregano)              | Carvacrol, Thymol         | Antibacterial, anti-biofilm                        | Strong inhibition vs. Gram-positive bacteria; synergistic with drugs           | 16        |
| <i>Cinnamomum zeylanicum/cassia</i> (Cinnamon) | Cinnamaldehyde, Eugenol   | Antibacterial, biofilm disruption, membrane damage | Disrupts bacterial walls, biofilm inhibition, enhances antibiotic penetration  | 15,16     |
| <i>Melaleuca alternifolia</i> (Tea Tree)       | Terpinen-4-ol             | Antibacterial, anti-inflammatory                   | Effective vs. resistant <i>S. aureus</i> ; topical potential                   | 16        |
| <i>Eugenia caryophyllata</i> (Clove)           | Eugenol                   | Antibacterial, membrane disruption                 | Enhances drug penetration; inhibits <i>E. coli</i> and <i>S. aureus</i> growth | 15,16     |
| <i>Rosmarinus officinalis</i> (Rosemary)       | 1,8-Cineole, Alpha-pinene | Antibacterial, antioxidant, drug synergist         | Reduces milk bacterial load; increased efficacy when combined with antibiotics | 16        |
| <i>Citrus limonum</i> (Lemon)                  | Limonene, Citral          | Antibacterial, antioxidant                         | Biofilm inhibition; improved milk stability                                    | 16        |
| <i>Mentha piperita</i> (Peppermint)            | Menthol, Menthone         | Antibacterial, mild anti-inflammatory              | Cooling and soothing effect; modest activity but useful as adjunct therapy     | 16        |
| <i>Salvia officinalis</i> (Sage)               | Thujone, Camphor          | Antibacterial                                      | Moderate activity vs. <i>S. aureus</i> ; suitable for blends                   | 16        |
| <i>Lavandula angustifolia</i> (Lavender)       | Linalool, Linalyl acetate | Antibacterial, anti-inflammatory, soothing         | Local calming effect plus bacterial inhibition; ideal for sensitive cows       | 16        |

|                                             |                                 |                                                      |                                                                                   |    |
|---------------------------------------------|---------------------------------|------------------------------------------------------|-----------------------------------------------------------------------------------|----|
| <i>Foeniculum vulgare</i><br>(Fennel)       | Anethole, Fenchone,<br>Limonene | Antibacterial,<br>antioxidant                        | Inhibits Gram-positive bacteria;<br>improves milk quality, anti-<br>bloat effects | 15 |
| <i>Matricaria chamomilla</i><br>(Chamomile) | Chamazulene,<br>Alpha-bisabolol | Anti-inflammatory,<br>antibacterial                  | Reduces udder inflammation;<br>soothing, immune-enhancing                         | 15 |
| <i>Zingiber officinale</i><br>(Ginger)      | Gingerol, Shogaol               | Antibacterial,<br>antioxidant, anti-<br>inflammatory | Lowers SCC and inflammation;<br>inhibits <i>S. aureus</i>                         | 15 |
| <i>Ocimum basilicum</i><br>(Basil)          | Linalool, Eugenol               | Antibacterial,<br>antioxidant                        | Strong inhibition vs. Gram-<br>positive bacteria; promising for<br>topical use    | 15 |

Discussion and Conclusion

This review highlights that medicinal plants and essential oils possess significant potential as alternative or complementary therapies for managing bovine mastitis. Active compounds such as allicin, flavonoids, terpenoids, and essential oils exhibit antibacterial, anti-inflammatory, antioxidant, and immune-boosting properties. These phytochemicals effectively inhibit major mastitis pathogens like *Staphylococcus aureus* and *E. coli*, and some are capable of disrupting bacterial biofilms, thereby preventing chronic infections.

When used alone or in combination with conventional antibiotics, several plant-based treatments have shown synergistic effects, reducing bacterial resistance and minimizing drug residues in milk. Medicinal plants such as turmeric, holy basil, and Indian gooseberry have demonstrated the ability to lower somatic cell counts and improve milk quality.

Although most evidence stems from in vitro studies, the promising results suggest practical applicability in dairy farms. However, further in vivo research is necessary to establish optimal dosages, safety profiles, and economic feasibility.

In conclusion, phytotherapeutic agents offer a natural, safe, and sustainable strategy for mastitis control. Integrating these alternatives into herd health programs may reduce antibiotic dependence while supporting animal welfare and the production of safer dairy products.

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## A REVIEW ON THE USE OF MEDICINAL PLANTS IN THE TREATMENT OF RETAINED PLACENTA IN DAIRY COWS

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### Abstract

Retained placenta (RP) is one of the most common reproductive disorders in dairy cows, characterized by the failure to expel the placenta within 12 to 24 hours after calving. This disorder is often associated with complications such as metritis, delayed return to estrus, reduced fertility, and decreased milk production, posing a considerable economic burden on the dairy industry. Its prevalence ranges from 5% to 15% across different herds and may exceed 25% in cases involving twinning, dystocia, or premature delivery. While conventional treatments such as oxytocin administration, antibiotics, and manual removal are commonly used, they may present limitations such as uterine damage or antimicrobial resistance. As a result, herbal medicine has attracted attention as a complementary and low-risk approach. Traditional formulations such as Sheng Hua Tang, GYS herbal powder, alcohol-based herbal tinctures, post-calving tonics, and indigenous medicinal plants have demonstrated promising results in reducing the incidence of RP and improving reproductive indices. These treatments are generally administered orally or via injection shortly after parturition, and exert their effects by stimulating uterine contractions, enhancing local blood flow, reducing inflammation, and boosting immunity. Clinical findings indicate that rational and evidence-based use of herbal remedies can offer an effective strategy in RP management.

**Keywords:** Dairy cows, Fertility, Medicinal plants, Retained placenta

### Introduction

Retained placenta (RP) is among the most prevalent reproductive complications in dairy cows, defined as the failure to expel the fetal membranes within 12 to 24 hours postpartum.<sup>1</sup> RP negatively affects reproductive performance and can lead to secondary conditions such as metritis, endometritis, delayed resumption of estrus, reduced conception rates, and prolonged calving-to-conception intervals.<sup>2</sup>

The incidence of RP varies from 5% to 15% in general herds and can exceed 25% in cases of twinning, dystocia, or premature delivery.<sup>3</sup> Several factors contribute to the development of RP, including:

- Dystocia
- Nutritional deficiencies (e.g., selenium, vitamin E)
- Uterine or placental infections
- Impaired uterine contractions or immune dysfunction
- Twinning or stillbirth



Due to the significant clinical and economic consequences of RP, effective treatment is essential. Common therapeutic strategies involve antibiotic therapy, oxytocin injection, and manual removal, all of which carry risks such as microbial resistance and uterine trauma.<sup>4</sup>

In recent years, interest has grown in using traditional herbal remedies and plant-based compounds as safe, accessible, and cost-effective alternatives. Medicinal plants are rich in bioactive substances such as flavonoids, saponins, alkaloids, and terpenes, which exert effects through uterotonic, anti-inflammatory, antimicrobial, and immunomodulatory mechanisms.<sup>5</sup> Herbs such as *Angelica sinensis*, *Leonurus Artemisia*, and *Carthamus tinctorius* have shown beneficial outcomes in reducing RP prevalence and improving reproductive recovery.<sup>6-8</sup>

Recent clinical trials confirm that traditional formulations and plant extracts can shorten placental expulsion time, enhance fertility outcomes, and reduce reliance on synthetic drugs.<sup>8</sup>

### **Medicinal Plants and Formulations Used in the Treatment of RP**

#### **1. Sheng Hua Tang**

This traditional Chinese formula includes *Radix Angelicae sinensis*, *Ligustici rhizoma*, *Semen persicae*, *Zingiberis rhizoma*, and *Radix glycyrrhizae*. In the study 50 mL of the herbal decoction was administered orally to Holstein cows once daily for three days postpartum. The incidence of RP decreased from 17% to 4%, and improvements in return to estrus and conception rate were reported. The formulation promotes uterine contractions and improves uterine blood flow.<sup>6</sup>

#### **2. GYS Herbal Powder**

Huang et al. evaluated a powder composed of *Leonurus Artemisia*, *Angelica sinensis*, *Ligusticum chuanxiong*, *Sparganium stoloniferum*, *Curcuma zedoaria*, *Cyperus rotundus*, and *Glycyrrhiza uralensis*. The powder (200 g/day) was given orally for five days postpartum. It reduced placental retention time, lowered the risk of metritis, and improved reproductive performance in cows with RP.<sup>7</sup>

#### **3. Herbal Alcoholic Tincture**

Cui et al. studied an injectable tincture containing *Herba Leonuri*, *Angelica sinensis*, *Flos Carthami*, *Myrrha*, and *Rhizoma Cyperi*. Administered intramuscularly in two 20 mL doses 24 hours apart, the treatment resulted in 73% placental expulsion within 72 hours, compared to 0% in the control group. It also reduced days to first service and increased conception rates.<sup>6</sup>

#### **4. Indigenous Medicinal Plants in Ethiopia**

Abdisa et al. documented the use of local herbs such as *Flax seed*, *Vernonia amygdalina*, *Dodonea angustifolia*, *Solanum acaule*, and *Momordica spp*. These were administered as infusions or feeds (50–150 g/day depending on plant and case). Their anti-inflammatory, antimicrobial, and uterotonic properties facilitated uterine cleansing and placental expulsion without harming the endometrium.<sup>5</sup>

#### **5. Post-Calving Tonic (PCT)**

Kumarswamy et al. evaluated a tonic containing *Carica papaya*, *Peganum harmala*, and *Gloriosa superba*. Administered orally at 100 mL/day for two days postpartum, it reduced mean RP expulsion time to approximately 10 hours and improved return to estrus and milk yield.<sup>8</sup>

**Table 1.** Effective Medicinal Plant Formulations for RP in Dairy Cows

| Formulation Name           | Main Herbal Components                                                                                                                                                                                        | Administration Route | Dosage          | Duration of Use   | Reference |
|----------------------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|----------------------|-----------------|-------------------|-----------|
| Sheng Hua Tang             | <i>Angelica sinensis</i> , <i>Ligustici rhizoma</i> ,<br><i>Semen persicae</i> , <i>Zingiberis rhizoma</i> ,<br><i>Glycyrrhizae radix</i>                                                                     | Oral                 | 50 mL/day       | 3 days postpartum | 6         |
| GYS Herbal Powder          | <i>Leonurus Artemisia</i> , <i>Angelica sinensis</i> ,<br><i>Ligusticum chuanxiong</i> , <i>Curcuma zedoaria</i> , <i>Sparganium stoloniferum</i> ,<br><i>Cyperus rotundus</i> , <i>Glycyrrhiza uralensis</i> | Oral                 | 200 g/day       | 5 days postpartum | 7         |
| Herbal Alcoholic Tincture  | <i>Herba Leonuri</i> , <i>Angelica sinensis</i> , <i>Flos Carthami</i> , <i>Myrrha</i> , <i>Rhizoma Cyperi</i>                                                                                                | Intramuscular        | 20 mL × 2 doses | 24-hour interval  | 6         |
| Indigenous Ethiopian Herbs | <i>Flax seed</i> , <i>Vernonia amygdalina</i> ,<br><i>Dodonea angustifolia</i> , <i>Solanum acaule</i> ,<br><i>Momordica spp.</i>                                                                             | Oral / Infusion      | 50–150 g/day    | Case-dependent    | 5         |
| Post-Calving Tonic (PCT)   | <i>Carica papaya</i> , <i>Peganum harmala</i> ,<br><i>Gloriosa superba</i>                                                                                                                                    | Oral                 | 100 mL/day      | 2 days postpartum | 8         |

**Discussion and Conclusion**

Given the relatively high prevalence and serious economic and physiological consequences of retained placenta in dairy cows, the development of safe, effective, and cost-efficient treatment options is essential. Herbal remedies, especially those grounded in traditional Eastern and ethnoveterinary knowledge, have shown promising results in enhancing placental expulsion and reproductive recovery. Formulations containing *Angelica sinensis*, *Leonurus Artemisia*, and *Carthamus tinctorius* offer anti-inflammatory, uterotonic, and immunomodulatory benefits that make them suitable as complementary or alternative therapies. However, further clinical trials and standardization of dosage and composition are necessary to ensure safety, efficacy, and economic viability in broader commercial use.

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## A REVIEW OF MEDICINAL PLANTS EFFECTIVITY ON POULTRY *EIMERIA* SPECIES

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### Abstract

Avian coccidiosis has a high economic impact on the poultry industry worldwide. The etiology of this intestinal disease are pathogenic *Eimeria* species that belong to the phylum Apicomplexa. Differences between these *Eimeria* species include the invasion of specific sites of the intestine, pathogenicity and type of lesion produced. The clinical disease in broilers includes diarrhea, reduction in weight gain and feed ingestion, and in severe cases, mortality. The strategies for the control of the disease includes the use of vaccination and drugs for prophylaxis. Nevertheless, the continuous use of different coccidiostats have led to drug resistance by *Eimeria*. Among alternative approaches, traditional treatments using medicinal plants have been investigated and researched in recent years. Broiler chicks at 1 d of age (n = 150) should be arbitrarily distributed into 6 groups. Birds from groups 1–3 should receive the starter and finisher diets plus 2, 4, and 6 g of desired herbal compound/kg of the diet, respectively. Birds from group 4 should be fed the starter and finisher diets plus 66 mg of salinomycin, group 5 constitute the positive control (PC), with the coccidial challenge, and group 6 constitute the negative control (NC), without the coccidial infection, which are both maintained on diets without any herbal compound. As it was mentioned, anticoccidial effects of herbal compounds were investigated by many studies. In some of these studies, it was discovered that herbal compounds might show good anticoccidial effects, especially on *E. tenella*. So perhaps it is time for more research to be conducted on the use of medicinal plants in the treatment of important diseases such as coccidiosis so that they can be officially used as new generation drugs in the treatment of this disease before drug resistance to current commercial anticoccidials reaches a point where they become useless.

**Keywords:** Anticoccidial effects, Coccidiosis, *Eimeria*, Medicinal plants, Poultry

### Introduction

Apicomplexan parasites of the genus *Eimeria* are organisms which invade the intestinal tract, causing coccidiosis, an enteric disease of major economic importance worldwide. The disease causes high morbidity ranging from an acute, bloody enteritis with high mortality, to subclinical disease. However, the presence of intestinal lesions depends on the *Eimeria* species. The most important poultry *Eimeria* species are: *E. tenella*, *E. necatrix*, *E. acervulina*, *E. maxima*, *E. brunetti*, *E. mitis*, and *E. praecox*.<sup>1</sup> Coccidiosis has a high economic impact on the poultry industry worldwide.<sup>2, 3</sup> The etiology of this intestinal disease are pathogenic *Eimeria* species that belong to the phylum Apicomplexa, in particular *E. maxima*, *E. tenella*, and *E. acervulina*.<sup>4</sup> Differences between these *Eimeria* species include the invasion of specific sites of the intestine, pathogenicity and type of lesion produced.<sup>5</sup>

The clinical disease in broilers includes diarrhea (from mucoid and watery to hemorrhagic), reduction in weight gain and feed ingestion, and in severe cases, mortality.<sup>6</sup> Most chickens are infected with coccidias at some

point in their life, but only a few will develop clinical manifestations of coccidiosis. The clinical symptoms tend to occur in young animals, but occasionally affect adults.<sup>4, 7</sup> Due to the self-limiting nature of the life cycle and enhanced resistance to reinfection, coccidiosis is rarely a problem in extensively raised systems, but it becomes important in closely confined and highly intensive production systems. The strategies for the control of the disease includes the use of vaccination and drugs for prophylaxis. Nevertheless, the continuous use of different coccidiostats have led to drug resistance by *Eimeria*.<sup>8</sup>

Coccidiosis has a high impact in the poultry industry where parasite transmission is favoured by high-density housing of large numbers of susceptible birds. Coccidiosis control in poultry is achieved by careful husbandry combined with in-feed anticoccidial drugs or vaccination with live parasites. However, outbreaks of coccidiosis still occur and subclinical infections, which significantly impact on productivity and food security, are common due to widespread drug resistance, high parasite prevalence, and environmental persistence.<sup>9</sup> There have been numerous reports of resistance in poultry *Eimeria* species to a variety of anticoccidial drugs, including clopidol, diclazuril, maduramycin, monensin, salinomycin, toltrazuril, sulfachloropyrazine, amprolium, sulfaquinoxaline/sulfadimethoxine, nicarbazin, and halofuginone.<sup>10, 11, 12</sup>

According to mentioned reports, severe drug resistance is observed in field studies. Overall, coccidiosis is a major and recurring problem for livestock productivity and food security, current control methods are not perfect, progress on third generation anticoccidial vaccines has been slow and new approaches based on genetics, genomics, and biology offer some renewed opportunities. Among alternative approaches, traditional treatments using medicinal plants have been investigated and researched in recent years. In the present study, the subject of discussion is a review of a number of studies conducted to conclude whether herbal compounds can be counted on as an effective alternative in the treatment of *Eimeria* infections in poultry in the not too distant future.

### Materials and Methods

For example, a total of 150 day-old broiler chicks from a local hatchery should be reared in a controlled coccidian-free house until experimentation. The birds should be randomly assigned to 6 treatment groups. Each treatment group consists of 25 birds with 5 replicates per group and 5 birds per replicate. Birds in groups 1, 2, and 3 should be infected with certain *Eimeria spp.* and receive 2, 4, and 6 gr of desired herbal compound/ kg of the diet, respectively, and those in group 4 should receive 66 mg salinomycin/ kg diet. Birds in groups 5 and 6 should be infected, unmedicated as the PC, and uninfected, unmedicated as the NC, respectively.<sup>13</sup>

A starter diet was provided for day 1–21 and a finisher diet was provided for day 22–28. Feed and water should be accessible ad libitum. The desired medicinal plants should be purchased from a local supplier. The plants should be ground into a fine powder for chemical analysis. The approximate analysis of plants powder should be conducted to determine moisture, CP, total crude fiber, gross energy, ash, ether extract, total fiber fraction: acid detergent fiber, and neutral detergent fiber.<sup>14</sup> The dry powder of desired plants should be mixed with the basal diet. On day 21, birds in each replicate must be weighed and have similar weights and sizes, which are recorded as the initial BW before the parasitic challenge.<sup>13</sup>

Unsporulated oocysts of certain *Eimeria spp.* should be obtained from the feces of naturally infected chickens on the 7 d postinfection (DPI). These unsporulated oocysts must be sporulated, purified, and preserved using a 2.5% potassium dichromate solution at 25 °C for 72 h.<sup>15</sup> The identification of sporulated oocysts can be done by the observation of lesions and use of morphologic characteristics and morphometry.<sup>16</sup> The sporulated



oocysts should be kept at 4 °C until use. In addition, sporulated oocysts should be passaged and propagated 2 to 3 times in 2-week-old healthy broiler chickens to confirm the site of lesions and activate parasites before use. After the confirmation of the parasite species, oocysts should be collected, sporulated, and washed, and the dose adjusted to  $4 \times 10^4/100 \mu\text{L}/\text{bird}$ . At 21 d of age, all the birds, except for group 5, should be orally gavaged with a 1 mL suspension of distilled water containing 40,000 desired *Eimeria spp.* sporulated oocysts.<sup>13</sup>

The anticoccidial effectivity of plants powder could be assessed using diarrhea, relative BW gain (BWG), survival rate, oocyst output/g excreta, and lesion scores. Clinical symptoms and mortality should be recorded every DPI. The anticoccidial index (ACI) of each treatment must be calculated;  $\text{ACI} = (\text{relative ratio of BWG} + \text{survival rate}) - (\text{lesion scores} + \text{oocyst value})$ .<sup>17</sup> An ACI >180 is considered to have an excellent anticoccidial impact, 160–180 is considered marked, 140–160 is considered moderate, 120–140 is considered as mild, and an ACI <120 is considered inactive.<sup>18</sup> On day 4–7 after infection, the birds should be examined for diarrhea/ bloody diarrhea by enumerating excreta 2 times a day. The degree of diarrhea/ bloody diarrhea is assigned to at least 1 of 4 categories, from zero to 3, based on the average of excreta pieces rounded to the closest integer.<sup>19</sup> Excreta should be collected from all replicates of each group from 4–7 DPI. The oocysts are counted using a hemocytometer. In short, 1 g of each well-mixed feces sample is spread in 10 mL water. Oocysts are counted under an optical microscope at suspension of 10  $\mu\text{L}$ . The results should be expressed as oocysts/g output. The oocyst value and oocyst reduction rate are calculated.<sup>17</sup>

$$\text{Oocyst value (\%)} = \frac{\text{Oocysts/g output of every group}}{\text{Oocysts/g output of the PC group}} * 100$$

$$\text{Oocyst reduction rate (\%)} = \frac{\text{OPG of PC group} - \text{OPG of experimental group}}{\text{OPG of PC group}} * 100$$

$$\text{Survival rate} = \frac{\text{Number of surviving chickens}}{\text{Initial No. of chickens}} * 100$$

At 0 and 7 DPI, all the chickens in each replicate are weighed to get the starting and final BW, respectively. The BWG is calculated as the final BW minus the initial BW.

$$\text{Relative ratio BWG} = \frac{\text{Average BWG in each group}}{\text{average BWG of the CN group}} * 100$$

At day 7 DPI (day 28 of age), 1 bird from each replicate (5 bird/group) should be slaughtered by cervical dislocation, and the specific parts of small and large intestine of each bird must be removed and inspected. Lesion scores are ranked as 0 to 4 based on the disease severity. Zero represented the normal position and 4 corresponded to each of the foremost severe and dead birds.<sup>13</sup> Performance measures of the experimental birds include an increase in BWG and feed consumption (FI). All birds are weighed individually, and the BWG should be calculated by subtracting the initial BW (at 0 DPI) from the final BW (at 7 DPI). At the end of the trial, the FI of each replicate is measured by subtracting the residual feed weight from the offered feed weight at the start of the trial. The FCR for each treatment is computed using the data obtained from FI and BW and the formula  $\text{FCR} = \text{feed intake (g)}/\text{BWG (g)}$ . The European production efficiency factor (EPEF) should be calculated using the method:<sup>20</sup>

$$EPEF = \frac{\text{Livability} * \text{Live weight (kg)}}{\text{Age in days} * \text{FCR}} * 100$$

The Statistical Analysis System statistically analyzes data collected. The anticoccidial and performance indicators are expressed as mean ± SEM and a 1-way variance analysis is used to determine differences in those parameters between groups. Using the nonparametric Kruskal-Wallis H test, the diarrhea/ bloody diarrhea and lesion scores of each treatment are compared. The means of data should be separated using Ryan-Einot-Gabriel-Welsch's multiple comparison test for statistical differences. A comparison with P-values <0.05 is considered to be significantly different.<sup>21</sup>

### Findings

As it was mentioned, anticoccidial effects of herbal compounds were investigated by many studies. In some of these studies, it was discovered that herbal compounds might show good anticoccidial effects, especially on *E. tenella*. In a study by Zaman et al. (2012), the anticoccidial effect of different concentrations of the herbal complex of 4 plants on *E. tenella* was examined. It was discovered that treatment with 6 gr of the herbal complex significantly reduced the negative performance and pathogenic effects associated with *E. tenella* challenge at a level that was comparable with amprolium when using a largely susceptible recent field isolate.<sup>22</sup> In a study conducted by Habibi et al. (2016), anticoccidial effects of herbal extracts on *E. tenella* infection in broiler chickens were examined. In conclusion, the study showed that herbal extracts were effective in control of coccidiosis caused by the *E. tenella* infection.<sup>23</sup> In another study by Qaid et al. (2021), anticoccidial effectivity of *Cinnamomum verum* in broiler chickens infected with *E. tenella* was investigated. It was concluded that *C. verum* at level 6 gr cinnamon/kg diet moderately reduced coccidiosis and attempted to improved BW, feed conversion ratio, and production efficiency at 7 DPI compared with the unmedicated chickens.<sup>13</sup> In another study also conducted by Qaid et al. (2022), anticoccidial effect of *Cinnamomum verum* bark and *Rumex nervosus* leaves in experimentally infected broiler chickens with *E. tenella* was evaluated. The results showed that *R. nervosus* leaves were not able to reduce weight loss caused by coccidiosis, *C. verum* bark at 6 g improved FCR and production efficiency index (PEI) at 7 DPI compared to the unmedicated chickens. In conclusion, *R. nervosus* leaves at 5 gr and *C. verum* bark at 6 gr have moderate anti-coccidial activity and could be used to treat poultry coccidiosis in the field.<sup>24</sup> In a study by Jiang et al. (2024), the effects of natural extracts from nine medicinal herbs on the growth performance, immunity, and intestinal integrity of broilers experimentally infected with *E. tenella* were evaluated. Results showed that feeding extracts could significantly reduce the number of oocysts in infected chickens, especially 1.0 g/kg extracts, which exhibited moderate anticoccidial efficacy. When infected with *E. tenella*, the supplementation of 1.0 g/kg extracts increased the renal index; restored the hepatic, splenic, and bursal indexes to BC levels; increased the levels of immunoglobulin A (IgA), IgM, and IgY; and reduced the contents of tumor necrosis factor (TNF-α), interferon-γ (IFN-γ), interleukin-6 (IL-6), and IL-10 of the infected chickens.<sup>25</sup> Some anticoccidial medicinal plants with their bioactive components are mentioned in Table 1 (designed by Jamil et al.).

**Table 1.** Anticoccidial medicinal plants with their bioactive components and applications.<sup>26</sup>



| Scientific Name                               | Common Name        | Secondary Bioactive Metabolite                      | Applications                                                                                       |
|-----------------------------------------------|--------------------|-----------------------------------------------------|----------------------------------------------------------------------------------------------------|
| <i>Camellia sinensis</i><br><i>kuntze</i>     | Green tea          | Polyphenolic compounds                              | Inactivate the enzyme for coccidian sporulation                                                    |
| <i>Pinus radiata</i> D. Don                   | Pine bark          | Tannins                                             | Effective against <i>E. tenella</i> , <i>E. maxima</i> , <i>E. acerulina</i>                       |
| <i>Cyamopsis</i><br><i>tetragonoloba</i> Taub | Guar bean          | Saponins which might lyse oocyst                    | Reduce the chance of coccidiosis in chicken                                                        |
| <i>Berberis lycium</i> Royle                  | Barberry root bark | Isoquinoline alkaloid berberine                     | Inhibit the sporozoites of <i>E. tenella</i> in chicken through the initiation of oxidative stress |
| <i>Vitis vinifera</i>                         | Grape seed         | Proanthocyanidin                                    | Reduces the coccidiosis via downregulation of oxidative stress                                     |
| <i>Olea europaea</i>                          | Olive tree         | Maslinic acid                                       | Enhances the anticoccidial index                                                                   |
| <i>Quisqualis indica</i>                      | Rangoon creeper    | gallic acid and ellagic acid                        | Decreased lesion score, reduced oocyst and mortality                                               |
| <i>Morinda lucida</i>                         | brimstone tree     | alkaloids, anthraquinones, and anthraquinols        | Decreased oocyst count                                                                             |
| <i>Artemisia afra</i>                         | African wormwood   | Flavonoids, terpenes, coumarins, and phenolic acids | Decreased oocyst count, increased feed consumption, reduced lesion score                           |
| <i>Echinacea purpurea</i><br><i>Moench</i>    | Coneflower         | Flavonoid echinolone, chloric acid                  | Provoke the humoral immune response against the coccidiosis in poultry                             |
| <i>Curcuma longa</i>                          | Turmeric rhizome   | Curcumin (diferuloylmethane)                        | Inhibition of sporozoites of <i>E. tenella</i> and reduced gut damage in poultry                   |
| <i>Aloe vera</i> (L.) Burm. f.                | Aloe leave         | Acemann sugars anthraquinones                       | Aloe vera supplemented group exhibited considerably lesser intestinal lesions                      |
| <i>Phyllanthus emblica</i>                    | Emblic fruits      | Tannins                                             | Against coccidiosis                                                                                |
| <i>S. flavescens</i>                          | Shrubby sophora    | Sophorae Radix                                      | Reduced oocyst count, decreased lesion score and decreased mortality                               |
| <i>Moringa oleifera</i>                       | Drumstick tree     | Flavanol, rutin and glycoside                       | Reduced oocyst count and increased body weight                                                     |

### Discussions and Conclusion

In industrial poultry production, especially in broiler chickens, coccidiosis, caused by *Eimeria* protozoa parasites in the digestive tract, continues to be a serious health and economic problem. In economic terms, the losses are mainly caused by the costs of prevention and treatment, as well as the reduced weight gain and reduced condition of the birds. Coccidiosis is a well-known infectious disease. It causes terrible bloody diarrhea in birds by destroying intestinal epithelial cells. Currently, the prophylactic program of this disease unit is mainly based on the use of chemotherapeutics. Another way to control coccidiosis is active immunoprophylaxis, based on the vaccination of chickens for fattening, and passive immunoprophylaxis, based on vaccination of the parent flock. An alternative, effective way to reduce the negative effects of protozoan infestation by *Eimeria spp.* may be the use of herbs and their extracts (as it was aforementioned), pre- and probiotics and betaine.

Overall, what we can learn from the studies results, is that herbal medicine's mechanism of action is not fully understood; if an analysis is performed to fill this hole, they would be able to suggest nontoxic and effective dosage determination methods, drug preservation, and value addition. The advances in genomics, proteomics, metabolomics, bioinformatics, and chemoinformatics should be used to detect and improve medications. Native medicinal plant products should be tested using biotechnological advancements as a high-throughput screening platform. Furthermore, it will allow for further practices, such as preserving herbal extracts for longer shelf life, forming tablets, herbal teas, and infusions, lyophilization (freeze dried products), or even fortifying food with herb extracts. Ethnoveterinary reports should be used carefully, and approve with controlled experiments if medicinal plants increase the parasite's resistance. Performance and behavior of parasitized hosts should be monitored. Local and systemic immune responses should be tracked, and host health and performance during experiments must be monitored. Which components are active should be determined and the bioavailability should be calculated and methodologies must be established.

Thus, according to the results obtained from many studies, the use of herbal medicines may be a good alternative to treat parasitic infections. Several parasitic infections can be cured and controlled using herbal medicine, coccidiosis included. Owing to their wide distribution, economical nature, and ease of use, some of these medicinal plants could serve as a moderate alternative anticoccidial agent and provide a new tactic for the controlling and preventing of poultry coccidiosis. According to the World Health Organization, over 80% of the population relies on plants to treat common ailments. Although traditional medicine information is diverse, no major steps have been taken to interpret and promote its use for clinical purposes, so perhaps it is time for more research to be conducted on the use of medicinal plants in the treatment of important diseases such as coccidiosis so that they can be officially used as new generation drugs in the treatment of this disease before drug resistance to current commercial anticoccidials reaches a point where they become useless.

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## THE USE OF MEDICINAL PLANTS IN THE AQUACULTURE INDUSTRY AND FISH HEALTH

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### **Abstract**

Medicinal plants are a group of plants that, in addition to their medical and pharmaceutical uses in humans, are also used in livestock, poultry, and aquatic animals. Many plants contain compounds that play an effective role in improving, treating, or preventing diseases, and these compounds are called active ingredients. Herbal medicines are used for various purposes in the aquaculture industry, including stimulating the immune system of fish and other farmed aquatic animals, acting as probiotics, treating infectious diseases, sedating and anesthetizing fish, using them to treat fish poisoning, healing superficial wounds in the bath therapy method with herbal immune stimulants, and as a coating to increase the shelf life of fishery products in aquatic animal processing. Nowadays, plant-based immune stimulants have attracted more attention due to their advantages such as availability, lower risk to the environment and animals, and lower cost.<sup>1</sup> Also, plant compounds and products extracted from plants are added to the feed to improve the nutritional acceptability of foods and growth performance in farmed animals. Active plant compounds (such as phenolic compounds and flavonoids) can have several effects on living organisms. These effects include improving feed efficiency and digestion, reducing nitrogen excretion, improving intestinal flora and animal health status.<sup>2</sup> Therefore, the use of immunostimulants in fish farming has become widespread in order to increase the immune system and non-specific immune responses and protect the body against diseases. For this reason, the use of immunostimulants is a suitable solution for controlling aquatic disease.<sup>3</sup>

**Keywords:** Medicinal plants, Aquaculture, Fish health, Immune system stimulants

### **Definition of Medicinal Plants**

Medicinal plants are a group of plants that are used for medical, therapeutic, clinical and pharmaceutical purposes in humans, livestock, poultry and aquatic animals. There are compounds in plants that play an effective role in improving, treating or preventing diseases, and these compounds are called active ingredients. The active ingredients of medicinal plants include complex chemical compounds that are produced and stored in various organs of medicinal plants and are usually used as raw materials for pharmaceuticals. The active ingredients of medicinal plants are of two types: the first type is the substances resulting from primary metabolism (mainly saccharides) or the necessary and vital substances that are produced in all green plants by photosynthesis. The second type of secondary metabolites are produced by the plant as a result of nitrogen absorption. These products are apparently often useless for the plant, but on the contrary, their therapeutic effects are significant. Generally, these substances are not found in pure form in nature and are combined with other elements that complement and enhance their effects.<sup>4</sup>



## New Aspects of Medicinal Plant Use

Recently, the use of compounds that are generally considered harmless has attracted much attention. Natural bioactive compounds derived from plants are among the most important of these compounds because substances derived from essential oils and plant extracts can be used for food preservation and in pharmaceuticals and as new therapeutic agents against diseases and microbial infections.<sup>5</sup> The major trend in the world towards the use of medicinal plants in the treatment of various diseases is that about 20,000 species of plants are used for medicinal purposes in the world.<sup>6</sup> About 3,000 types of plant essential oils have been identified, of which 300 types are commercially important and are mainly used to create aroma and flavor. However, currently, about 25-50% of the commonly used drugs in the world are of plant origin. According to the 1999 FDA statistics, more than 25% of the American population uses herbal medicines and spends about \$5 billion on herbal medicines to treat depression, enhance sexual function, reduce weight, and enhance memory.<sup>7</sup>

### Active ingredients of medicinal plants

The active ingredients of medicinal plants are of two types:

- 1- Substances resulting from primary metabolism or necessary and vital substances that are produced in all green plants by photosynthesis.
- 2- Substances resulting from secondary metabolism that are produced as a result of nitrogen absorption by the plant. These products are apparently often useless for the plant, but their therapeutic effects are significant. These compounds include essential oils, resins and various alkaloids.

The classification of active (medicinal) substances of plants, which is accepted today, is into four main groups: alkaloids, glycosides, volatile oils, and other active substances. Other active substances include compounds such as: bitter substances, flavones, flavonoids, mucilages, and similar specific carbohydrates, vitamins, tannins, silicic acid and similar specific acids, and finally other compounds like them that do not fall into the previous three groups due to the inconsistency and breadth of their chemical structure.

#### 1- Alkaloids

Alkaloids produce strong physiological reactions in humans with specific effects, especially on the nervous system. Some types of alkaloids have very pronounced and significant pharmacological effects and are therefore very useful and valuable. In this case, caffeine, strychnine, quinine, morphine, and the like can be mentioned. Since alkaloids are mostly toxic and deadly, the extraction and use of alkaloid-containing plants should only be carried out in relevant industries and under the supervision of technical experts.

#### 2- Glycosides

Glycosides are produced in various forms in different metabolic pathways. Glycosides are converted into sugar compounds (glycones) and non-sugar compounds (aglycones) after hydrolysis (by acids, some enzymes, etc.). The latter compounds (aglycones) have many uses in pharmaceuticals. One of the most important glycoside compounds is cyanogenic glycosides, which are products of glycoside compounds after their hydrolysis with hydrocyanic acid. One of the most important cyanogenic glycosides is amygdalin, which is widely found in plants of the *Rosaceae* family (apple, pear, tomato, almond, etc.), *Leguminosae* (clover, bean, etc.), *Aquifolium*, *Flax*, and some other families. Some of these substances are used in the preparation of cough medicines, laxatives, and painkillers. Another important glycoside compound is anthraquinone glycosides. The aforementioned glycosides produce anthraquinone after hydrolysis. Anthraquinones play a major role in the treatment of constipation. Plants

containing anthraquinones include senna, cyathose, rhubarb, and several other plants. Finally, another important glycoside is cardiac glycosides. The chemical structure of cardiac glycosides is different from other glycoside compounds. These glycosides have specific effects on the heart muscles. Cardiac glycosides are found in some flowering plants such as the *Oleander*, *Foxglove*, *Alale*, and several other families.

### 3- Essential oils

The third group of active ingredients found in plants is essential oils. Essential oils are not homogeneous in terms of chemical composition, but are observed as different compounds. However, they are generally from the chemical group known as terpenes or have terpene origin. These compounds usually have a pungent odor and taste and their specific gravity is often less than water (rarely, some of them have a specific gravity higher than water). These substances are also known as volatile oils.

### 4- Bitter substances

These substances are known as bitter substances due to their bitter taste. Bitter substances have the ability to significantly stimulate the digestive glands to secrete digestive juices. In particular, they cause the secretion of digestive juices in the stomach. These substances also affect the taste buds in the mouth. Bitter substances also increase the movements of the gallbladder and the motility of the digestive tract (stomach and intestines). Bitter substances can not only be used as appetite stimulants, but they also cause rapid digestion of food and accelerate metabolic activities.

### 5- Flavones and flavonoids

Flavones and their derivatives (flavonoids) are substances that occur free in many plants or in combination with glycosides. These substances belong chemically to the phenols. Some of the compounds belonging to this group include coumarins and anthocyanins. The latter substance (anthocyanins) is responsible for the blue and red colors of some flowers. The most important derivatives of flavones are usually yellow (the name flavones is derived from the Latin word flavus, meaning yellow).

### 6- Mucilages

Mucilages are carbohydrates with a very complex chemical structure and high molecular weight. These substances are insoluble in alcohols. Mucilages dissolve in water and swell and become bulky after absorbing water. Plants that contain mucilage compounds and are of particular importance in the pharmaceutical industry include *Althea* (almond), *Cinum*, and *Certaria*. The most important medicinal properties of mucilages are their anti-irritant properties. Mucilage produces a thin protective layer on the gastric mucosa and prevents the effect of irritating agents on the surface. Therefore, these substances are used to treat ulcers in the digestive tract and infections of the throat and pharynx. The water-absorbing properties of mucilages are used to reduce the water in the digestive tract. Mucilages can also be used externally to treat certain skin conditions. Since mucilage is not absorbed or distributed throughout the body, its cutaneous effects are always limited to the area of application.

### 7- Saponins

Saponins are glycosides with high molecular weight. The most important physical property of these substances is that they produce foam when dissolved in water. Therefore, saponins have cleansing properties. Saponins cause hemolysis of red blood cells when they enter the bloodstream, so it is better to monitor their consumption as a relatively toxic substance. Of course, eating these substances is not very harmful, but they are



also diuretic, laxative and expectorant, and they can even be used as an antitussive. Saponins are used as an agent that helps intestinal absorption of other medicinal substances along with them.

### **8- Silicic acid**

Silicic acid is used to treat some diseases. The presence of this substance is very important for the growth of hair and fingernails and toenails, and the negative consequences of a deficiency of this substance in the daily human diet are clearly visible on the aforementioned organs.

### **9- Tannins**

Tannins refer to different compounds. These compounds are generally hard, loose, and astringent and have the ability to bind to proteins and precipitate them. The properties of hardness and astringency can only be used to combat common diarrhea, chronic bloody diarrhea, heal wounds, and infections related to the throat and trachea (and the digestive and respiratory tract in general). Since tannins cause protein deposition in the mucous membranes, they deprive bacteria of nutrients in the aforementioned membranes. Therefore, they also have antibacterial properties.

### **Importance of medicinal plants in aquaculture:**

Herbal and natural medicines have increased the desire to use natural products of plant origin due to factors such as low side effects compared to chemical drugs, lack of relative resistance of pathogens to herbal medicines, exclusive treatment of some diseases with medicinal plants, as well as recent bans and restrictions on the use of growth-promoting antibiotics.<sup>8</sup> Today, the use of medicinal plants together or with modern drugs in combination to reduce drug side effects has been welcomed for therapeutic purposes. Herbal medicines are used for various purposes in the aquaculture industry, including stimulating the immune system of fish and other farmed aquatic animals, acting as probiotics, treating infectious diseases, sedating and anesthetizing fish, and healing superficial wounds in the bath therapy method with herbal immune stimulants.

### **Application of medicinal plants in aquaculture**

#### **1- Medicinal plants as immune system stimulants**

In the aquaculture industry, pathogens are factors that reduce production, and given the greater development of non-specific immunity in fish compared to specific immunity and the special role of immune stimulants in stimulating non-specific immunity, the use of these stimulants in aquatic animals is more preferable than in warm-blooded animals. Among the different immune stimulants, plant-derived immune stimulants have advantages such as availability, lower risk to the environment and the animal, and lower price.<sup>1</sup>

#### **2- Use of medicinal plants in hatcheries**

The rapid and significant development of the aquaculture industry in the country, especially the reproduction and farming of rainbow trout, and the major problems of its reproduction centers, especially the severe losses during egg incubation, have led to efforts to find suitable natural alternatives, including various plant extracts and essential oils, instead of malachite green.

#### **3- Use of medicinal plants as anesthetics in the aquaculture industry**

Anesthetics in fish are of particular importance in reducing stress caused by handling fish during research activities, vaccination, sexual maturity detection and egg collection from broodstock in artificial propagation operations, moving and transporting fish from one place to another, surgical procedures and other cases. Several studies have been conducted on the anesthetic effects of clove flowers in some fish species.

#### 4- Medicinal plants in the treatment of fish poisoning

In studies, the seeds of *Impatiens balsamina* have been found to be useful in the treatment of fish poisoning. It has been reported that the seeds of this plant contain six types of glycosides. Also, the acetone extract of the dried leaves of *Sophora tomentosa* has been used in the treatment of fish poisoning.

#### 5- Use of medicinal and aromatic plants in aquatic animal processing

Due to consumer concerns about the use of artificial preservatives in recent years, the trend towards the use of natural preservatives in food has increased. In a study, researchers showed that marjoram extract had antimicrobial activity in culture medium and food model of cod and shrimp fillets.

#### 6- Use of medicinal plants against pathogens and infectious diseases of aquatic animals

Medicinal plants, with their various antioxidant and immune system stimulant compounds, have significant effects in strengthening the immune system of fish and increasing the body's resistance to pathogens. Glycyrrhizin is a plant immunostimulant and is known as an anti-inflammatory and antitumor substance. Edahiro et al. reported in 1990 and 1991 that the oral administration of Glycyrrhizin to yellowtail fish increased the resistance of this fish to *Edwardsiella seriola*.

#### Review of research

During the study of the effect of saffron petal powder (*Crocus sativus* L.) on growth performance, nutrition, and antioxidant enzyme activity of grass carp larvae (*Ctenopharyngodon idella*), it was determined that the amount of malondialdehyde and catalase in larvae with different levels of supplementation was significantly reduced compared to the control group, and a significant increase in glutathione peroxidase and superoxide dismutase parameters was observed compared to the control group. As a result, this study shows that the use of saffron petals can be used as a feed additive in aquaculture.

By investigating the effect of hydroalcoholic extracts of oregano (*Origanum vulgare* L.) and safflower (*Hypericum perforatum*) on growth indices, innate immunity, digestive enzyme activity, and antioxidant activity in rainbow trout, it was determined that a significant difference was observed between immune parameters, digestive enzyme activity, and antioxidant enzyme activity between the 3% oregano treatment and the control treatment. Overall, the results of this study showed that the use of hydroalcoholic extract of oregano significantly improved the growth and immune system of this fish under commercial farming conditions.

The effect of extracts of *Conocarpus* (*Conocarpus erectus* L.), *Paulownia* (*Paulownia fortunei*) and their mixtures on growth and feeding efficiency, digestive enzymes, and non-specific immunity of salmon was studied, and the results showed that the levels of digestive enzymes showed a significant increase in the treatment of fish fed with one percent of *Paulownia* extract compared to other treatments and the control, and the levels of glucose and hemoglobin in fish consuming the plant extract increased significantly.

By examining the separate and combined effects of chicory (*Cichorium intybus*) and tea grass extracts on immune indices and antioxidant enzyme activity of rainbow trout, they found that the separate and combined addition of extracts of both plants to the diet led to an increase in the activity of SOD and GPX enzymes compared to the control diet, and as a result, adding 3% chicory plant extract to the diet improved immune indices and 3% tea grass led to an increase in the level of antioxidant enzyme activity of rainbow trout.

Researchers investigated the effect of dietary supplementation of *Artemisia annua* L. extract on some hematological and biochemical indices of blood serum of common carp and concluded that glucose levels



increased significantly in the fed treatments, and the results of liver enzymes also showed that the fish diet did not have a significant difference in the activity of aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase compared to the control group, and as a result, *Artemisia annua* L. extract in the diet of common carp can have significant effects on the health status of the fish compared to the control treatment.<sup>9</sup>

In a study, the effects of hydroalcoholic extracts of barberry and sumac on growth factors, immunity, and stress responses of rainbow trout were investigated, and the results showed that adding barberry and sumac plants at a level of 100 mg/kg of food to the diet of rainbow trout is effective in improving and enhancing growth, stress, and immunity factors of fish and will lead to increased production efficiency in aquaculture.

By examining the separate effects of garlic and ginger powder (*Zingiber officinale*) on liver indices, carcass chemical composition, digestive enzyme activity, and resistance of fry of Sabiti, they found that the combination of garlic and ginger improves carcass chemical composition, increases digestive enzyme activity, and increases resistance to environmental stresses in this fish.

By examining the effect of soy lecithin on some immune and blood biochemical indices of Caspian salmon progenitors, researchers found that the results of immune parameters, activity of glutathione transferase and superoxide dismutase enzymes, along with some blood biochemical indices, indicated statistical superiority of the treatments over the control treatment, and soy lecithin can be used due to its positive effects and improvement of physiological parameters of Caspian salmon.

By examining the effect of water extract of tea grass on blood and serum indices and survival of rainbow trout, it was determined that the levels of liver enzymes AST and ALT increased in the second stage compared to other groups. Based on the results, tea grass has the ability to control the effects of stress and increase the survival of trout when exposed to chronic heat stress. By studying the effects of garlic and Echinacea medicinal plants on growth indices, blood parameters, and resistance to bacterial contamination in rainbow trout, it was determined that the use of 1% garlic extract and 0.5% Echinacea extract stimulates the immune system and increases the resistance of rainbow trout to pathogens.

Based on the findings of the study aimed at evaluating the effect of chamomile and yarrow extracts on rainbow trout, it can be concluded that the presence of various compounds, including flavonoids, tannins, terpenoids, etc. in the hydroalcoholic extract of chamomile flowers and also the hydroalcoholic extract of yarrow flowers had antimicrobial effects, especially antifungal effects against fungal infections during the incubation period of rainbow trout eggs. By examining the effect of garlic and Echinacea extracts on some non-specific immune responses and resistance to heat stress in rainbow trout, it was determined that the level of lysozyme activity was higher than the control treatment, in terms of total serum immunoglobulin levels, treatment 3 had a significant difference with other treatments, and in terms of glucose levels, there was no statistically significant difference between the treatments. Based on the findings of this study, it can be concluded that the use of garlic and Echinacea extracts improves immune responses in rainbow trout.

A study of the digestive physiology of river yellow carp fed with different levels of alfalfa meal showed that the activity of protease and amylase enzymes initially increased and then decreased with increasing alfalfa meal. The length of intestinal villi also increased with increasing alfalfa meal by 10%. Overall, the highest levels of digestive enzyme activity and intestinal villi length were observed in the treatment containing 10% alfalfa meal.<sup>10</sup>

A study was conducted to evaluate the effects of garlic on antioxidant activities in tilapia fish by adding garlic powder to the diet, and the results showed that the antioxidant activities of the treatments increased significantly compared to the control group, and adding garlic to the fish diet increased growth rate, reduced mortality rate, and increased antioxidant activity in the plasma of this fish.<sup>11</sup>

Digestive enzyme activity and growth of shoefish (*Paralichthys olivaceus*) were investigated after feeding with a diet containing Chinese herbs and it was found that pepsin and protease activities as well as specific growth rate were higher in the experimental treatments than in the control group. Also, the use of these herbs improved the feed conversion ratio compared to the control group, while the activity of amylase enzyme was not significantly different compared to the control group.<sup>12</sup>

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# THE EFFICACY OF MEDICINAL PLANTS: A SYSTEMATIC REVIEW OF THEIR ROLE IN DISEASE PRESERVATION AND CONTROL FOR LARGE LIVESTOCK, WITH A FOCUS ON RUMINANT HEALTH AND PRODUCTIVITY

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## Abstract

The sustainable intensification of global livestock production is critically dependent on effective animal health management. In large ruminants (cattle, sheep, goats), the reliance on antimicrobials for disease treatment and growth promotion has contributed significantly to the escalating crisis of antimicrobial resistance (AMR), driving the urgent need for natural, sustainable alternatives. Medicinal plants, with their vast array of bioactive secondary metabolites such as tannins, saponins, flavonoids, and essential oils, present a profoundly promising solution. These phytochemical compounds exhibit a wide spectrum of biological activities, including antimicrobial, antiparasitic, anti-inflammatory, immunomodulatory, and rumen modulatory properties. This systematic review synthesizes a global body of evidence, integrating significant findings from international and Iranian research, to critically evaluate the multifaceted effects of various medicinal plants on the health, productivity, and disease resistance of large livestock. The consolidated findings robustly demonstrate that dietary supplementation with extracts, essential oils, or whole plants—such as sainfoin (*Onobrychis viciifolia*), garlic (*Allium sativum*), thyme (*Thymus vulgaris*), wormwood (*Artemisia absinthium*), and tannin-rich forages—can significantly enhance rumen fermentation, reduce methane emissions, control gastrointestinal nematodes, mitigate metabolic disorders, and improve overall productivity. The mechanisms are pleiotropic, involving direct anthelmintic and antimicrobial effects, modulation of the rumen microbiome, enhancement of host immune function, and reduction of oxidative stress. This review concludes that phytochemical feed additives are viable, natural, and effective tools for integrated health management in ruminant production, aligning with the principles of sustainable agriculture and the "One Health" paradigm. However, challenges regarding the standardization of active compounds, determination of optimal dosages, and evaluation of long-term efficacy under varied farming systems must be addressed through future interdisciplinary research.

**Keywords:** Health and productivity, Large livestock, Ruminant

## Introduction

The global livestock sector stands as a critical pillar of food security, economic development, and agricultural sustainability, with large ruminants (cattle, sheep, and goats) playing an indispensable role in providing high-quality protein, dairy products, fiber, and draught power to billions of people worldwide. The Food and Agriculture Organization (FAO) projects that to meet the demands of a growing and increasingly affluent global population, the production of meat and milk will need to increase by nearly 70% and 55% respectively by 2050. <sup>1</sup> This necessary intensification of production, however, brings with it a host of profound challenges, paramount among them being the increased risk and prevalence of infectious diseases, metabolic

disorders, and parasitic infestations. These health challenges are exacerbated by high stocking densities, physiological stress, and the constant pressure of production, creating environments where pathogens and parasites can thrive. For over half a century, the primary arsenal against these threats has consisted of antimicrobials, anthelmintics, and other synthetic pharmaceuticals. Used prophylactically and therapeutically, these compounds have been instrumental in safeguarding animal health and ensuring productivity. However, their widespread and often indiscriminate use has unleashed a silent pandemic: antimicrobial resistance (AMR). The World Health Organization (WHO) has declared AMR one of the top ten global public health threats facing humanity, a crisis fueled in significant part by the overuse of these drugs in animal agriculture.<sup>2</sup> The emergence of multi-drug resistant bacteria and anthelmintic-resistant parasites not only renders treatments ineffective, leading to animal suffering and economic losses, but also poses a direct and severe risk to human health through the transmission of resistant pathogens via the food chain and the environment.

Concurrently, a growing wave of consumer awareness and concern regarding drug residues in animal products, animal welfare, and the environmental footprint of conventional farming has shifted market dynamics. There is now a powerful and growing demand for naturally produced, organic, and "antibiotic-free" meat, milk, and dairy products. This consumer-driven shift, combined with increasingly stringent regulatory restrictions on the use of antibiotics as growth promoters exemplified by the European Union's complete ban in 2006 and similar movements worldwide has created an urgent and unprecedented imperative for the livestock industry. The industry must now navigate the complex challenge of maintaining animal health, welfare, and productivity while drastically reducing its reliance on conventional antimicrobials. This paradigm shift has catalyzed a fervent search for safe, effective, sustainable, and natural alternatives that can support animal health without contributing to AMR.

Within this pressing context, medicinal plants and their derivatives, often termed "phytonics" or "botanicals," have surged to the forefront of scientific and commercial interest as a promising solution. The utilization of plants for healing purposes is arguably as old as animal husbandry itself, forming the cornerstone of ethnoveterinary medicine the traditional knowledge and practice of animal healthcare—for millennia across all continents.<sup>3</sup> From the ancient texts of Ayurveda in India to the extensive pharmacopeia of Traditional Chinese Medicine, and notably within the rich and sophisticated traditions of Persian medicine, as documented by scholars like Avicenna (Ibn Sina) in *The Canon of Medicine*, plants have been revered for their therapeutic properties.<sup>4</sup> This deep-rooted historical wisdom now provides an invaluable foundation for modern scientific inquiry. Unlike synthetic drugs, which typically feature a single active ingredient targeting a specific pathway, medicinal plants present a complex matrix of secondary metabolites (such as tannins, flavonoids, alkaloids, saponins, and essential oils) that evolved as the plant's own defense mechanisms against pathogens, parasites, and predators. This complex phytochemical synergy offers a multi-targeted, "multi-pronged" approach to combating diseases, making it significantly more difficult for pathogens to develop resistance compared to single-target synthetic drugs. The biological activities of these phytochemicals are remarkably diverse and directly address the key challenges in ruminant health. Condensed tannins (CTs), found in plants like sainfoin (*Onobrychis viciifolia*), birdsfoot trefoil (*Lotus corniculatus*), and various browse trees, have demonstrated potent anthelmintic properties, offering a natural strategy for controlling gastrointestinal nematodes (GINs) whose resistance to commercial dewormers is now a global crisis.<sup>5</sup> These same tannins can modulate rumen fermentation by forming complexes



with dietary proteins, protecting them from microbial degradation in the rumen and increasing the flow of high-quality "bypass protein" to the small intestine, thereby improving nitrogen utilization efficiency.<sup>6</sup> Furthermore, tannins and essential oils from plants like garlic (*Allium sativum*), cinnamon (*Cinnamomum verum*), and clove (*Syzygium aromaticum*) have been shown to selectively inhibit methanogenic archaea and protozoa in the rumen, thereby reducing enteric methane production (a potent greenhouse gas) and potentially redirecting dietary energy towards productive purposes, enhancing feed efficiency.<sup>7</sup>

Beyond the gut, the immunomodulatory and antioxidant capacities of many medicinal plants are of critical importance. The transition period for dairy cows (the weeks immediately before and after calving) is a state of immense physiological stress and immunosuppression, making animals highly susceptible to metabolic disorders like ketosis and fatty liver disease, as well as infectious diseases like mastitis. Plants such as milk thistle (*Silybum marianum*), whose active complex silymarin is a powerful hepatoprotectant, can support liver function and mitigate the negative effects of negative energy balance.<sup>8</sup> Similarly, plants like *Echinacea* and *Urtica dioica* (nettle) can act as immunostimulants, enhancing the animal's own innate and adaptive immune responses and reducing the incidence and severity of infections.<sup>9</sup> The anti-inflammatory and antimicrobial properties of essential oils also show great promise for the topical and systemic treatment of mastitis, the most costly disease in the dairy industry, offering an alternative to intramammary antibiotics.<sup>10</sup> The scientific evidence supporting these applications is growing exponentially, with a significant and valuable contribution coming from researchers in regions with rich traditions of herbal medicine, such as Iran. Iranian researchers have extensively studied the effects of native plants like *Zataria multiflora* (Shirazi thyme), *Artemisia absinthium* (wormwood), *Trachyspermum ammi* (ajwain), and *Carum carvi* (caraway) on ruminant health, providing crucial insights into their effects on rumen metabolism, parasite control, and milk production.<sup>11, 12</sup> This body of work enriches the global knowledge base and highlights the importance of exploring locally adapted plant species.

However, the path towards the widespread and effective integration of medicinal plants into commercial livestock production is not without its obstacles. The very nature of plants introduces variability; the concentration and profile of bioactive compounds can be dramatically influenced by a multitude of factors including the plant's genetics, geographical origin, climate, soil conditions, stage of maturity at harvest, and post-harvest processing and storage methods.<sup>13</sup> This variability poses a major challenge for standardization, making it difficult to ensure consistent efficacy and safety in commercial feed formulations. Furthermore, the potent bioactive compounds that confer health benefits can also be anti-nutritional if not dosed correctly. High concentrations of certain tannins can suppress voluntary feed intake and inhibit digestive enzymes, while some essential oils can disrupt the rumen microbiome to the detriment of fiber digestion if their application is not carefully optimized.<sup>14</sup> Palatability can also be a concern with strong-flavored herbs and oils. Therefore, determining the optimal dosage (the narrow window between efficacy, inertness, and toxicity) is paramount and must be established for each plant and each production context. Moreover, while generally recognized as safe (GRAS), more extensive toxicological studies are needed to fully understand the long-term effects of feeding specific plants, potential interactions with other feed additives or veterinary drugs, and the pharmacokinetics of plant compounds to ensure there are no undesirable residues in milk and meat that could affect food safety or sensory quality. The economic viability of large-scale cultivation, processing, and incorporation into feed must also be demonstrated to convince producers of their value.

Therefore, this systematic review aims to synthesize the vast and growing body of global scientific literature, with particular attention to research from Iran and other regions active in this field, to provide a comprehensive and critical assessment of the efficacy of medicinal plants in managing diseases and improving the health and productivity of large livestock. It will explore their roles in parasite control, rumen modulation, metabolic health, immunostimulation, and mastitis management. By critically evaluating the evidence, elucidating mechanisms of action, and honestly addressing the challenges of application, this review seeks to provide a solid scientific foundation for the rational and effective use of phytogetic feed additives. Ultimately, the successful integration of these natural compounds into sustainable livestock production systems holds the promise of enhancing animal welfare, reducing the environmental impact of agriculture, providing safer food products for consumers, and mitigating the global threat of antimicrobial resistance, thereby aligning with the holistic principles of "One Health" that interconnect animal, human, and environmental well-being.

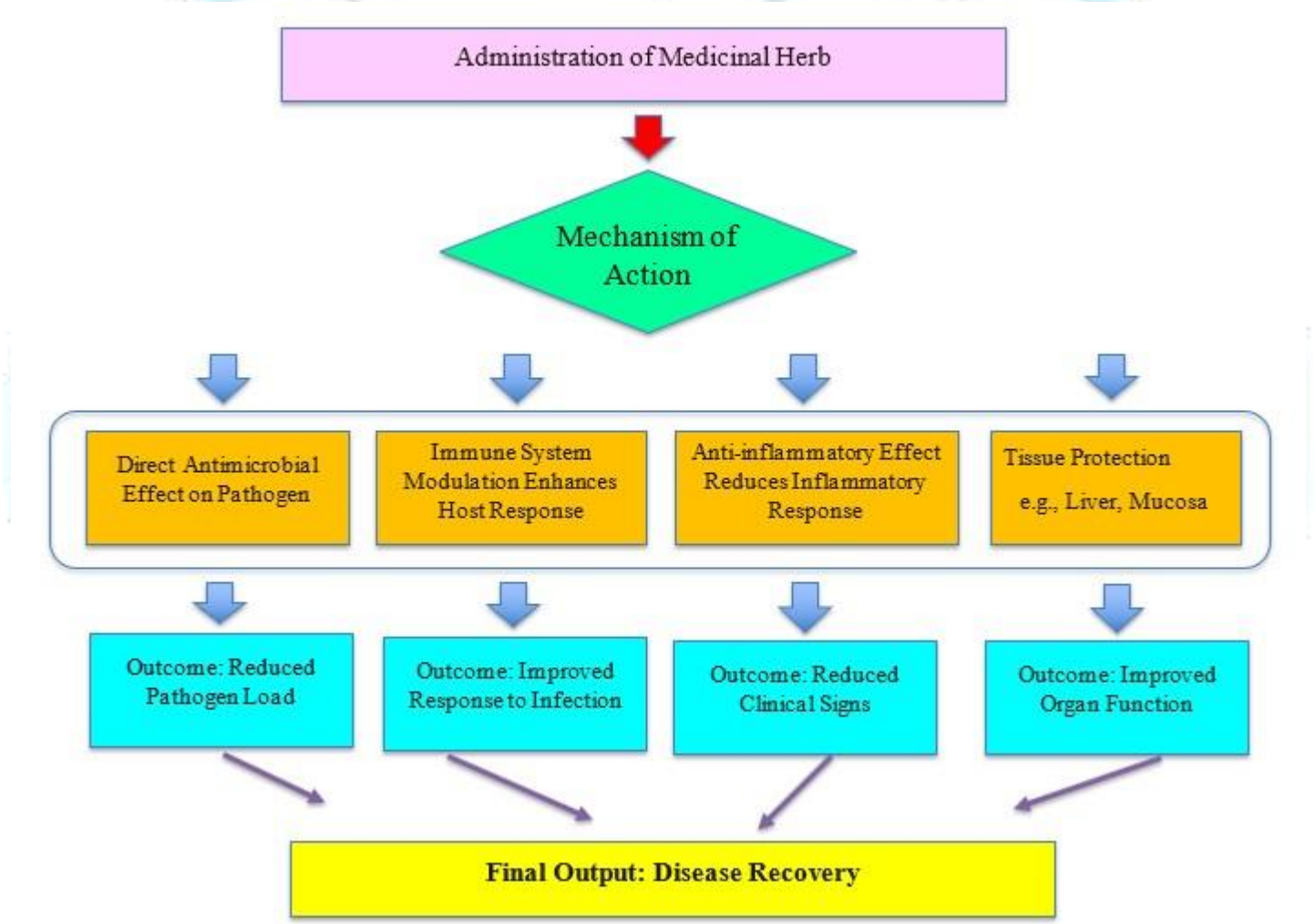


**Figure 1.** Schematic Diagram of impact of Medicinal Herbs on Diseases of Large Animals

**Methodology: Systematic Literature Review Protocol**

This systematic review was conducted in accordance with the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) guidelines to ensure methodological rigor, transparency, and reproducibility. A comprehensive and systematic electronic search was performed using major international databases (Scopus, PubMed, Web of Science, CAB Abstracts) and Persian scientific databases (Magiran, Scientific Information Database (SID), IranDoc) for relevant articles published between January 2000 and December 2023. The search strategy utilized a structured combination of keywords and Boolean operators: ("medicinal plant" OR "phytogetic" OR "herbal extract" OR "essential oil" OR "tannin" OR "forage legume")



AND ("large ruminant" OR "cattle" OR "dairy cow" OR "sheep" OR "goat" OR "livestock") AND ("disease" OR "parasite" OR "nematode" OR "rumen fermentation" OR "methane" OR "mastitis" OR "metabolic disorder") AND ("anthelmintic" OR "antimicrobial" OR "immunomodulat"). The search was adapted for Persian databases using equivalent terms. Inclusion criteria were: (a) original peer-reviewed experimental studies conducted “in vivo” on cattle, sheep, or goats; (b) interventions involving any form of medicinal plant (crude extract, essential oil, purified compound, powdered form, fresh forage) administered via diet; (c) measurement of outcomes related to health, disease resistance, parasitism, rumen function, methane production, or productivity; and (d) articles published in English or Persian. Exclusion criteria encompassed studies not performed on large ruminants, purely “in vitro” studies, review articles, and non-peer-reviewed literature. The initial database search yielded 345 potential articles. After removing duplicates and screening titles and abstracts, 140 studies were selected for full-text eligibility assessment. Ultimately, 102 studies that fully met the inclusion criteria were synthesized and analyzed in this systematic review.

### **Control of Gastrointestinal Nematodes (GINs) and Antiparasitic Effects**

Gastrointestinal nematode (GIN) infections represent one of the most significant health and economic challenges in grazing ruminants worldwide. Widespread resistance to synthetic anthelmintics has rendered many traditional dewormers ineffective, creating a critical need for alternative control strategies. Condensed tannin (CT)-containing forages and plants have emerged as among the most promising natural anthelmintics. Athanasiadou et al. (2001) demonstrated that feeding CT-rich plants like sainfoin (*Onobrychis viciifolia*) and sulla (*Hedysarum coronarium*) significantly reduced fecal egg counts and adult worm burdens in sheep infected with *Haemonchus contortus* and *Trichostrongylus colubriformis*. Corroborating these findings, significant research from Iran has shown the efficacy of native plants.<sup>15</sup> Azizi et al. (2016) reported that dietary inclusion of *Artemisia absinthium* (wormwood) extract reduced the fecal egg count of GINs in goats and improved packed cell volume, indicating a reduction in the pathogenic blood loss caused by *Haemonchus*. The mechanisms of action are multifaceted. Tannins can directly bind to glycoproteins on the cuticle of the larvae and adult worms, causing death or reduced fecundity. They also indirectly improve the host's resilience by forming complexes with dietary proteins, leading to increased bypass protein and improved nitrogen utilization, which allows the animal to better withstand the pathogenic effects of parasitism.<sup>16</sup> Furthermore, some essential oils, such as those from thyme and oregano, have shown ovicidal and larvicidal effects against nematode eggs and larvae.

### **Modulation of Rumen Fermentation and Mitigation of Methane Emissions**

The rumen microbiome is central to a ruminant's ability to convert fibrous plant material into energy, but it also produces methane (CH<sub>4</sub>), a potent greenhouse gas, as a by-product of fermentation. Modulating rumen fermentation to improve efficiency and reduce environmental impact is a key goal. Medicinal plants and their extracts, particularly essential oils and saponins, have demonstrated significant potential as natural rumen modifiers. Benchaar et al. (2008) conducted a comprehensive review showing that essential oils from plants like garlic, cinnamon, and clove can selectively inhibit gram-positive bacteria and protozoa, leading to a shift in fermentation patterns. This often results in reduced ammonia nitrogen concentrations (indicating improved nitrogen efficiency) and a decrease in methane production.<sup>17</sup> Tavendale et al. (2005) found that condensed tannins from *Lotus corniculatus* reduced methane production by directly inhibiting methanogenic archaea and by redirecting hydrogen away from methanogenesis towards alternative metabolic pathways. This dual benefit of

improving feed efficiency while reducing the environmental footprint of livestock production makes phyto-genics highly attractive for sustainable intensification. The effects are dose-dependent, however, and excessive amounts can potentially suppress overall microbial activity and fiber digestion, highlighting the need for precise dosing.<sup>18</sup>

### Prevention and Management of Metabolic Disorders

Transition dairy cows are particularly susceptible to metabolic disorders such as ketosis, milk fever, and fatty liver disease due to the immense metabolic demands of late gestation and early lactation. Medicinal plants with hepatoprotective, anti-inflammatory, and appetite-stimulating properties can play a supportive role in managing this critical period. Shamsavari et al. (2013) investigated the effects of *Artemisia dracuncululus* (tarragon) supplementation in transition dairy cows and observed improved dry matter intake and a tendency for reduced beta-hydroxybutyrate (BHB) levels, a key indicator of subclinical ketosis. Silymarin, a complex of flavonolignans extracted from milk thistle (*Silybum marianum*), is one of the most well-researched hepatoprotective agents.<sup>19</sup> Tedesco et al. (2004) demonstrated that supplementation with silymarin improved liver function parameters in dairy cows by reducing oxidative damage to hepatocytes and promoting liver regeneration. This helps the liver better process the massive influx of non-esterified fatty acids (NEFAs) mobilized from body fat reserves around calving, thereby reducing the risk of fatty liver syndrome and associated metabolic complications. The use of such plants offers a prophylactic, natural strategy to support metabolic health and maintain productivity during periods of high physiological stress.<sup>19</sup>

### Management of Mastitis and Antimicrobial Effects

Bovine mastitis, an inflammation of the udder typically caused by bacterial infection, is the most costly disease in the dairy industry. The overuse of intramammary antibiotics for treatment has contributed to antibiotic resistance and concerns about milk residues. Medicinal plants with broad-spectrum antimicrobial and anti-inflammatory properties offer an alternative or complementary approach for both treatment and prevention. “In vitro” studies have shown that essential oils from thyme, oregano, and tea tree possess strong bactericidal activity against major mastitis pathogens, including “*Staphylococcus aureus*”, “*Escherichia coli*”, and “*Streptococcus uberis*”. The phenolic compounds in these oils, such as carvacrol and thymol, disrupt the bacterial cell membrane. Hashemi et al. (2015) reported that a topical formulation containing *Zataria multiflora* (Shirazi thyme) essential oil reduced somatic cell count (SCC) and clinical signs of mastitis in dairy cows, comparable to a standard antibiotic treatment. Beyond direct antimicrobial effects, many plants also possess potent anti-inflammatory properties that help reduce the pain, swelling, and tissue damage associated with mastitis. Administering these plants through the diet could potentially improve overall udder health and resilience, reducing the incidence of new infections.<sup>20</sup>

### Enhancement of Immune Function and Antioxidant Status

The immunomodulatory and antioxidant properties of medicinal plants are fundamental to their overall health-promoting effects in livestock. Chronic stress, poor nutrition, and disease challenges can suppress the immune system and increase oxidative stress, leading to increased susceptibility to infections. Many plants contain bioactive compounds that can enhance both innate and adaptive immunity. Bakht et al. (2013) found that dietary supplementation with *Andrographis paniculata* extract improved immune parameters in broilers, and similar mechanisms are likely in ruminants.<sup>21</sup> Persian literature also contributes to this field; a study on *Urtica dioica* (nettle) supplementation in sheep showed an increase in antibody titers following vaccination.



Furthermore, the high antioxidant capacity of plants rich in polyphenols, flavonoids, and vitamins helps neutralize reactive oxygen species (ROS) that cause cellular damage. Salami et al. (2016) demonstrated that feeding lambs with a diet containing sainfoin tannins improved the oxidative stability of meat, indicating a systemic antioxidant effect. A robust antioxidant status supports immune cell function, reduces inflammation, and improves overall animal vitality, making livestock more resilient to environmental and physiological stressors.<sup>22</sup>

**Table 1:** Impact of Medicinal Herbs on Diseases of Large Animals

| Disease (System)                                | Medicinal Herb                             | Main Effects (Pharmacology)                                                                               | Reference |
|-------------------------------------------------|--------------------------------------------|-----------------------------------------------------------------------------------------------------------|-----------|
| Mastitis (Mammary)                              | Thyme ( <i>Thymus vulgaris</i> )           | Potent antibacterial activity against <i>Staphylococcus aureus</i> and <i>E. coli</i> , anti-inflammatory | 23        |
|                                                 | Common Mallow ( <i>Malva sylvestris</i> )  | Reduces mammary inflammation, demulcent                                                                   | 23        |
| Diarrhea & GI Disorders (Digestive)             | Chamomile ( <i>Matricaria chamomilla</i> ) | Antispasmodic, anti-inflammatory, soothes intestinal mucosa                                               | 24        |
|                                                 | Peppermint ( <i>Mentha piperita</i> )      | Reduces gas and bloating, relieves abdominal pain, antispasmodic                                          | 24, 25    |
| Respiratory Infections (Respiratory)            | Thyme ( <i>Thymus vulgaris</i> )           | Antitussive, expectorant, antibacterial, and broncho-antispasmodic                                        | 23, 26    |
|                                                 | Eucalyptus ( <i>Eucalyptus globulus</i> )  | Decongestant, antimicrobial, opens respiratory airways                                                    | 26        |
| Internal Infections & Immunity Boost (Systemic) | Garlic ( <i>Allium sativum</i> )           | Immune system enhancer, antibacterial, antifungal, antiparasitic                                          | 25, 27    |
|                                                 | Echinacea ( <i>Echinacea purpurea</i> )    | Stimulates immune system, increases phagocytosis                                                          | 27        |
| Internal Parasites (Digestive)                  | Milk Thistle ( <i>Silybum marianum</i> )   | Protects liver from toxins (anti-hepatotoxic)                                                             | 28        |

**Effects on Production Performance and Product Quality**

The ultimate validation of any feed additive is its effect on productivity and product quality. The collective benefits of improved health, reduced parasitism, better rumen function, and enhanced metabolic status often translate into tangible improvements in production parameters. Numerous studies have reported increases in milk yield and milk composition (fat and protein content) in dairy cows supplemented with garlic, fenugreek, or other botanicals.<sup>29, 30</sup> In meat production, the effects are seen in improved average daily gain (ADG) and feed conversion efficiency.<sup>31</sup> Importantly, phytochemicals can enhance the quality of animal products. As mentioned, tannin-rich diets can increase the concentration of beneficial polyunsaturated fatty acids (PUFAs) like conjugated linoleic acid (CLA) in meat and milk due to a shift in rumen biohydrogenation pathways.<sup>32</sup> They also improve the oxidative stability of products, extending shelf life.<sup>33</sup> This creates a valuable opportunity for product differentiation in the market, allowing producers to offer "natural," "omega-3 enriched," or "antioxidant-rich" meat and milk to health-conscious consumers, potentially commanding a premium price.

**Challenges and Considerations for Commercial Application**

Despite the compelling evidence from experimental trials, the widespread adoption of medicinal plants in commercial ruminant production faces several significant challenges. The foremost issue is the lack of standardization. The concentration and profile of active compounds (e.g., tannins, essential oils) can vary dramatically within the same plant species due to genetics, geography, climate, soil, harvest time, and processing methods. This variability makes it extremely difficult to establish consistent, effective, and safe dosages for

commercial feed production, leading to unpredictable results. Secondly, the practical aspects of incorporation pose difficulties. The strong flavors and aromas of some essential oils can negatively affect palatability and dry matter intake if not properly dosed or encapsulated. Furthermore, while generally recognized as safe, more extensive toxicological studies are needed to determine the long-term effects of certain plants, potential interactions with other feed additives or medications, and the transfer of compounds into milk and meat to ensure consumer safety. Therefore, future research must focus on standardizing extracts based on active compound content, developing protected delivery systems (e.g., encapsulation) to enhance stability and mask odors, and conducting long-term, large-scale field trials to validate economic efficacy and practical viability under diverse commercial farming conditions.

### Conclusion and Future Perspectives

This systematic review consolidates a formidable body of global evidence, underscoring that medicinal plants and their derivatives are effective, multi-functional, and sustainable tools for advancing the health and productivity of large livestock. They offer a viable natural strategy to control parasites, modulate rumen fermentation for improved efficiency and reduced methane emissions, mitigate metabolic disorders, support immune function, and enhance the quality of animal products. Their application aligns perfectly with the growing demands for sustainable agriculture, animal welfare, and safe, naturally produced food, embodying the "One Health" approach that connects animal, human, and environmental health. Looking forward, the future of phytogenics in ruminant production depends on a concerted effort to bridge the gap between experimental proof-of-concept and practical, on-farm application. Research priorities must include: the bioassay-guided identification and standardization of active compounds; the development of advanced delivery technologies to ensure stability and efficacy in the rumen environment; exploration of synergistic effects between different plants or with other nutritional strategies; a deeper understanding of their effects on the rumen microbiome through metagenomics and metabolomics; and comprehensive cost-benefit analyses. By addressing these challenges through interdisciplinary collaboration, medicinal plants can transition from a promising alternative to a mainstream, indispensable component of sustainable, profitable, and resilient ruminant production systems worldwide.

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# SECTION 4

## Abstract Poster Papers



## UROLITHIN A SHIELDS AGAINST DIABETES-INDUCED PATHOSPERMIA AND EMBRYOTOXICITY IN MATURE RATS

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### Abstract

Diabetes mellitus (DM) as a chronic disease can negatively affect male fertility due to endocrine and metabolic pathways disruption. This study was implemented to appraise the possible beneficial effects of urolithin A (C<sub>13</sub>H<sub>8</sub>O<sub>4</sub>; UA), a natural polyphenol produced by gut microbiota from ingested ellagitannins and ellagic acid, against DM adverse effects on epididymal sperms and pre-implantation embryo development in mature rats. Forty mature male Wistar rats were randomly allotted to five equal groups, including non-treated control group, DM group administered streptozotocin (50.00 mg/kg; IP), and three treated diabetic groups received 25.00 mg/kg, 100.00 mg/kg, and 400.00 mg/kg of UA for 60 days orally, respectively. Thereafter, serum levels of testosterone, follicle-stimulating hormone (FSH), and luteinizing hormone (LH), and testicular total anti-oxidant capacity (TTAC) and malondialdehyde (TMDA), as well as sperm quantity and quality, *in vivo* fertility, and *in vitro* fertilization (IVF) success rate were rigorously assessed. It was disclosed that UA exerted dose-dependent protective effects against DM-related negative impacts on biochemical (testosterone, FSH, LH, TTAC, and TMDA), spermatological (sperm count, motility, viability, and chromatin and DNA qualities), *in vivo* fertility, and embryological (IVF, and two-cell embryos, morulae, and blastocysts formation rates) parameters. This study's outcomes spotlighted the promising protective functions of UA in DM-associated male reproductive disorders in rats, owing its weighty anti-oxidative properties, positioning it as an encouraging candidate to manage DM-induced organopathies.

**Keywords:** Diabetes, Fertility, Rat, Sperm, Urolithin A

## THE EFFECT OF DIFFERENT RATIOS OF $\text{NH}_4^+:\text{NO}_3^-$ ON THE PHYTOCHEMICAL AND ANTIOXIDANT OF *CANNABIS SATIVA* L. MEDICINAL PLANT

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### Abstract

The cultivation, processing, and nutritional profile of the cannabis plant are intrinsically linked to its veterinary applications. Cultivation practices (e.g., organic farming, specific harvesting times) and extraction methods (e.g.,  $\text{CO}_2$  vs. ethanol) directly influence the purity, potency, and final cannabinoid profile of hemp-derived products, which is critical for ensuring consistent and safe dosing in animals. Furthermore, the plant's nutritional value, particularly from hemp seed meal which is rich in omega-3 and omega-6 fatty acids, proteins, and fiber, supports animal health by improving skin/coat condition and providing a sustainable protein source in feed, thereby connecting agronomic practices directly to veterinary nutraceuticals and therapeutics. *Cannabis sativa* L. is a one-year plant from the hemp family and important in industry, pharmaceutical-medicine and agriculture. Cannabis is very complex in terms of phytochemical compounds and more than 480 different chemical compounds have been identified. Plants need enough nutrients in the right proportions to grow. Nitrogen is absorbed in the form of  $\text{NO}_3^-$  or  $\text{NH}_4^+$ , and the total absorbed N is the combination of these two forms. So, the ratio of these two is important in plants and affects it. In this research, the effect of  $\text{NH}_4^+:\text{NO}_3^-$  ratios including 20:80, 40:60 and 60:40 with full and half nutrient concentration with a randomized complete block experimental design on the phytochemical properties including chlorophyll a and b as well as its antioxidant activity investigated. According to the results, the highest amount of Chlorophyll a in the ratio was 20:80 in half nutrient concentration and, the Chlorophyll b in the ratio was 20:80 in full-ionic strength. The highest antioxidant activity of both leaves and flowers was in the ratio of 60:40 in half nutrient concentration the highest antioxidant activity of both leaves and flowers was in the ratio of 60:40 in half nutrient concentration and the highest antioxidant activity in the root was in the ratio of 20:80 in full nutrient concentration.

**Keywords:** Antioxidant, *Cannabis sativa*, Phytochemical



## REPRO-PROTECTIVE EFFECT OF GLABRIDIN IN DIABETIC MATURE MALE MICE: SPERMATOLOGICAL AND EMBRYOLOGICAL EVIDENCE

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### Abstract

Diabetes mellitus (DM) as a metabolic disorder is associated with male and female reproductive complications. Glabridin ((4-[3R)-8,8-dimethyl-3,4-dihydro-2H-pyrano[2,3-f] chromen-3-yl] benzene-1,3-diol, C<sub>20</sub>H<sub>20</sub>O<sub>4</sub>; GBD), a prenylated isoflavan being extracted from *Glycyrrhiza glabra* L., has been reported to have a protective effect against DM-elicited organopathies. This study aimed to examine the effects of GBD on testicular oxidative status, sperm characteristics, and early embryo development in diabetic mature mice. Forty mature male mice were assigned into five equal groups, including non-treated control group, diabetic group received streptozotocin (50.00 mg/kg; IP), and three experimental groups receiving 12.50 mg/kg, 50.00 mg/kg, and 200.00 mg/kg GBD orally *per day* for 30 days, respectively. Serum levels of testosterone, sperm parameters, and testicular malondialdehyde (MDA), total antioxidant capacity (TAC), and catalase (CAT), as well as pre-implantation embryo development were determined. The diabetic group exhibited significantly reduced sperm motility, viability, and count, testosterone level, and testicular TAC and CAT, and increased testicular MDA level, and DNA-damaged and immature sperms along with poor *in vitro* fertilization outcomes compared to the control group. In contrast, the GBD administration, particularly at the highest dose, caused a pronounced improvement in the aforementioned parameters. These findings postulate that GBD may play a beneficial role in impeding DM-induced male reproductive complications through oxidative stress repression, and sperm cells and early embryos protection. Nonetheless, further research is imperative to delve into the precise underlying mechanisms, laying the groundwork for the development of GBD-based therapeutic strategies to promote fertility status in diabetic patients.

**Keywords:** Diabetes mellitus, Glabridin, *In vitro* fertilization, Mice, Sperm

## EFFECTS OF CROCETIN ON HISTOPATHOLOGICAL CHANGES OF HIPPOCAMPUS INDUCED BY LONG TERM MORPHINE INJECTION

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### Abstract

Opioid addiction is one of the major problems of the world. Morphine is the main component of opium and directly affected the opioid receptors and has harmful effects by the production of free radicals and oxidative stress. Chronic administration of morphine causes several adverse effects such as neurotoxicity, especially in the brain structures. Crocetin (CRT), an active ingredient in saffron, has been shown to have potent antioxidant and neuroprotective properties. The present study evaluated the protective effects of crocetin on the morphine-induced structural pathology of hippocampus. Twenty-four male rats were randomly divided into 4 groups (n=6) and were treated intraperitoneally for 28 days with normal saline, morphine (20 mg/kg/day), and morphine (20 mg/kg/day) + crocetin (2.5 and 10 mg/kg/day). Histological parameters were evaluated using hippocampal specimens. Plasma levels of total antioxidant capacity (TAC) and malondialdehyde (MDA) were measured. The number of hippocampal neurons was also counted. Morphine decreased TCA, increased MDA and reduced the number of neurons in the hippocampus. Although Morphine produced vacuolization and severe shrinkage of hippocampal neurons. Blood TCA and MDA changes induced by morphine were improved with long-term i.p. injection of crocetin at doses of 10 mg/kg. Crocetin prevented hippocampal neurons number loss and shrinkage in morphine group rats. We concluded that crocetin produced a neuroprotective effect through antioxidant activity.

**Keywords:** Crocetin, Hippocampus, Histopathology, Morphine, Rat



## EFFECT OF INTRAPERITONEAL ADMINISTRATION OF SILYMARIN ON CERVICAL LINEAR ESOPHAGOTOMY WOUND HEALING IN RATS

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### Abstract

Wound healing is a complex, multifactorial biological process critical for restoring tissue integrity and function, particularly in specialized organs such as the esophagus. Due to its distinct anatomical and functional properties, esophageal wound repair presents unique clinical challenges. *Silybum marianum*, a medicinal plant widely utilized in traditional Iranian and global folk medicine, contains bioactive flavonolignans collectively known as Silymarin. This study aimed to investigate the effects of Silymarin on esophageal wound healing using an experimental rat model involving cervical esophagotomy. Following surgical esophagotomy, twenty-one rats were randomized into three groups (n=7 each) for postoperative management. The control group received water for the initial 48 hours followed by standard dietary regimens, without further treatment. The solvent group underwent the same dietary protocol but received daily intraperitoneal injections of the vehicle (DMSO plus distilled water) for five consecutive days. The treatment group was administered Silymarin dissolved in the same solvent under identical dietary and injection schedules. Wound healing was assessed 14 days post-surgery through clinical observation, necropsy, and histopathological evaluation. Tissue samples underwent hematoxylin and eosin (H&E) and Masson's Trichrome staining to quantify fibroblast proliferation, neovascularization (capillary buds), collagen deposition, and inflammatory response. Results indicated that the treatment group exhibited a statistically significant increase in fibroblast density, capillary bud formation, and organized collagen matrix compared to both control and solvent groups ( $p<0.05$ ). In contrast, control animals displayed marked submucosal inflammation and edema, with inflammatory cell infiltration ranking as control > solvent > treatment ( $p<0.05$ ). Collagen formation was notably enhanced in the Silymarin-treated group, with significant improvements in both quantity and structural consistency ( $p<0.001$ ). These findings support that intraperitoneal administration of Silymarin facilitates esophageal wound healing by promoting fibroblast activity, collagen organization, and reducing inflammation. The study suggests potential therapeutic applications of Silymarin in veterinary medicine for improving recovery following esophageal injuries.

**Keywords:** Silymarin, Linear esophagotomy, Healing, Anti-inflammatory, Rat

## PHYTOCHEMICAL RESPONSES OF THYME TO BIO- AND NANO-FERTILIZER UNDER WATER DIFICIT STRESS

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### Abstract

Thyme (*Thymus daenensis* Celak.), a medicinal plant rich in phenolic compounds is widely used in traditional medicine, pharmaceutical, food, cosmetic and health industries. The biosynthesis and accumulation of secondary metabolites, including phenolic compounds, which are integral to plants defense mechanisms, are influenced by environmental factors such as water deficit and nutritional stress. To investigate these effects, a three-year split-plot study was conducted using a randomized complete block design with three replications at Urmia University during the 2014-2017 cropping years. The main factor was irrigation regimes at three levels: normal irrigation (once every two weeks), supplementary irrigation (once a month), and rainfed conditions (without irrigation). The sub-factor consisted of six fertilizer treatments: biofertilizer (Azotobaror + Phosphate-Barvar 2), complete chemical fertilizer, and complete Nano fertilizer, each with and without vermicompost. Sampling and trait analysis were performed during the third year of planting (2016-2017). The results showed that total phenol content was significantly effect by irrigation regimes and fertilizer treatments. supplementary irrigation and rainfed conditions increased total phenol content. The combined application of Nano fertilizer and vermicompost had the most significant effect on enhancing total phenol content. The interaction between irrigation regimes and fertilizer treatments indicated that low irrigation conditions increased anthocyanin content, total flavonoids, antioxidant activity, and essential oil yield while reducing total dry weight. Furthermore, the combined application of Nano fertilizer and vermicompost under normal and rainfed irrigation conditions, as well as the application of Nano fertilizer alone under supplementary irrigation, significantly improved the thyme essential oil yield.

**Keywords:** Anthocyanin, Essential oil yield, Thymol, Total flavonoid, Total phenol



## EVALUATION OF NUTRIENTS OF THYME IN RESPONSE TO FERTILIZER SOURCES AND DIFFERENT IRRIGATION REGIMES

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### Abstract

Drought is one of the most important environmental stresses that in addition to affecting a wide range of morphological, physiological and biochemical characteristics of plants, will also cause plant nutrition disorders. Since the biosynthesis and improvement of essential oil content of medicinal plants as one of the most important goals of their cultivation is affected by environmental factors such as water deficit stress and nutritional stress conditions. In this regard, three years split plot study were done as a randomized complete block design with three replications in Urmia University at the 2014-2017 cropping years. The main factors include irrigation regimes in three levels {normal irrigation (once every two weeks as control), supplementary irrigation (once a month) and rainfed conditions (without irrigation)}, and the sub-factor at six levels included fertilizer resources {biofertilizer (*Azotobaror 1* + *Phosphate-Barvar 2*), complete chemical fertilizer and complete Nano fertilizer with and without vermicompost. Sampling and analysis of traits were done in the cropping year 2016-2017 (third year of planting). The interaction results of irrigation regime with fertilizer sources showed that low irrigation conditions lead to decreasing concentrations of nitrogen, phosphorus, potassium and copper, and increasing concentrations of iron, zinc and essential oil yield, as well as reducing total dry weight. According to the results, the concentration of manganese in the supplementary irrigation conditions decreased and then increased again with increasing stress intensity in rainfed conditions. Combined application of nano fertilizer with vermicompost under normal irrigation conditions, and application of nano fertilizer in supplementary and rainfed irrigation conditions led to improved essential oil yield. In general, the improvement of essential oil yield in water deficit stress, during the application of nano fertilizer, indicates the efficiency of this fertilizer in providing nutrients and achieving sustainable agricultural goals.

**Keywords:** Biofertilizer, Chemical fertilizer, Essential oil yield, Nano fertilizer, Vermicompost

## CAFFEINE-LOADED POLY LACTIC CO-GLYCOLIC ACID NANOPARTICLES FOR TREATMENT OF TRIPLE NEGATIVE BREAST CANCER

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### Abstract

Caffeine is a methylxanthine alkaloid commonly found in beverages like coffee and tea, as well as in pharmaceutical formulations. Current evidence indicates that caffeine possesses multiple bioactive properties, such as neuroprotection, antioxidative and anti-inflammatory actions, performance enhancement, and possible antitumor activity. However, its therapeutic efficacy is limited by rapid metabolism, short half-life, and variable bioavailability. To overcome these challenges, caffeine was incorporated into Poly(lactic-co-glycolic acid) (PLGA) nanoparticles to enhance its stability and enable sustained drug release, optimizing its therapeutic effectiveness. This study focused on encapsulating caffeine in PLGA nanoparticles to improve its physicochemical characteristics, followed by an assessment of its anticancer potential against 4T1 murine breast cancer cells. Poly (D, L-lactide-co-glycolide) (PLGA) nanoparticles (NPs) containing caffeine were prepared using a double-emulsion solvent evaporation technique, and then surface morphology and size distribution were assessed by scanning electron microscopy (FESEM), and also encapsulation efficiency was measured. To investigate the anticancer effects of caffeine and caffeine nanoparticles, 4T1 cancer cells were treated with various concentrations (25, 50, 100, and 200  $\mu$ M) of each compound. Cell viability was assessed using the MTT assay. The growth rate and morphological changes were observed with an inverted light microscope. After treating the cells with the IC<sub>50</sub> concentration of the compounds and incubating them for 48 hours, they were examined at 400X magnification, and images were captured.  $75 \pm 3.7$  nm of average diameters and uniform morphology of the particles were confirmed by FESEM, and the encapsulation efficiency was  $83.4 \% \pm 1.2$ . The results of the MTT assay demonstrated a notably higher cytotoxic effect of the caffeine-loaded nanoparticles on 4T1 cells than the free caffeine. So that the IC<sub>50</sub> concentration of Free caffeine was 103  $\mu$ M, and the IC<sub>50</sub> concentration of Nano caffeine was 33  $\mu$ M. In morphological analysis, the results showed that caffeine nanoparticles killed more cancer cells than free caffeine, and the remaining cells also underwent apoptosis. Notably, the caffeine nanoparticles exhibited significantly stronger anticancer effects on mouse mammary cancer cell line compared to free caffeine. Further studies in mouse models are needed to confirm the results.

**Keywords:** Caffeine, Nanoparticles, Cytotoxicity, 4T1 cell line



## BIOCHEMICAL EVALUATION OF QUERCETIN EFFECTS ON CLOTHIANIDIN (CTD)-INDUCED RENAL TOXICITY IN RATS

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### Abstract

Clothianidin (CTD), a widely used neonicotinoid insecticide, has raised environmental and health concerns due to its potential toxicity. Quercetin, a flavonoid abundant in various dietary sources, exhibits potent antioxidant properties and effectively scavenges reactive oxygen species (ROS). The present study aimed to evaluate the potential protective effects of quercetin against clothianidin-induced renal toxicity in a rat model. Thirty-five male Wistar rats were divided into six groups as follows: control (NS), control (DMSO), CTD (20 mg/kg/IP) every 3 days, CTD + quercetin (2.5mg/kg/IP), CTD + quercetin (5mg/kg/IP), CTD + quercetin (10 mg/kg/IP) for 21 days. Serum levels of Urea, Creatinine and total protein were measured by an automatic biochemical analyzer using commercial kits. Malondialdehyde and total antioxidant capacity were measured in homogeneous kidney samples of animals using commercial kits. There was a statistically significant change in kidney tissue TAC values in different groups ( $P > 0.05$ ). The TAC values of kidney tissue in the clothianidin + quercetin group (10 mg/kg) indicated that quercetin treatment significantly increased the total antioxidant capacity of kidney tissue compared to the CTD group ( $P < 0.05$ ). The levels of TAC in the treatment groups showed an upward trend. Clothianidin caused a significant increase in the level of malondialdehyde in kidney tissue in the CTD group in comparison with the control groups ( $P < 0.05$ ). In contrast, administration of quercetin (10 mg/kg) caused a significant decrease in the level of MDA in kidney tissue of rats compared to the group receiving the CTD ( $P < 0.05$ ). A significant increase in urea and creatinine were observed in the CTD group compared to the control groups and a significant decrease was observed in the CTD+quercetin (10 mg/kg/daily) group in comparison with the clothianidin group ( $P < 0.05$ ). Also, there was no significant change in serum total protein between treatment groups ( $P < 0.05$ ). These results indicated that quercetin at 10 mg/kg can partly prevent the renal injury induced by clothianidin.

**Keywords:** Biochemical evaluation, Clothianidin, Kidney, Quercetin, Rat

## QUERCETIN AMELIORATES CLOTHIANIDIN-INDUCED CARDIOTOXICITY IN RATS

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### Abstract

Clothianidin, a widely used neonicotinoid insecticide, has raised environmental and health concerns due to its potential toxicity. Quercetin, a flavonoid abundant in various dietary sources, exhibits potent antioxidant properties and effectively scavenges reactive oxygen species (ROS). The present study aimed to evaluate the potential protective effects of quercetin against clothianidin-induced cardiotoxicity in a rat model. Forty-two rats were randomly assigned to six groups: (1) Normal control group: rats received 0.9% NaCl intraperitoneally (IP); (2) Vehicle control group: rats were administered 5% dimethyl sulfoxide (DMSO) IP daily; (3) Clothianidin group: rats received clothianidin (20 mg/kg/day) every three days for 21 days (seven injections); (4–6) Treatment groups: rats received clothianidin as in group 3, concurrently with quercetin at doses of 2.5, 5, or 10 mg/kg IP daily for 35 days. Serum enzyme activities of creatine kinase-MB (CK-MB) and lactate dehydrogenase (LDH) were measured. In cardiac tissue, total antioxidant capacity (TAC), malondialdehyde (MDA), and nitrate/nitrite levels were assessed. Histopathological evaluations of cardiac tissue were also conducted. Clothianidin administration induced significant cardiac injury, evidenced by decreased TAC and increased levels of MDA, nitrate/nitrite, CK-MB, and LDH. Quercetin at a dose of 10 mg/kg significantly mitigated the cardiotoxic effects of clothianidin, as indicated by reduced CK-MB and LDH levels. Furthermore, quercetin enhanced TAC and reduced MDA and nitrate/nitrite concentrations in cardiac tissue compared to the clothianidin-only group. Clothianidin administration induced significant cardiac injury, evidenced by decreased TAC and increased levels of MDA, nitrate/nitrite, CK-MB, and LDH. Quercetin at a dose of 10 mg/kg significantly mitigated the cardiotoxic effects of clothianidin, as indicated by reduced CK-MB and LDH levels. Furthermore, quercetin enhanced TAC and reduced MDA and nitrate/nitrite concentrations in cardiac tissue compared to the clothianidin-only group. Histopathological and biochemical analyses suggest that quercetin at 10 mg/kg exerts cardioprotective effects against clothianidin-induced toxicity, likely through its antioxidant mechanisms.

**Keywords:** Cardiotoxicity, Clothianidin, Oxidative stress, Quercetin, Rat



## QUERCETIN'S PROTECTIVE ROLE AGAINST CLOTHIANIDIN-INDUCED LIVER INJURY IN RATS

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### Abstract

Clothianidin (CTD) is a member of the neonicotinoid group of insecticides. This study was performed to determine the effect of quercetin on clothianidin-induced liver injury (CTD) in rats. Rats were randomly assigned to a normal control group (Normal saline), Vehicle control group (5% DMSO), CTD group (20 mg/kg) every 3 days for 21 days, and CTD + quercetin-treated groups (2.5, 5, and 10 mg/kg) for 35 days intraperitoneally. Enzyme activity, including alanine aminotransferase (ALT) and aspartate aminotransferase (AST), was measured in serum samples by an automatic biochemical analyzer using commercial kits. Total antioxidant capacity (TAC), malondialdehyde (MDA), and nitrate-nitrite were measured in homogeneous liver tissue samples of animals. A significant increase in ALT and AST enzyme activity was observed in the CTD group in comparison with that of the control groups. In the clothianidin + quercetin (10 mg/kg) group, the ALT and AST enzyme levels decreased compared to the clothianidin control group significantly ( $P<0.05$ ). The MDA value of the liver increased in the clothianidin-treated group compared to that of the control groups ( $P<0.05$ ). Decreased tissue TAC level was observed in the CTD-treated group in comparison with that of the control groups ( $P<0.05$ ). The MDA level of the liver decreased in the clothianidin + quercetin (10 mg/kg) group compared to that of the CTD control group ( $P<0.05$ ). Quercetin significantly raised the level of TAC in the liver tissue of the clothianidin + quercetin (10 mg/kg) treated group compared to that of the clothianidin control group ( $P<0.05$ ). Liver nitrate-nitrite measurement showed a significant increase in the clothianidin group compared to that of the normal control group ( $P<0.05$ ). Nitrate-nitrite level in the liver was decreased in clothianidin + quercetin (10 mg/kg) compared to that of the clothianidin control group significantly ( $P<0.05$ ). Histopathological investigation revealed that contact to the CTD induced tissue disorganization and inflammatory cell infiltration, but minor histopathological alterations in the liver tissues of rats treated with CTD and quercetin (10 mg/kg) were detected. Quercetin (10 mg/kg) can partially prevent liver damage from CTD toxicity. In terms of public health, if our diet is likely to be contaminated by pesticides, we should eat more foods rich in quercetin.

**Keywords:** Clothianidin, Liver, Oxidative stress, Quercetin, Rat

## THE EFFECT OF *STACHYS LAVANDULIFOLIA* ON PROSTATE CANCER CELL LINE (LNCAP)

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### Abstract

Prostate cancer is one of the leading causes of cancer-related deaths among men, and despite advances in treatment, challenges such as drug resistance and recurrence remain significant. *Stachys lavandulifolia*, a medicinal plant known for its bioactive compounds, has demonstrated potential in various biological activities, including anticancer effects. This study aims to evaluate the cytotoxicity and anticancer potential of *Stachys lavandulifolia* on the LNCap prostate cancer cell line. The cytotoxic effect of *Stachys lavandulifolia* was assessed using the MTT assay, which determined the IC<sub>50</sub> value to be 1.09 mg/mL. Five different treatment groups were established with varying concentrations of the plant extract: 0.125, 0.25, 0.5, 1, and 2 mg/mL. The cells were seeded in 96-well plates, and after 48 hours of exposure to the extract, the MTT assay was performed to assess cell viability. The IC<sub>50</sub> value was calculated based on the concentration that inhibited 50% of cell viability. Subsequently, flow cytometry evaluated apoptosis induction, revealing that a significant percentage of LNCap cells underwent apoptosis following treatment with the plant extract at the IC<sub>50</sub> concentration (1.09 mg/mL). Flow cytometry analysis was performed using Annexin V-FITC and propidium iodide (PI) staining, and the percentage of apoptotic cells was calculated based on the data. Additionally, acridine orange staining was performed to confirm apoptosis induction further. These findings suggest that *Stachys lavandulifolia* exhibits promising anticancer activity against prostate cancer cells by inhibiting cell proliferation and promoting apoptosis.

**Keywords:** Prostate cancer, Cell line, Apoptosis, Cytotoxic effect, *Stachys lavandulifolia*



## AN INTEGRATED APPROACH TO CANINE WELLNESS: DEVELOPMENT OF A NOVEL LINE OF THERAPEUTIC TREATS COMBINING PHYTOTHERAPY AND POSTBIOTICS

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### Abstract

To address the lack of scientific validation in many companion animal supplements, this research developed a cohesive line of six functional products under the Mastiff™ brand. These products uniquely combine phytotherapy with targeted postbiotics to support common canine conditions: anxiety (Calmara), joint degradation (Flexava), dermatological issues (Dermara), gastrointestinal distress (Gutrina), urinary tract health (Urovella), and dental hygiene (Dentava). Formulations were based on a systematic review of veterinary literature to identify botanicals and compounds with established safety and efficacy. Our dual-platform strategy combined: (1) polyherbal synergism to target multiple pathways, enhancing bioavailability with compounds like piperine for curcuminoids (*Curcuma longa*); and (2) strategic incorporation of a postbiotic derived from hydrolyzed *Saccharomyces cerevisiae*, which provides inactivated yeast, intracellular contents, and the functional cell wall, to support the gut microbiome and modulate the gut-brain axis. Active ingredient doses were calibrated to veterinary standards (mg/kg), and a low-temperature (40 °C) dehydration process was employed to preserve the integrity of thermosensitive phytochemicals and postbiotics. The methodology yielded six optimized, palatable formulations, each enhanced with a tailored postbiotic profile. For instance, the Calmara formulation combines *Valeriana officinalis*, *Passiflora incarnata*, and *Matricaria recutita* to modulate the GABAergic system. This effect is complemented by postbiotics that support the gut-brain axis via neurotransmitter synthesis. Similarly, the Flexava formulation combines the anti-inflammatory properties of curcumin (bioavailability enhanced by piperine) with natural chondroprotective agents to address both inflammation and structural joint support. This research establishes a robust framework for the development of evidence-based companion animal supplements that effectively integrate phytotherapy and postbiotics, effectively bridging the gap between traditional medicine and modern science. These optimized formulations represent strong candidates for in-vivo clinical trials to further validate their safety, palatability, and therapeutic efficacy.

**Keywords:** Biotics, Canine, Feed formulation, Medicinal plants, Veterinary phytotherapy

## IMMUNOMODULATORY BENEFITS OF FUCOIDAN IN AN ANIMAL MODEL OF COMPLETE FREUND'S ADJUVANT-INDUCED INFLAMMATION ARTHRITIS

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### Abstract

Fucoidan, a complex sugar found mainly in brown seaweed, is known for its various health-related properties. This study investigated fucoidan extracted from the Persian Gulf seaweed *Sargassum tenerrimum*, examining its effects on arthritis induced in Wistar rats using complete Freund's adjuvant. Rats were given Freund's adjuvant to trigger arthritis. The rats (10 per group) then received daily treatments of either a placebo, fucoidan at different doses (50, 100, 150 mg/kg), or prednisolone (10 mg/kg) from day 5 to day 23. Fucoidan effectively lessened inflammation and eased the symptoms of arthritis in proportion to the dosage. Immunohistochemical analysis revealed that fucoidan significantly decreased the levels of inflammatory substances IL-1 $\beta$  and TNF- $\alpha$  in the arthritic rats' joint tissue. Moreover, fucoidan reduced multiple inflammatory markers, including myeloperoxidase, nitric oxide, malondialdehyde, and C-reactive protein, in a dose-dependent manner in joint tissue. Fucoidan also lowered the activity of genes that promote inflammation (T-bet, ROR $\gamma$ t), which led to a decrease in Th1 and Th17 cell development, while increasing the activity of GATA3 and FOXP3 genes, encouraging the development of Th2 and Treg cells. Unlike prednisolone, fucoidan increased the levels of Nrf2 and HO-1 in the rats' paws. Fucoidan from *S. tenerrimum* exhibits significant immune-regulating capabilities, suggesting it could be a valuable treatment option for managing complex inflammatory conditions like arthritis.



## THE BENEFICIAL EFFECT OF FREEZE-DRIED SUMAC EXTRACT ON INFLAMMATION INDUCED BY COMPLETE FREUND'S ADJUVANT

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### Abstract

Given the anti-inflammatory and antioxidant properties of brown sumac extract, it is hypothesized that it may mitigate inflammation associated with complete Freund's adjuvant. To investigate this possibility, the present study employed a model of arthritis to examine the effects of freeze-dried sumac extract. The effects of sumac extract were evaluated in both healthy and Wistar rats with induced arthritis. Healthy rats received oral administrations of the extract at dosages of 0, 100, 200, or 400 mg/kg for a period of one month. Arthritis was induced via Freund's adjuvant injection, followed by administration of the same extract dosages from days 12 to 32 post-induction, specifically once the arthritis index exceeded a value of one. The analysis of the extract revealed a total phenolic content of  $179.45 \pm 3.12$  mg/g (expressed as gallic acid equivalents) and a FRAP antioxidant activity of  $101.9 \pm 0.98$  mmol Trolox equivalents/100 g. In healthy rats, the administration of the extract resulted in a dose-dependent increase in serum TAC levels, without any observed effects on RBC, WBC, glucose, or cholesterol levels. In arthritic rats, the progression of the disease was attenuated with the administration of the treatment, particularly at dosages of 200 and 400 mg/kg, with the latter demonstrating the lowest arthritis index. The administration of the extract at 200/400 mg/kg led to improvements in anemia through the normalization of WBC counts and the elevation of RBC counts, Hb levels, and Hct. TAC levels were restored in a manner dependent on the administered dose. Furthermore, CRP and MPO levels exhibited a decrease correlating with increased extract dosage, notably at 200/400 mg/kg. mRNA ratios (T-bet/GATA-3, T-bet/Foxp3, ROR $\gamma$ t/Foxp3, and ROR $\gamma$ t/GATA-3) were diminished in arthritic rats, with further reductions observed in ROR $\gamma$ t/Foxp3 and ROR $\gamma$ t/T-bet at the 400 mg/kg dosage. The findings suggest that sumac extract may have the potential to mitigate inflammatory effects.

**Keywords:** Sumac extract, Complete freund's adjuvant, Immunomodulation, Anti-inflammatory, T-cell transcription factors

## CHLOROGENIC ACID AND ALUM SYNERGISTICALLY ENHANCE CELLULAR AND HUMORAL IMMUNITY AGAINST HEAT-KILLED *SALMONELLA* *TYPHIMURIUM* VACCINE

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### Abstract

Chlorogenic acid (CGA), a naturally occurring polyphenolic compound, is present in various plants, notably green coffee beans. This study aimed to evaluate the impact of combining CGA with alum on cellular and humoral immunity after vaccination with heat-killed *Salmonella typhimurium* (HKST). Male Balb/c mice were given two administrations of the HKST vaccine, either with or without alum, CGA, or both, spaced two weeks apart. Two weeks after the final immunization, immune responses and protection levels against *S. typhimurium* were assessed. The combination of alum and CGA improved the HKST vaccine's capacity to promote lymphocyte proliferation, delayed-type hypersensitivity responses, and antibody levels, as well as to shift the IgG2a/IgG1 ratio toward IgG2a. These results were associated with a greater Th1 immune response and enhanced protective immunity against *S. typhimurium*. Additionally, the group treated with the combined adjuvant exhibited an increased survival rate following exposure to the acute dose of *S. typhimurium* and a decreased bacterial load in the liver after exposure to the subacute dose of *S. typhimurium*, compared to other immunization strategies. The combination of alum and CGA considerably enhanced cellular and humoral immunity following HKST immunization.

**Keywords:** Adjuvant, Alum, Chlorogenic acid, *Salmonella typhimurium*, Humoral and cellular immunity, Vaccine



## COMBINED NICOTINE AND THYMOL IN AMELIORATING EXPERIMENTAL RHEUMATOID ARTHRITIS

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### Abstract

Considering the anti-inflammatory properties of both Nicotine and Thymol, this study aimed to assess the combined effects of these compounds on clinical manifestations and immune responses in Wistar rats with Freund's complete adjuvant (FCA)-induced RA. The study included 50 male Wistar rats, weighing  $150 \pm 7$  g, in whom RA was induced using FCA. The rats were randomly divided into five groups ( $n = 10$ ): RA rats treated with PBS (100 mg/kg orally), RA rats treated with Thymol (100 mg/kg orally), RA rats treated with Nicotine (2.5mg/kg-orally), and RA rats treated with a combination of Nicotine and Thymol (each at half doses orally). All treatments commenced on day seven post-induction (p.i.), once all rats exhibited a clinical score of  $\geq 1$ . Clinical symptoms were monitored every other day until day 23 p.i. The data indicated that the combination therapy reduced disease severity and improved weight gain more effectively than either agent alone. Furthermore, the combination therapy led to a more significant reduction in certain hematological and biochemical parameters associated with RA, including Rheumatoid factor, C-Reactive Protein, Nitric oxide, Myeloperoxidase, IL-1, and IL-17, compared to individual treatments. Notably, the combination therapy with half doses of Nicotine and Thymol did not show any synergistic advantage in terms of anti-proliferation effect, and consequently, immunosuppression side effects were not exacerbated compared to using each agent separately. Combined Nicotine and Thymol may represent a valuable approach for managing RA.

**Keywords:** Combination therapy, Nicotine, Rheumatoid arthritis, Thymol, Wistar rat

## COMBINED GLYCYRRHIZIC ACID AND ALUM LIPOSOMES ENHANCE IMMUNE RESPONSE TO INACTIVATED *SALMONELLA TYPHIMURIUM*

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### Abstract

Killed microorganisms combined with alum typically do not elicit strong cellular immune responses. Glycyrrhizic acid, a triterpenoid saponin, possesses immunomodulatory properties. This study investigated the synergistic effects of glycyrrhizic acid (GL) and alum liposomes on immune responses against heat-killed *Salmonella typhimurium* (HKST). In this experimental study, male Balb/c mice were divided into 5 groups of 15 and immunized with HKST vaccine alone or in combination with alum, GL, or alum-GL combination twice, with a two-week interval. Fourteen days post-last vaccination, immune responses against *S. typhimurium* and the protective potential of the vaccines were evaluated. Survival rate was monitored using Kaplan-Meier analysis. Other findings were analyzed via one-way analysis of variance and Tukey's post hoc test, with significance set at  $P < 0.05$ . The group receiving combined adjuvant and HKST showed significant increases in delayed-type hypersensitivity reaction, lymphocyte proliferation, IgG2a antibody titer, and improved survival against live challenge compared to other groups. Furthermore, the combined adjuvant group exhibited a more pronounced increase in IFN- $\gamma$  levels and decrease in IL-4 levels than the other groups. The alum-GL combination, used as an adjuvant, synergistically enhanced cellular and humoral immunity following immunization with the HKST vaccine.

**Keywords:** Adjuvant, Alum, Glycyrrhizic acid liposome, *Salmonella typhimurium*, Vaccine



## **BUNIAM PERSICUM ESSENTIAL OIL MODULATES LIPID PROFILE IN HYPERLIPIDEMIC RATS: FOCUS ON HDL ENHANCEMENT AND ATHEROGENIC RISK REDUCTION**

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### **Abstract**

Hyperlipidemia is a pivotal modifiable risk factor for cardiovascular diseases (CVD), contributing to atherosclerosis and related complications worldwide. Despite advances in pharmacotherapy, natural alternatives with fewer side effects are increasingly essential. In this study, we explored the hypolipidemic potential of *Bunium persicum* essential oil (BPEO), derived from a medicinal plant traditionally used in Iranian folk medicine, in a rat model of diet-induced hyperlipidemia. Lovastatin served as the positive control to benchmark efficacy. BPEO was obtained through hydrodistillation using a Clevenger apparatus. For the animal feeding, a standard commercial chow was used as the base diet. This powdered feed served as the basis for preparing the high-fat diet in the laboratory. The hyperlipidemia-inducing diet consisted of the following components: standard chow (47%), cholesterol (2%), sodium cholate (1%), dextrose (40%), and olive oil (10%), formulated into a solid cake. Forty-two male Wistar rats were divided into six groups (n=6 each): normal control, hyperlipidemic control (fed a 2% cholesterol diet), lovastatin-treated (10 mg/kg), and BPEO-treated at doses of 5, 10, and 20 mg/kg. Hyperlipidemia was induced over 21 days, followed by 14 days of oral treatment. Serum lipids—including total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were measured, alongside atherogenic indices (TC/HDL ratio, LDL/HDL ratio, and atherogenic index). Our results demonstrated dose-dependent benefits of BPEO. All doses significantly elevated HDL levels compared to the hyperlipidemic control (p<0.05), with the 20 mg/kg dose yielding the greatest increase (32.7%). Notably, only the highest dose reduced LDL by 28.4% (p<0.05), approaching lovastatin 34.1% reduction. Atherogenic indices improved across all BPEO groups: TC/HDL ratio decreased by 18.9-26.3%, LDL/HDL ratio by 22.1-30.8%, and atherogenic index by 24.7-33.5% (p<0.05). However, unlike lovastatin, which lowered TC and TG (p<0.05), BPEO did not significantly affect these parameters (p>0.05). In conclusion, although *Bunium persicum* essential oil (BPEO) does not completely reverse diet-induced hyperlipidemia, its significant HDL-raising and atheroprotective properties—particularly at the 20 mg/kg dose—underscore its potential as a cardioprotective agent. This effect may be attributed to the presence of bioactive compounds such as flavonoids, which enhance LDL receptor activity and promote reverse cholesterol transport, as well as antioxidant components that inhibit lipid peroxidation and reduce oxidative stress. Unlike statins, which primarily target LDL reduction, BPEO's HDL-centric action supports its use as a complementary therapy for managing dyslipidemia. Future studies should focus on isolating and characterizing key bioactive constituents (e.g., cuminaldehyde,  $\gamma$ -terpinene), evaluating their effects on endothelial function, and assessing long-term impacts on atherosclerosis progression in vivo.

**Keywords:** *Bunium persicum*, HDL cholesterol, Atherogenic indices, Dyslipidemia, Phytotherapy

## THE POTENTIAL OF HERBAL MEDICINE IN MANAGING RECURRENT AIRWAY OBSTRUCTION IN HORSES

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### Abstract

Recurrent airway obstruction (RAO) is a chronic, reversible airway disease affecting stabled adult horses, characterized by exercise intolerance, coughing, nasal secretions, and increased respiratory effort. Conventional treatments, including environmental management and pharmacotherapy with anti-inflammatories (e.g., dexamethasone) and bronchodilators (e.g., clenbuterol), are limited by logistical challenges, such as restricted outdoor access in equestrian facilities, and side effects, including laminitis, pneumonia, and impaired athletic performance. This study explores herbal medicines as a promising alternative for RAO management due to their antimicrobial, antioxidant, anti-inflammatory, antihistamine, and antitussive properties. A comprehensive literature review was conducted to evaluate the efficacy of herbal medicines in treating RAO in horses. The review focused on both Chinese and Western herbal formulations, assessing their pharmacological properties and therapeutic outcomes in equine models. Key herbs and combinations, including Schisandra 5, Bai He Gu Jin Tang, Bu Fei San, Ren Shen Ge Jie San, and a Western herbal blend (yellow gentian, garden sorrel, cowslip, verbena, and common elder), were analyzed for their impact on RAO symptoms and pathophysiology. Chinese herbal formulations demonstrated significant anti-inflammatory, antimicrobial, and antitussive effects, alleviating RAO symptoms in affected horses. The Western herbal combination reduced histamine sensitivity and maximal intrapleural pressure difference, key indicators of airway hyperresponsiveness. These findings suggest that herbal medicines can modulate airway inflammation and improve respiratory function, offering a viable adjunct or alternative to conventional therapies. Herbal medicines present a promising approach for managing RAO in horses, addressing limitations of standard treatments. However, their application remains underexplored. Future research should prioritize standardized formulations, mechanistic studies, and controlled clinical trials to establish efficacy and safety. Integrating herbal therapies could enhance equine welfare and performance while minimizing adverse effects.

**Keywords:** Equine, Herbal medicine, Recurrent airway obstruction, Anti-inflammatory, Phytotherapy



## HESPERIDIN PLUS BERBERINE INDUCES POTENT ANTICANCER EFFECTS ON MOUSE BREAST CANCER

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### Abstract

Phytochemicals play a crucial role in the pharmaceutical and food industries due to their significant properties. Hesperidin is a flavanone glycoside that is found abundantly in citrus fruits. It is recognized as a promising agent with anti-inflammatory, antioxidant, and anticancer properties. Berberine is an isoquinoline alkaloid commonly extracted from the medicinal plant *Rhizoma Coptidis*. Recent evidence indicates that berberine exhibits multiple therapeutic properties, including antimicrobial, antioxidant, anti-inflammatory, antidiabetic, hepatoprotective, and anticancer activities. In this research, we used both berberine and hesperidin simultaneously and separately to determine their anticancer effects on murine breast cancer cells (4T1 cells). To investigate the anticancer effects of hesperidin and berberine, different concentrations of them (20, 40, 60, and 100 ug/ml) and also, both berberine and hesperidin simultaneously in half of their concentration, were affected on the breast cancer cells and the viability of the cancer cells was assessed by the MTT technique. The expression of genes involved in apoptosis was estimated by the qRT-PCR method. Both hesperidin and berberine reduced the viability of breast cancer cells in a dose-dependent manner. However, this reduction was more significant in cells treated with a combination of both hesperidin and berberine. Additionally, an evaluation of genes involved in the induction of apoptosis in cancer cells revealed that the Bax gene was expressed more frequently in cells treated with both hesperidin and berberine than in separate treatments, which was statistically significant. In contrast, the expression of the Bcl-2 gene was decreased in all treatment groups, and this was not statistically significant. Overall, this study's results demonstrated that co-treatment with hesperidine and berberine exhibited the most significant anticancer activity and could serve as a therapeutic agent for murine breast cancer cells (4T1 cells).

**Keywords:** Berberine, Cancer, Hesperidin, Phytotherapy

## EFFECT OF INTRAPERITONEAL ADMINISTRATION OF BROMELAIN ON HEALING OF COLON ANASTOMOSIS IN RATS

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### Abstract

Intra-abdominal adhesions are known as a major complication following colon surgeries which may lead to abdominal pain and intestinal obstruction. Bromelain is a mixture of proteolytic enzymes extracted from pineapple (*Ananas cosmosus*) and possesses anti-inflammatory, anti-microbial, and analgesic properties. Reportedly, bromelain promotes wound healing, reduce inflammation and improve biomechanical parameters in diabetic wounds. The aim of this study was to investigate the effects of bromelain on healing of experimental colon anastomosis in rats. Twenty male rats were anesthetized using intaperitoneal injection of 80 mg/kg ketamine and 10. mg/kg xylazine. Aseptically, descending colon was exteriorized through a caudal abdomen incision. Then, colonic resection and anastomosis using absorbable suture was performed and then abdominal incision was sutured (Figure 1). Control and treatment rats were received intraperitoneal injection of normal saline and 10 mg/kg bromelain respectively, for five consecutive days. On day six, the rats were euthanized by anesthetic overdose and the anastomosis sites were evaluated for adhesion formation, obstruction, and leakage. Then, samples were harvested for histopathological studies using hematoxylin and eosin staining. Data were analyzed in SPSS using Mann-Whitney and independent t-test, respectively. Intraabdominal adhesions between the anastomotic sites and the greater omentum/spleen/small intestines/bladder (4 cases vs. 1), leakage (3 cases; vs. 0), and intestinal obstruction and distension (4 cases vs. 1) were observed in the control group at significantly higher levels compared to the treatment group ( $p < 0.05$  for all comparisons; Figure 2 and 3). Histopathologically, a significant increase in infiltration of inflammatory cells was found in the control group compared to the treatment group ( $p < 0.05$ ), indicating a stronger inflammatory response in the untreated condition. In contrast, the treatment group demonstrated a significant enhancement in angiogenesis and fibroplasia ( $p < 0.05$ ), reflecting improved tissue repair (Figure 4).

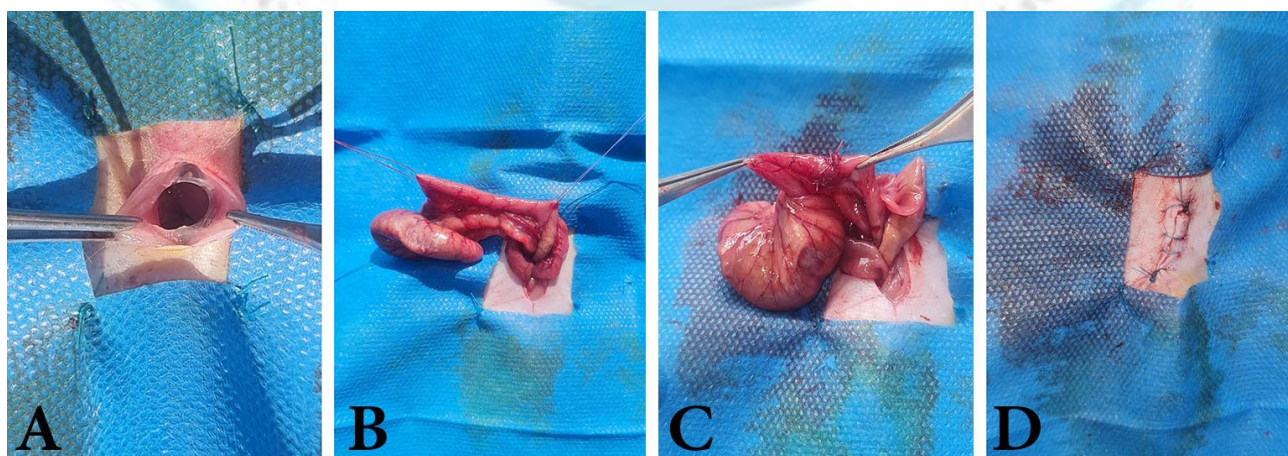


**Figure 1.** A) Caudal abdomen incision, B) exteriorization of descending colon and stay suture placemen, C) colonic anastomosis using 4/0 Dexon suture, and D) ventral midline suture using 3/0 nylon suture.



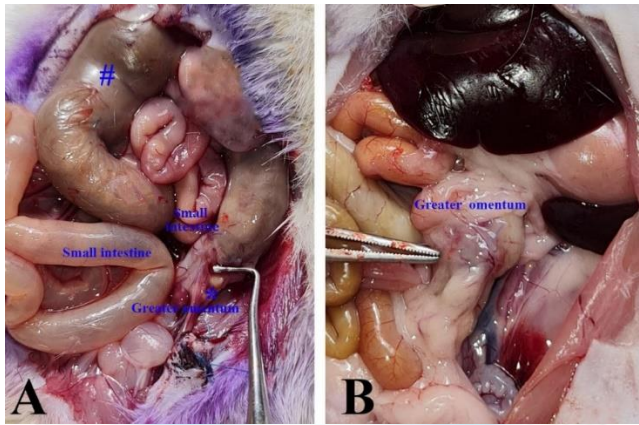


**Figure 2.** A) Intraabdominal adhesion was observed between the anastomosis and omentum, small intestine and bladder in control group. \* and # indicate leakage and colonic distention due to obstruction at the anastomotic site. B) In treatment group, only greater omentum was found attached to the anastomotic site.

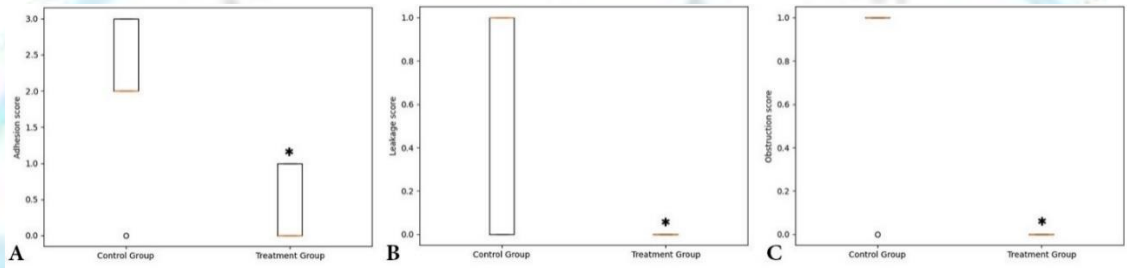


**Figure 3.** A) Boxplot of adhesion score, B) Boxplot of leakage score, and C) Boxplot of obstruction score at day 6 after anastomosis.

\* indicates significant difference between control and treatment group ( $p < 0.05$ ).

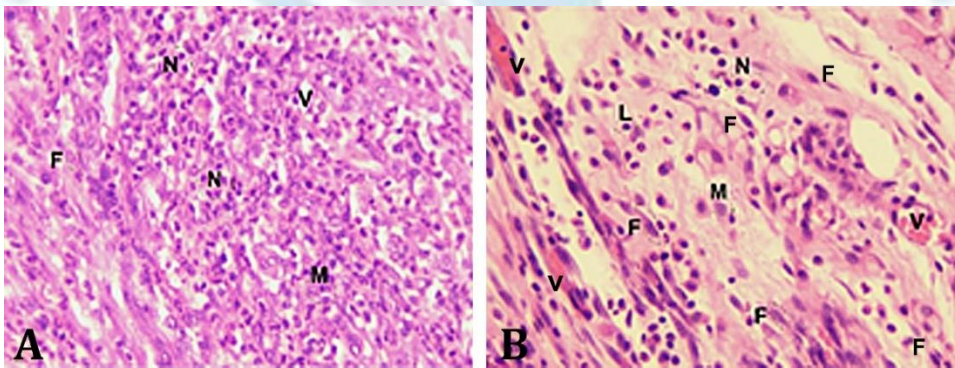


**Figure 4.** Micrographs of anastomotic sites in A) control group and B) treatment group. Infiltration of inflammatory cells was superior in control group. In contrast, number of fibroblast and newly formed vessels were was significantly higher in treatment group. N: Neutrophil, F: Fibroblast, M: Macrophage, V: Blood vessel.

The results of the present study showed that intraperitoneal administration of bromelain following colonic anastomosis in rats improves the structural indices of wound healing and decreases the rates of anastomotic complications. It is recommended to further investigate and consider the administration of bromelain in clinical settings, as it demonstrates a significant capacity to attenuate postoperative complications.

**Keywords:** Bromelain, Colon, Complication, Healing, Pineapple

## EFFECT OF CROCETIN ON FUNCTIONAL RECOVERY IN THE RAT MODEL OF SCIATIC NERVE CRUSH INJURY: COMPARISON WITH VITAMIN C

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### Abstract

Crocetin (CRT) is one of the active chemical compounds of saffron and has many biological effects such as antioxidant property. The present study investigated the effects of CRT on crushed sciatic nerve function. Vitamin C (Vit C) was used as an antioxidant drug. Material and Methods: Thirty rats were divided into six groups (n=5) including intact, sham, crush, CRT 7.50, CRT 30.00 and Vit C 100. In anesthetized rats, right sciatic nerve was crushed using a small hemostatic forceps. With no significant differences, SFI at the test days was around 0.00 in intact and sham groups. In the crush group, SFI was  $-114.72 \pm 11.28$  at days 5 and reached to  $-82.46 \pm 12.04$  at day 20 after crush injury and showed significant differences ( $p < 0.001$ ) with intact and sham groups. The CRT ( $7.50 \text{ mg kg}^{-1}$ ) did not affect time-point SFI. The CRT ( $30.00 \text{ mg kg}^{-1}$ ) significantly increased the decreased SFI to  $-44.43 \pm 5.42$  ( $p < 0.05$ ) at day 5 and reached it to the intact and sham groups value ( $-7.09 \pm 2.68$ ,  $p > 0.05$ ) at day 20. The Vit C ( $100 \text{ mg kg}^{-1}$ ) significantly increased the decreased SFI to  $-54.71 \pm 6.08$  ( $p < 0.05$ ) at day 5 and reached it to the intact and sham groups value ( $-6.32 \pm 3.13$ ,  $p > 0.05$ ) at day 20 (Figure 1A). When the average of SFI was analyzed by one-way ANOVA, a significant ( $p < 0.0001$ ) difference was observed between groups. Subsequent analysis by Türkiye's test revealed that CRT ( $30.00 \text{ mg kg}^{-1}$ ) and Vit C ( $100 \text{ mg kg}^{-1}$ ) significantly ( $p < 0.001$ ) decreased the increased SFI, whereas CRT ( $7.50 \text{ mg kg}^{-1}$ ) was without effect. The severities of sciatic nerve degeneration (edema, vacuolation and ellipsoid bodies) and gastrocnemius muscle atrophy were ameliorated, and the increased MDA level and the decreased SOD activity in the serum were restored by 20 consecutive days oral administration of  $30.00 \text{ mg kg}^{-1}$  CRT and  $100 \text{ mg kg}^{-1}$  Vit C. No significant differences were observed between  $30.00 \text{ mg kg}^{-1}$  and  $100 \text{ mg kg}^{-1}$  Vit C. It is concluded that CRT and Vit C produce similar improving effects on crushed-injured sciatic nerve function. Inhibition of oxidative stress, enhancement of endogenous antioxidant activity might be involved in improving effects of CRT and Vit C.

**Keywords:** Crocetin, Gastrocnemius muscle, Histopathology, Sciatic nerve, Vitamin c



## THE ANTIFUNGAL EFFECT OF PROPOLIS ETHANOLIC EXTRACT ON *ASPERGILLUS FLAVUS* AND *ASPERGILLUS PARASITICUS*

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### Abstract

Propolis, also known as bee glue, is a resinous mixture that honeybees create by combining beeswax with collected tree resins, saps, and other botanical sources. Anti-fungal effect of propolis ethanolic extract on the major aflatoxin producing fungi, *Aspergillus flavus* and *Aspergillus parasiticus* were studied. Morphological changes of growth and spore production were investigated. To evaluate the antimicrobial properties of ethanolic extract of propolis against the fungi *Aspergillus flavus* and *Aspergillus parasiticus*, Minimum Inhibitory Concentration(MIC) and Minimum Fungicidal Concentration(MFC) were determined by using micro-dilution test method. The Disc Diffusion Test method for were used for determining of growth inhibition zone. The results showed that ethanolic extract of propolis with 2000 µg/ml delayed the growth of *Aspergillus flavus* in three days and the delayed sporulation until the tenth day. For *Aspergillus parasiticus*, delaying of growth and sporulation with 2000 µg/ml of propolis extract 3 and 9 day respectively. The Minimum Inhibitory Concentration (MIC) of ethanol extract of propolis against *Aspergillus flavus* and *Aspergillus parasiticus* 250 and 125 µg/ml were determined respectively. The MFC of this extract for *Aspergillus flavus* 500 µg/ml and *Aspergillus parasiticus* 250µg/ml were determined. In Conclusion this study showed that ethanolic extract of propolis can be used as effective antifungal agent against *Aspergillus flavus* and *Aspergillus parasiticus*.

**Keywords:** *Aspergillus flavus*, *Aspergillus parasiticus*, Ethanolic extract of propolis, Morphological change

## IN VITRO STUDY OF OVICIDAL EFFECT OF LAVENDER (*LAVANDULA ANGUSTIFOLIA*) EXTRACT ON *FASCIOLA HEPATICA* EGGS

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### Abstract

Fasciolosis is of considerable economic and public health importance worldwide. In controlling this disease, the ovicidal effects of anthelmintic drugs can be quite effective. Additionally, natural herbal products offer promising potential, as they contain a wide variety of medicinal ingredients that have been shown to be effective against various parasitic diseases. In the present study, in vitro effectiveness of the extract of Lavender (*Lavandula angustifolia*) on the eggs of *Fasciola hepatica* was investigated. *Fasciola hepatica* eggs were collected from the gall bladders of naturally infected sheep at Urmia slaughterhouse. The eggs were exposed to varies concentrations of Lavender extract (1, 5, 10, 25 and 50 mg/mL) for 24, 48 and 72 h. To investigate the effect of the Lavender extracts on miracidial formation, the treated eggs were incubated at 28°C for 14 days. The results indicated that *F. hepatica* eggs were susceptible to the extract of Lavender. The ovicidal effect of Lavender extract at concentration of 1 mg/mL in 24h, 48h, and 72h was 44.02%, 52.6% and 68.09%, respectively (compared with 21.50 % for control group). One hundred percent ovicidal efficacy was obtained through application of Lavender extract at concentrations of 5 mg/mL and 10 mg/mL in 72 h and 48h, respectively. The in vitro ovicidal effect of the methanolic extract of Lavender was satisfactory in this study, however, in vivo efficacy of this extract, recommended for further studies.

**Keywords:** Egg, *Fasciola hepatica*, In vitro, Lavender (*Lavandula angustifolia*) extract



## THE ROLE OF PHYTOESTROGENS FROM MEDICINAL PLANTS IN MODULATING REPRODUCTIVE PERFORMANCE IN LIVESTOCK: A VETERINARY PHARMACOLOGY PERSPECTIVE

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### Abstract

Phytoestrogens constitute a heterogeneous class of plant-derived compounds whose chemical framework closely resembles that of estradiol-17 $\beta$  (E2). Owing to this structural mimicry, they are often described as estrogen-like molecules or non-steroidal estrogens. Livestock production underpins both global food security and economic stability (accounting for about 40 % of agricultural output worldwide and supporting the livelihoods of roughly 1.3 billion people), and in developing countries it can contribute 15–80 % of agricultural GDP; within this sector, improving reproductive performance is essential for sustaining and enhancing profitability. Multiple studies show that some of the phytoestrogens exposure markedly influences reproductive health, with effects varying according to dose and exposure route. Administered at 50–200 mg kg<sup>-1</sup> of feed, the legume phytoestrogen daidzein boosts reproductive performance in multiple livestock species. It raises embryo numbers, litter weight, and milk yield in sows and rats; hastens lay onset and improves egg and bone quality in poultry; and yields comparable gains in ruminants. These benefits arise from estrogen-receptor modulation plus antioxidant and anti-inflammatory effects that elevate reproductive hormones, adjust key ovarian genes ( $\uparrow$  ER $\beta$ , NR5A2;  $\downarrow$  PRLR), and strengthen antioxidant defenses collectively enhancing gamete quality, fetal growth, and early, high-quality egg production. Peer-reviewed evidence shows that supplementing a Tris-based semen extender with roughly 10  $\mu$ M genistein during freezing or thawing markedly elevates the quality of cryopreserved ram sperm: the additive increases post-thaw motility, protects acrosomal structure, suppresses caspase-3 activity, and significantly decreases DNA fragmentation, thereby raising the success rate of artificial insemination. Although many studies have demonstrated the positive effects of phytosterols on animal reproduction, others have reported conflicting results. Formononetin, a phytoestrogen prevalent in red clover, is metabolized to equol (a compound with even stronger estrogenic activity) both of which can markedly disturb livestock reproduction. In sheep, sustained exposure causes structural changes in the uterus and cervix that lead to infertility and erratic estrous cycles, while in cattle it has been associated with the formation of ovarian cysts and short-term infertility. The present review explores the prospective application of medicinal herbs to enhance reproductive performance in animals.

**Keywords:** Livestock, Phytoestrogens, Reproductive performance

## IN VITRO STUDY ON INTERFERING EFFECTS OF *CARUM COPTICUM* ESSENTIAL OIL, PROPOLIS EXTRACT, AND SILVER NANOPARTICLES ON GRAM-NEGATIVE BACTERIA

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### Abstract

Propolis or bee glue is considered to be one of the most useful products of the hive. It is a colloidal and resinous matter that its structural composition composed of various components such as aromatic acids and their esters, flavonoids and terpenoids. *Escherichia coli*, *Salmonella typhimurium* and *Pseudomonas aeruginosa* as Gram-negative bacteria are among important causes of bacterial infections. The aim of this study was to determine the interfering effects of ethanolic extract of Propolis, *Carum Copticum* essential oil and Silver nanoparticles on Gram-negative bacteria. The ajowan oil (*Carum Copticum* essential oil) and propolis extract prepared in the microbiology lab. The Silver nanoparticle was prepared from Nanomaterials Company, USA. The in vitro study was done for determining of MIC for each treatment by using broth microdilution method. Gentamicin used as a positive control in this study. The results for Minimum Inhibitory Concentration (MIC) of propolis extract with Ajowan essential oil on *Escherichia coli* and *Salmonella typhimurium* was 250 and 125 µg/ml respectively. These agents had no deterrent effect on *Pseudomonas aeruginosa*. The MIC of propolis with silver nanoparticles on *Escherichia coli* and *Salmonella typhimurium* was respectively 500 and 50 µg/ml and on *Pseudomonas aeruginosa* was respectively 250 and 25 µg/ml. The results of MIC for Ajowan essential oil with silver nanoparticles on *Escherichia coli* and *Salmonella typhimurium* was respectively 125 and 25 µg/ml and on *Pseudomonas aeruginosa* 62/5 and 12/5 µg/ml was respectively. Due to synergistic anti-bacterial properties of ethanolic extract of propolis, Ajowan essential oil and silver nanoparticles, it can be concluded that these materials are able to have several usage in pharmaceuticals. Therefore, an ethanolic extract of propolis, Ajowan essential oil and silver nanoparticles can be appropriate replacements for chemical agents.

**Keywords:** Ajowan essential oil, Ethanolic extract of propolis, Silver nanoparticles, *Escherichia coli*, *Salmonella typhimurium*, *Pseudomonas aeruginosa*



## EFFECTS OF *CERATONIA SILIQUA* L., EXTRACT-LOADED NANOLIPOSOMES ON A RAT MODEL OF EXCISIONAL WOUND HEALING

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### Abstract

Wound healing consists of inflammatory, proliferation and remodeling phases. The objective of the current study was to investigate the effect of *Ceratonia siliqua* L., extract-loaded liposomes on wound healing. *Ceratonia siliqua* L., commonly called carob, is an evergreen tree that belongs to the Leguminosae family widely cultivated in Mediterranean countries. Twenty-five healthy adult male Wistar rats were randomized into five groups of five animals each: SHAM group with only wound creation. EUCRN group: As a control group with wound creation as well as 5 g eucerin administered topically to the wound bed. NLPSM group: As a group with wound creation as well as 5 mL nanoliposome (20 mg). EUCRN/NLPSM group: As a group with wound creation as well as 5 g eucerin containing 5 mL nanoliposome (20mg). TREATMENT group: As a group with wound creation as well as 5 mL eucerin (5 g) containing nanoliposome (20 mg) loaded with hydroalcoholic extract of *Ceratonia siliqua* L. The excisional wound model was used for biochemical, histopathological and planimetric assessments. The wound area was significantly reduced in the TREATMENT group compared to other groups ( $P < 0.05$ ). Biochemical and quantitative histopathological analyses revealed a significant difference between TREATMENT and other groups ( $P < 0.05$ ). *Ceratonia siliqua* L., extract-loaded nanoliposomes showed the potential to improve wound healing significantly. This appeared to work by angiogenesis stimulation, fibroblast proliferation, inflammation reduction, and granulation tissue formation during the initial stages of the healing process. This accelerated healing led to earlier wound area reduction of the damaged area due to the reorganization of granulation tissue and collagen fibers. Topical administration of *Ceratonia siliqua* L., extract-loaded nanoliposomes could be recommended for wound healing due to reducing wound healing acceleration.

**Keywords:** *Ceratonia siliqua* L., Extract, Nanoliposomes, Wound healing, Rat

## PROKINETIC EFFECTS OF *TANACETUM BALSAMITA* EXTRACTS ON THE BOVINE ABOMASUM

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### Abstract

Impaired abomasal motility is a common disorder in dairy cattle, contributing significantly to displaced abomasum and abomasal volvulus, with considerable economic losses to the livestock industry. *In vitro* studies have demonstrated marked reductions in contractility in smooth muscle from cattle with displaced abomasum, highlighting the need for effective prokinetic agents to restore gastrointestinal motility. However, currently available pharmacological options for ruminants are limited. *Tanacetum balsamita* (costmary) has been traditionally used in Northwestern Iran for the treatment of human gastrointestinal disorders, yet its effects on ruminant gastrointestinal motility remain uninvestigated. This study evaluated the *in vitro* effects of aqueous and hydroalcoholic extracts of *T. balsamita* on bovine abomasal smooth muscle. Abomasal tissue samples from 30 healthy cattle were exposed to varying concentrations of *T. balsamita* extract, and contractile responses were measured using organ bath techniques. Hydroalcoholic extracts (100–1000 mg/L) and aqueous extracts (300–3000 mg/L) significantly enhanced contractility ( $p < 0.05$ ) of abomasal tissues with the strongest response observed at 3000 mg/L aqueous extract ( $182 \pm 29.3\%$  of control). Mechanistic evaluation using pharmacological antagonists showed that hexamethonium (nicotinic receptor blocker) and indomethacin (cyclooxygenase inhibitor) had no effect on the extract-induced contractions. In contrast, verapamil (calcium channel blocker), atropine (non-selective muscarinic antagonist), and 4-DAMP (M3-selective antagonist) completely inhibited these effects, indicating that the contractile response is mediated via M3 muscarinic acetylcholine receptors and is calcium-dependent. These findings suggest that *T. balsamita* extracts exert potent prokinetic effects on ruminant abomasal smooth muscle, supporting their potential as a natural therapeutic option for managing abomasal hypomotility in cattle.

**Keywords:** *Tanacetum balsamita*, Bovine, Abomasum, Prokinetic



## THE EFFECT OF CHITOSAN EDIBLE COATING CONTAINING (ZATARIA) THYME ESSENTIAL OILS ON PHYSICOCHEMICAL AND SENSORIAL PROPERTIES IN COMMON CARP AT REFRIGERATOR TEMPERATURE

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### Abstract

Chitosan is a water-soluble polysaccharide that has the properties of recyclability, antimicrobial, antifungal, antioxidant, gas barrier, and film and coating formation. Zataria thyme essential oil has antimicrobial properties that affect many pathogens and food spoilage organisms. The aim of the present study was to investigate the possibility of using an edible chitosan coating containing Zataria thyme essential oil on carp to increase the storage time at refrigerated temperature. The samples were stored at 4 °C for fifteen days and subjected to physical (weight loss), chemical (moisture, ash, fat, peroxide, pH), and sensory analysis at three-day intervals. All tests were performed in triplicate in a full factorial design. Mean difference comparison analysis was performed using Tukey's test ( $P < 0.05$ ). Sensory evaluation showed that Zataria thyme essential oil had a pleasant effect on all sensory characteristics of carp such as (aroma, color, texture, overall acceptability). The lowest weight loss was related to the treatments of 1% chitosan and 1.5% Zataria thyme essential oil. Also, the lowest ash content was related to the concentration of 1% chitosan containing 1.5% Zataria thyme essential oil. During the study, samples coated with edible chitosan coating containing Zataria thyme essential oil showed a lower pH compared to the control sample. Treatments with chitosan coating containing Zataria thyme essential oil had a better condition than the control sample in terms of peroxide level on day 15. The lowest fat loss was related to chitosan coating containing 1.5% Zataria thyme essential oil. Carp coated with Zataria thyme essential oil has better physicochemical properties than uncoated carp. This coating can be very effective in increasing the shelf life of carp stored in the refrigerator. The required amount of Zataria thyme and cinnamon were purchased from a medicinal plant store in the spring and their scientific names were confirmed by a reputable medicinal plant laboratory. Their essential oils were prepared by hydrodistillation using a Clevenger apparatus, and the essential oils of cinnamon and thyme were extracted by water distillation. Since essential oils are sensitive to light, oxygen, and temperature, and their compounds change and evolve under such conditions, the extracted essential oils were immediately transferred to a dark glass bottle in a seal and stored in the refrigerator until use. In this study, freshly caught farmed freshwater crayfish samples were tested. Their average weight was  $29 \pm 5$  grams. 300 samples were taken from freshwater crayfish by random sampling method. The crayfish samples were kept at 0-4 °C (ice powder) and placed in an insulated container. After necessary examinations, healthy and uniform samples were selected, washed, skinned, and decapitated, and were divided into 35 groups including 34 treatment groups and 1 control group. Each freshwater crayfish was completely coated based on the type of treatment (A: chitosan coating with a concentration of 0% + 3% gelatin (pure coating); B: chitosan coating with concentrations of 0, 0.5, 1, 1.5, and 2% + 3% gelatin + thyme essential oil with concentrations of 0.5 and 1%; C: chitosan coating with concentrations of 0, 0.5, 1, 1.5, and 2% + 3% gelatin + cinnamon essential oil with

concentrations of 0.5 and 1%; D: chitosan coating with concentrations of 0, 0.5, 1, 1.5, and 2% + 3%gelatin + thyme essential oil with concentrations of 0.5 and 1%; D: chitosan coating with concentrations of 0, 0.5, 1, 1.5, and 2% + 3% gelatin + thyme essential oil with concentrations of 0.5 and 1%, + cinnamon essential oil with concentrations of 0.5 and 1%) and then stored in polyethylene containers at refrigerator temperature. After 7 days of storage, 1 treatment was repeated every day and each treatment was repeated 3 times. In each repetition, 8 coated freshwater crab samples were tested. To form a coating on the surface of the freshwater crab samples, the samples were refrigerated for 2 hours and then packaged in polypropylene containers and stored at 4 °C andevaluated on the seventh day.

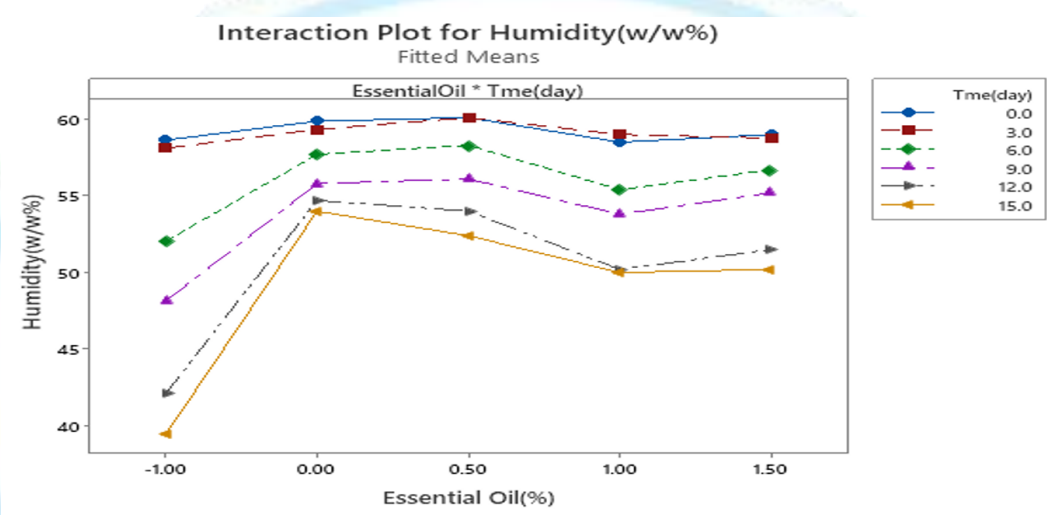


**Figure 1.** Results of moisture measurement of coated carp samples stored at refrigerator temperature for 15 days

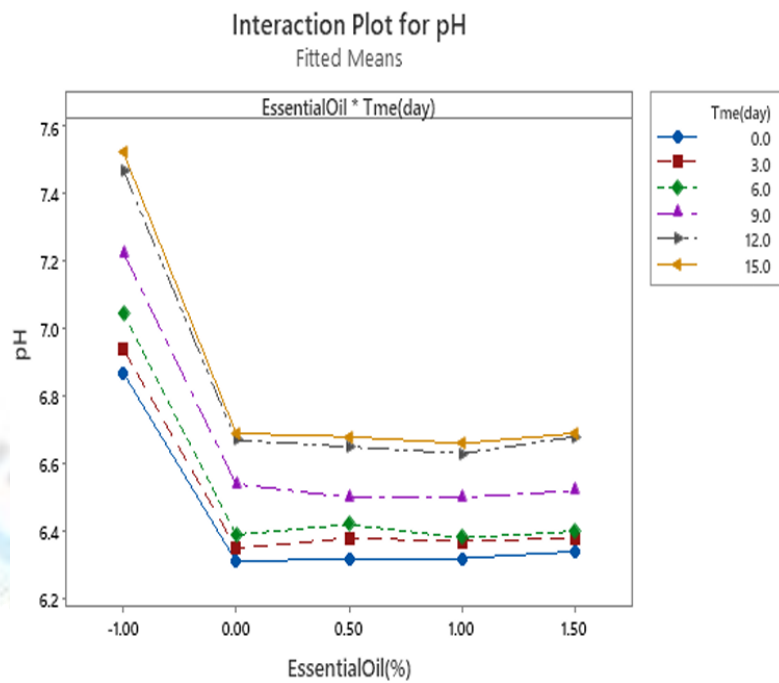


**Figure 2.** Results of pH measurement of coated carp samples stored at refrigerator temperature for 15 days



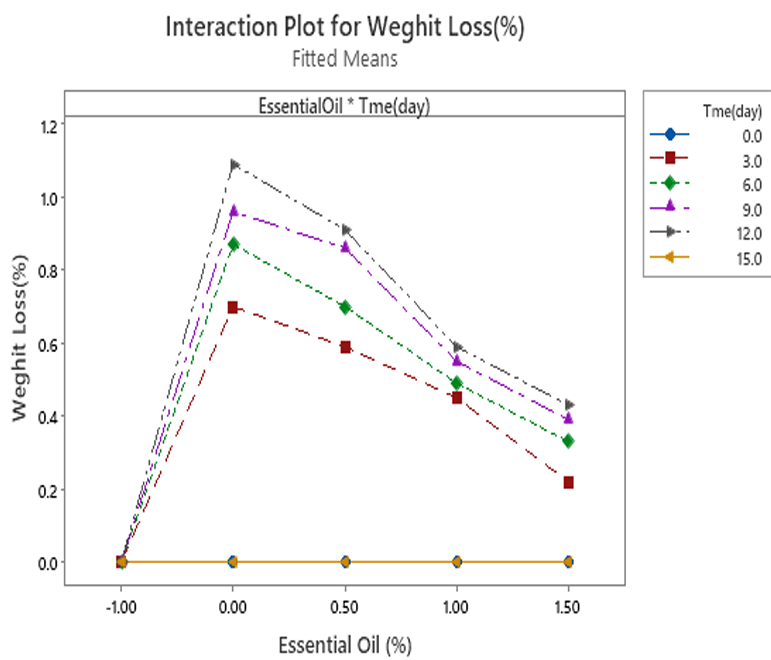


**Figure 3.** Results from measuring weight loss of coated carp samples during 15 days of storage at refrigerator temperature.

Considering the risks of chemical preservatives for consumers and also considering the high consumption of aquatic products including carp, it seems necessary to replace them with natural preservatives in coating formulations. The components of Zataria thyme essential oil are one of these natural compounds, and in this study, its antimicrobial effect on carp was investigated. Natural coatings combined with plant essential oils have antimicrobial properties and can reduce oxidation reactions, improve microbial characteristics, maintain product quality, and increase product shelf life, and provide a basis for the practical use of these compounds compared to synthetic compounds. Chitosan-based coatings combined with essential oils were better than coatings without essential oils in maintaining the quality characteristics of carp at refrigerator temperature, and 1% chitosan and 1% and 0.5% Zataria thyme essential oils combined coatings had the best optimal concentration in inhibiting microbial growth, chemical spoilage, controlling microbial spoilage, and improving physical properties.

**Keywords:** Chitosan, Edible coating, Zataria thyme essential oil, Carp

## INVESTIGATION OF THE PROTECTIVE EFFECTS OF PROTOCATECHUIC ACID ON DIAZINON-INDUCED LIVER DAMAGE IN RATS

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### Abstract

Pesticides are widely employed to control pests that threaten agricultural productivity by damaging crops and livestock. Among them, organophosphate (OP) compounds represent one of the most toxic groups used in modern agriculture. As a large and heterogeneous class of organophosphorus chemicals, OP pesticides exert their toxic effects through biotransformation mediated by cytochrome P450, which promotes oxidative stress and lipid peroxidation, ultimately leading to elevated protein carbonyl (PC) and malondialdehyde (MDA) levels. Beyond their well-documented ability to inhibit acetylcholinesterase, OP compounds also induce programmed cell death via both intrinsic and extrinsic apoptotic pathways. Within this group, diazinon (DZN) is a broad-spectrum pesticide extensively used as an insecticide, acaricide, and nematicide. DZN has been associated with a wide range of toxic outcomes, including acute biochemical alterations, tissue- and organ-specific damage, cytotoxic and genotoxic effects, reproductive toxicity, and negative ecological consequences. Experimental evidence further demonstrates that DZN exposure alters Hepatic enzyme activity, disrupts biochemical parameters, and provokes ultrastructural damage in hepatocytes, such as mitochondrial swelling. In contrast, natural antioxidant compounds have received increasing attention for their potential protective effects against xenobiotic-induced toxicity. Protocatechuic acid (PCA), a naturally occurring phenolic acid abundant in fruits, vegetables, cereals, teas, and medicinal plants, exhibits a wide pharmacological profile. PCA has been reported to exert antioxidant, anti-inflammatory, neuroprotective, antitumor, antibacterial, antidiabetic, and antiapoptotic properties. Its pharmacological effects are largely attributed to its potent in vitro and in vivo antioxidant activity. In addition, PCA protects against hepatotoxicity and nephrotoxicity by modulating enzyme activities, attenuating oxidative stress, and improving biochemical and histopathological alterations. Given these considerations, the present study was designed to investigate the protective potential of PCA against DZN-induced hepatotoxicity. Specifically, the study aimed to elucidate the effects of PCA on inflammation and apoptosis in rat liver tissue. A total of five groups were formed, each consisting of 12 rats. The experimental design was as follows:

Control group: Rats received physiological saline solution daily for 28 days via oral gavage.

PCA group: Rats were administered 100 mg/kg body weight of protocatechuic acid (PCA) daily for 28 days via oral gavage.

DZN group: To induce liver toxicity, rats were administered 20 mg/kg body weight of diazinon (DZN) daily for 28 days via oral gavage.

DZN + PCA (50 mg/kg) group: Rats were administered 20 mg/kg body weight of DZN daily for 28 days via oral gavage, followed 30 minutes later by 50 mg/kg body weight of PCA via oral gavage.

DZN + PCA (100 mg/kg) group: Rats were administered 20 mg/kg body weight of DZN daily for 28 days via oral gavage, followed 30 minutes later by 100 mg/kg body weight of PCA via oral gavage.

Twenty-four hours after the final DZN and PCA administrations (day 29), the rats were



decapitated under light sevoflurane anesthesia following adequate sedative induction. For biochemical analyses, blood samples were collected into vacuum tubes without anticoagulant, and subsequently centrifuged at 4000 rpm for 10 minutes at +4 °C to obtain serum samples. Alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities in the obtained serum samples were analyzed using an AU500 automated biochemistry analyzer (Beckman Coulter Inc., USA). Levels of inflammatory and apoptotic markers in liver tissues, including NF-κB, TNF-α, IL-1β, caspase-3, Bax, Bcl-2, and cytochrome c (CYC-C), were determined using the ELISA method. All analyses were performed with commercial kits according to the manufacturer's instructions. An ethical approval for the use of rats was obtained from the Atatürk University Medical Experimental Application and Research Center (ATADEM) Ethics Committee (approval no: E 75296309-050.01.04-2300372163, dated 21.11.2023). For the study, a total of 60 mal Sprague-Dawley rats, weighing 220–250 g and aged 10–12 weeks, were used. This study was supported by the Atatürk University Scientific Research Projects Coordination Unit under project number TDK-2024-13608.

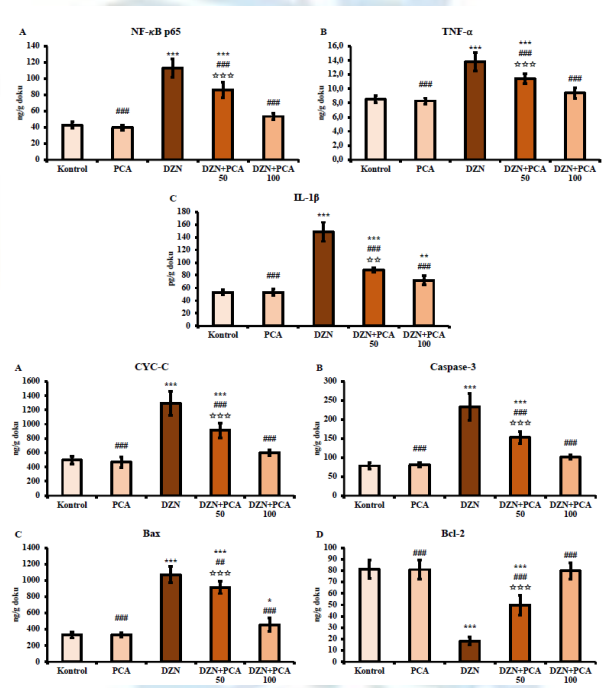


**Figure 1.** In the figures, identical symbols indicate no statistically significant differences between groups ( $p > 0.05$ ), whereas different symbols denote statistically significant differences ( $p < 0.05$ ,  $p < 0.01$ , and  $p < 0.001$ ). Statistical significance was defined as follows: Control vs. others: \*  $p < 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ ; DZN vs. others: #  $p < 0.05$ , ##  $p < 0.01$ , ###  $p < 0.001$ ; DZN + PCA 50 vs. DZN + PCA 100: ☆  $p < 0.05$ , ☆☆  $p < 0.01$ , ☆☆☆  $p < 0.001$ .

The findings of the present study demonstrated that diazinon (DZN) induces hepatic injury primarily by enhancing inflammatory responses and triggering apoptosis in liver tissues. In contrast, protocatechuic acid (PCA) exhibited significant anti-inflammatory and anti-apoptotic properties, thereby attenuating the detrimental effects of DZN. These results are in line with previous studies reporting the protective role of PCA against oxidative stress, hepatotoxicity, and cellular apoptosis in experimental models. Taken together, the current findings suggest that PCA may serve as a promising therapeutic agent for mitigating pesticide-induced hepatotoxicity, particularly through its ability to modulate oxidative stress, inflammatory mediators, and apoptotic pathways. Therefore, PCA could be considered a potential candidate for the development of novel therapeutic strategies aimed at protecting against DZN-induced liver injury.

## MECHANISTIC INSIGHT INTO ANTIMICROBIAL PROPERTIES OF QUERCETIN AGAINST COMMON BACTERIAL FOODBORNE PATHOGENS: A REVIEW

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### Abstract

Quercetin, a naturally occurring flavonoid compound mainly found in vegetables, fruits, and medicinal plants has garnered remarkable attention for its wide array biofunctions, including its well-known antimicrobial activities. This study critically explores previous literature that have been investigated the underling protective mechanisms of quercetin against various infections caused by foodborne bacterial pathogens including salmonellosis, listeriosis, campylobacteriosis, vibriosis, and *E. coli* and *S. aureus* infections. These Foodborne illnesses pose salient global health concerns given the increasing development of resistance to traditional antibiotics. A comprehensive literature search up to August 2025 was conducted using different scientific databases such as PubMed, Web of Science, Scopus and also Google Scholar web search engine, which investigated the protective mechanisms of quercetin against common bacterial foodborne pathogens. Quercetin, foodborne, pathogen, and antimicrobial were the main key words which used in search the literature. According to findings from current literature, the antibacterial properties of quercetin attributed to various mechanisms including disruption of bacterial cell-wall integrity, disruption of nucleic acid synthesis, and downregulation of virulence-related genes. Investigations have been demonstrated that quercetin endangers membrane permeability, leading to leakage of vital cytosolic contents and eventual bacterial cell death. Furthermore, it inhibits DNA gyrase, topoisomerase IV and other enzymes crucial for bacterial replication, thereby hindering bacterial proliferation. Also, quercetin's ability to regulate quorum sensing pathways influences biofilm formation and diminishes their pathogenic potency. In addition to direct actions, quercetin exhibits synergistic effects in combination therapy with traditional antibiotics, improves their efficacy, overcomes antibiotic resistance in certain strains. The anti-inflammatory and antioxidant properties of quercetin also contribute to its therapeutic capacity by modulating affected patient immune responses during bacterial food poisoning. This review highlights quercetin role as a promising natural-based and harmless candidate for food preservation and as an adjuvant to antimicrobial therapy. Despite satisfactory findings of *in vitro* and *in vivo* studies, challenges such as poor water- solubility, limited absorption, rapid biodisposition and variability in effectiveness across bacterial pathogens remain. Further and advanced investigation, particularly formulation studies and clinical trials, is necessary to fully clarify quercetin's application in food safety and bacterial foodborne illness management.

**Keywords:** Anti-microbial, Foodborne, Bacterial pathogen, Quercetin



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