1st International Congress of Veterinary Medicinal Plants and Traditional Medicine

21-22 October 2024
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Foreword

It is a great pleasure that we welcome you to the 1st International Congress of Veterinary Medicinal Plants and Traditional Medicine. This event marks a pivotal moment in veterinary science, where, traditional knowledge meets modern scientific advances, opening new horizons for animal health and well-being. This congress provides a unique platform for experts, researchers, practitioners and students to come together, share insights and foster collaboration across borders and disciplines. Our collective aim is to explore the potential of medicinal plants and traditional remedies in veterinary care, integrating these age-old practices into contemporary medicine to promote sustainable and holistic approaches to animal health.

The importance of this congress cannot be overstated, as it addresses one of the key challenges in modern veterinary care: The need for sustainable and effective alternatives to conventional pharmaceutical interventions. With the world increasingly turning to natural therapies, the role of medicinal plants and traditional healing methods has become more relevant than ever. This event seeks to raise awareness and promote research that can lead to new discoveries, preserve biodiversity and empower local communities with their dependence on the remedies to safeguard the animal health.

Beyond its scientific significance, this congress carries substantial economic importance. The use of medicinal plants in veterinary medicine offers cost-effective, sustainable alternatives to expensive pharmaceutical products contributing to a reduction in healthcare costs for both animals and the owners. Moreover, via promoting the research and development of herbal medicine, it opens new economic opportunities for local farmers, herbalists and communities to create pathways for growth of local economies and international markets. These economic benefits, coupled with preservation of traditional knowledge, highlight the dual advantage of using herbal remedies in veterinary care—improving animal health while also boosting economic resilience.

We extend our heartfelt congratulations to all participants, speakers and attendees for their dedication to advancing the field of veterinary medicine through research and collaboration. Your engagement in this congress reflects a shared determination to bridge the gap between traditional wisdom and modern veterinary practices. The diverse presentations,

discussions and workshops that will unfold here are certain to inspire fresh ideas, foster new partnerships and help shape the future of animal care worldwide.

Once again, we warmly welcome you to this inaugural congress. We look forward to the stimulating conversations and collaborative efforts that will emerge from this gathering, and we are excited about the lasting partnerships and innovations that will shape the future of veterinary medicine. Together, let us move forward with a vision of advancing veterinary care through the rich legacy of traditional knowledge, empowered by modern scientific inquiry and the economic potential of sustainable herbal solutions.

Prof. Masoud Maham

Head of Scientific Committee

and

Dean of Faculty of Veterinary Medicine

Urmia University

Foreword

The "**1st 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. Mustafa Sinan Aktaş
Dean of Faculty of Veterinary Medicine
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Section 1

Basic Sciences

Investigating the role of quercetin as an antioxidant in degenerative diseases: A review abstract

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Abstract

Cancer poses a major global challenge due to increasing incidence rates and limited treatment options. Thus, discovering new therapeutic targets and identifying potential therapeutic molecules for tumor treatment is crucial. Diet is crucial for maintaining health with natural products like flavonoids playing a role in cancer prevention. Flavonoids are plant-derived natural antioxidants found in fruits and vegetables, where they help trap free radicals. Quercetin, a notable bioactive flavonoid has garnered attention for its various health benefits. It is present in many foods including fruits, seeds, vegetables, tea, coffee, bracken fern, and natural dyes. Quercetin may protect against degenerative diseases by inhibiting lipid peroxidation. The extent and mechanism of quercetin *in vivo* absorption remain largely unknown. The primary glucoside form is thought to be converted into the aglycone which is then transformed into various quercetin metabolites. Studying quercetin metabolites is crucial for understanding its antioxidant mechanism. Multiple studies both *in vivo* and *in vitro* support its potential as an anticancer agent. Quercetin strong toxicity against cancer cells is linked to minimal or no side effects on normal cells. In recent decades, quercetin has been recognized for its anticancer properties including its involvement in cell signaling, pro-apoptotic activity, anti-proliferative effects, antioxidant capabilities and growth suppression. It is now understood that quercetin exerts various biological effects by inhibiting multiple enzymes involved in cell proliferation and signal transduction pathways. This review briefly discusses quercetin chemical and physical properties as well as its antioxidant and anticancer activities and mechanisms of action.

Keywords: Antioxidant, Degenerative diseases, Flavonoid, Quercetin

Introduction

The body combats oxidative stress through antioxidants which can be produced internally (endogenous) or obtained from food (exogenous). Antioxidants neutralize excess free radicals, protect cells from their harmful effects and help prevent disease. When an antioxidant neutralizes a free radical, it becomes oxidized itself, necessitating continuous replenishment in the body. Consequently, an antioxidant may be effective in one system but lose its effectiveness in another. Flavonoids are polyphenolic compounds found in most plants with over 4,000 different types identified including flavones, isoflavones, flavanones, and chalcones. Their primary health benefits stem from their strong antioxidant properties which may help prevent or delay various

chronic and degenerative diseases including cancer, cardiovascular disease, arthritis, aging, cataracts, memory loss, stroke, Alzheimer's disease, inflammation and infection. Quercetin, as a flavonoid, is a natural pigment found in many fruits, vegetables and grains serving as a potent dietary antioxidant. It helps combat free radical damage linked to chronic diseases and may reduce inflammation, allergies, blood pressure and associated health risks including heart disease, cancer and neurodegenerative disorders. The health benefits of flavonoids like quercetin stem from their antioxidant properties (1,11).

Quercetin is a yellow, crystalline substance that has a bitter flavor. It is insoluble in water, has slight solubility in alcohol and dissolves in glacial acetic acid and alkaline aqueous solutions. It belongs to the flavonoid family which consists of naturally occurring compounds characterized by a shared flavone structure made up of two benzene rings connected by a heterocyclic pyrone ring. Since animals cannot produce the flavones structure, flavonoids are exclusively found within the plant kingdom. Quercetin, along with more than 2,000 other flavonoids is derived from the condensation of p-glycosides. This compound is present in a variety of foods and plants including fruits, seeds, vegetables, tea, coffee, bracken fern and natural dyes. Typically, quercetin is extracted through the hydrolysis of rutin (quercetin-3-rutinoside), a naturally occurring flavonoid glycoside, though, it can also be synthesized artificially. An average person consumes 10-100 mg of quercetin daily from foods like onions, apples, grapes, berries, broccoli, citrus fruits, cherries, green tea and coffee. Quercetin is also available as a powdered or capsule supplement which people take to boost immunity, reduce inflammation, alleviate allergies, enhance athletic performance and maintain overall health (1, 10).

Health benefits of Quercetin

Reduce inflammation. Quercetin may help reduce inflammation which is linked to health issues such as certain cancers and heart and kidney diseases. High levels of free radicals can activate inflammatory genes leading to persistent inflammation, although some inflammation is necessary for healing. Studies have shown that quercetin reduces inflammatory markers in human cells, including tumor necrosis factor alpha (TNFα) and interleukin 6. In an 8-week study involving 50 women with rheumatoid arthritis, those taking 500 mg of quercetin experienced significant reductions in morning and post-exercise pain as well as decreased inflammatory markers like TNFα compared to a placebo group. Although these results are promising, further human research is necessary to fully understand quercetin anti-inflammatory potential (5, 6).

Alleviation of allergy symptoms. Quercetin may alleviate allergy symptoms due to its anti-inflammatory properties. Research indicates that it can inhibit enzymes related to inflammation and reduce inflammatory compounds like histamine. For instance, a study showed that quercetin supplementation decreased peanut-induced anaphylactic reactions in rats (13).

Reduced risk of chronic brain disorders. Research indicates that quercetin antioxidant properties may help protect against chronic brain disorders like Alzheimer's disease and dementia. In one study, mice with Alzheimer's received quercetin injections every two days for three months resulting in the reversal of several Alzheimer's markers and improved performance in learning tests. Another study found that a quercetin-rich diet reduced Alzheimer's markers and enhanced brain function in mice in early to middle stages of the disease. Additionally, coffee, a popular beverage linked to a lower risk of Alzheimer's, contains quercetin as opposed to caffeine has the key compound contributing to its potential protective effects (9).

Lower blood pressure. One in three American adults suffers from high blood pressure which raises the risk of heart disease, the leading cause of death in the U.S. Research indicates that quercetin can help lower blood pressure. In an *in vivo* study, hypertensive rats given daily quercetin for five weeks experienced average reductions of 18% in systolic and 23% in diastolic blood pressure. A review of nine human studies with 580 participants found that daily supplementation of over 500 mg of quercetin reduced systolic and diastolic blood pressure by an average of 5.8 mm Hg and 2.6 mm Hg, respectively (4).

Anti-cancer effects. Quercetin, known for its antioxidant properties, may also possess anti-cancer effects. Laboratory and animal studies indicate that it can suppress cell growth and induce cell death in various types of cancer cells including prostate, liver, lung, breast, bladder, blood, colon, ovarian, lymphoid and adrenal cancers. As a result, it is recommended as an alternative cancer treatment (1, 12).

Colon cancer. Numerous studies have examined the effects of quercetin on colon cancer. In an *in vitro* study, researchers utilized water-soluble tetrazolium salts assays, annexin V assays, real-time polymerase chain reactions, western blot analyses and gelatin zymography to evaluate quercetin inhibitory effects on colorectal lung metastasis. They found that quercetin can reduce cell viability in colon 26 (CT26) and colon 38 (MC38) cells and induce apoptosis in CT26 cells via the MAPK pathway, regulate the expression of epithelial-mesenchymal transition markers including E-cadherin, N-cadherin, β-catenin, and snail at nontoxic concentrations, and inhibit the migration and invasion of CT26 cells by regulating matrix metalloproteinases and tissue inhibitors of metalloproteinases. The researchers concluded that quercetin can impede the survival and metastatic capabilities of CT26 cells and suppress colorectal lung metastasis in mouse models, suggesting its potential as a therapeutic agent for metastatic colorectal cancer (7, 12).

Pancreatic cancer. Research indicates that quercetin triggers apoptosis in TRAIL-resistant pancreatic cancer cells. A BH3-only protein named BID significantly reduces the apoptosis induced by TRAIL and quercetin. Quercetin also decreases cellular FLICE-like inhibitory protein expression, offering pancreatic cancer cells protection from TRAIL/quercetin-induced apoptosis in a dose-dependent manner. Additionally, quercetin activates JNK which promotes the proteasomal degradation of FLICE-like inhibitory protein, further sensitizing pancreatic cancer cells to TRAIL-induced apoptosis. In human pancreatic cancer cell lines CFPAC-1 and SNU-213, quercetin-3-O glucoside inhibits migratory activity caused by TGF-β and vascular endothelial growth factor A even at low doses in CFPAC-1, though, not in bFGF-activated SNU-213 cells. Moreover, combining low doses of gemcitabine with quercetin-3-O-glucoside shows synergistic effects in inhibiting bFGF-induced migratory activity in both CFPAC-1 and SNU-213 cells (8).

Liver cancer. Liver cancer is primarily caused by cirrhosis from hepatitis B or C. Other factors include aflatoxin, fatty liver disease and liver flukes. The most prevalent types are hepatocellular carcinoma accounting for 80% of cases and cholangiocarcinoma. Quercetin has been shown to inhibit cancer cell growth through mechanisms like cell cycle arrest, apoptosis,and antioxidant activity. Zhao and colleagues investigated quercetin effects on human liver cancer HepG2 cells and found that it induces apoptosis by inhibiting fatty acid synthase. Their findings suggest quercetin may help prevent liver cancer. Moreover, research indicates that while dietary quercetin has a minor regulatory effect, higher supplement doses can significantly impact gene expression in hepatocytes (12, 14).

Lung cancer. Numerous studies have investigated quercetin as a chemotherapeutic agent for lung cancer. One study found that quercetin significantly enhances TRAIL-induced cytotoxicity in non-small cell lung cancer cells by increasing the expression of death receptor 5 (DR5) while not affecting other components of the death-inducing signaling complex. The researchers demonstrated that quercetin sensitizes lung cancer cells to TRAIL-induced cytotoxicity through two mechanisms: (a) induction of DR5 and (b) suppression of survivin expression which may account for its lung cancer preventive properties. Additionally, they observed that treating human lung cancer H-520 cells with quercetin increases cisplatin-induced apoptosis by 30.2%, down-regulates Bcl-XL and Bcl-2 and up-regulates Bax (3).

Absorption, metabolism and excretion of the quercetin. Quercetin glycosides have limited absorption in the small intestine. The microflora in the lower bowel hydrolyze these flavonoid-glycosides into quercetin and sugar enabling quercetin to enter the enterohepatic circulation. After administering quercetin orally to rabbits or rats, three metabolites were detected in urine: 3,4-dihydroxyphenylacetic acid, 3-methoxy-4-hydroxyphenylacetic acid (also known as homovanillic acid) and m-hydroxyphenylacetic acid which are likely produced in the liver through ring fusion. The distribution, metabolism and excretion of 4-[14C] quercetin in male ACI rats were investigated using autoradiography and radioactivity quantification. Following oral administration, 20% of the dose was absorbed from the gastrointestinal tract and subsequently excreted into the bile and urine within 48 hr as glucuronide or sulfate conjugates. Autoradiographic evaluation of a rat 3 hr after a single oral dose of 2.3 mg/kg quercetin indicated that the majority of radioactivity was remained in the digestive tract with minimal levels observed in the blood, liver, kidney, lung and rib (1, 2).

Quercetin is extensively investigated for its antioxidant, anti-inflammatory and anti-cancer properties in degenerative diseases. As a key component of flavonoids, quercetin exhibits strong antioxidant effects demonstrated in laboratory settings. Quercetin may play a significant role in combating chronic degenerative diseases and has shown potential in treating various cancers. Furthermore, quercetin may enhance the effectiveness of other anticancer medications allowing for reduced dosages and fewer side effects.

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Homeopathy in veterinary medicine

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Homeopathy, operating on the principle of "like cures like," is a traditional medicinal system known for many years. According to homeopathy, if any substance causes symptoms of illness in a healthy individual, then a patient showing similar symptoms can be treated with that substance. The vital force is important in homeopathy. Therefore, it can affect all living systems as humans, animals, and plants, yielding very effective results. For homeopathic purposes, substances derived from animal, plant, and mineral sources, as well as acids, salts, and materials obtained from healthy/sick tissues, can be used for treatment (1-3). Homeopathy can be used in the treatment of several diseases in animals; it is particularly beneficial in acute cases such as traumas, injuries, and insect bites. In such cases, homeopathic treatment reduces or completely prevents inflammation symptoms, such as swelling and pain, shortening the healing time. Additionally, it is successfully used in inflammatory conditions, such as acute and chronic diarrhea, gingivitis, respiratory diseases, and dermatitis. It has been reported that homeopathy also provides healing in chronic diseases in animals, such as arthritis and spondylitis. It is particularly effective in mastitis and colic in farm animals. Since the medicines are given in the form of small globules or dissolved in water, their application is easy, and they can be easily absorbed under the tongue (1).

Keywords: Homeopathy, Veterinary medicine, Traditional medicine

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Cardiovascular effects of pomegranate fruit

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The therapeutic properties of pomegranate (Pg) fruit (*Punica granatum* L.) have long been recognized. This report discusses some of the cardiovascular effects of this fruit. Research has shown that Pg juice has positive effects on cardiac mechanical activity, reducing myocardial cell death, improving redox status, and decreasing the incidence of arrhythmias following ischemia and reperfusion in isolated rat hearts (1). These effects were found to be linked to nitric oxide, as inhibition of nitric oxide synthase eliminated most of these benefits (2). Additionally, three weeks of intragastric gavage of mesocarp extract significantly improved ventricular performance and reduced the extent of the size of infarcted area in isolated rat hearts following ischemia and reperfusion. Clinical trials on patients with acute coronary syndrome demonstrated that five days of treatment with Pg juice resulted in significant reductions in the severity, duration, and frequency of angina pectoris, as well as decreased levels of serum malondialdehyde and troponin (3). Furthermore, ellagic acid, a prominent polyphenol in Pg, exhibited cardioprotective effects in isoproterenol-induced myocardial infarction and heart failure in rats. Specific inhibitors suggested the involvement of ATP-sensitive potassium channels and PI3-K-associated signaling pathways in these effects. Finally, ellagic acid was able to prevent endothelial dysfunction in the culture medium through adropin and nitric oxide. Overall, the research suggests that the powerful polyphenols in Pg fruit, particularly ellagic acid, may have significant preventive and therapeutic effects against cardiovascular damage, making Pg fruit a promising candidate for further exploration in the field of cardiovascular health.

Keywords: Pomegranate, *Punica granatum*, Ellagic acid, Heart, Myocardial ischemia and reperfusion

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Repro-protective effect of *Hypericum perforatum* in an experimentally Ketamine-induced schizophrenia

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Recreational ketamine (KET) abuse is extremely increasing worldwide due to its hallucinatory effects in human (1, 2). From this point of view, the present survey was designed to elucidate the repro-protective effects of *Hypericum perforatum* (HP) on mature rats testicular tissue and epididymal sperms characteristics in an experimentally KET-induced schizophrenia. Twenty adult male rats were assigned into four equal groups including non-treated control group, HP control group receiving HP (Kneipp® Johanniskraut Dragees H; 100 mg/kg/day) orally (PO) for 14 days, KET group receiving KET (20 mg/kg/day; intra-peritoneally [IP]) for 14 days, and KET/HP group receiving KET (20 mg/kg/day; IP) plus HP (100 mg/kg/day; PO) for 14 days. After that, testicular histo-architecture, histopathological changes and anti-oxidant/oxidant balance as well as epididymal sperms characteristics were examined at the end of experimental period. The HP administration caused significant promotion in testicular histological indices and total anti-oxidant capacity and epididymal sperms characteristics (count, motility and viability) along with significant decline in testicular tissue damage and total oxidant status compared to the KET group. These findings emphasize on protective function of HP against KET-instigated reproductive disorders in mature male rats, opening the novel way for future therapeutic approaches in the field of drug abuse adverse effects.

Keywords: *Hypericum perforatum*, Ketamine, Schizophrenia, Sperm, Testis

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Phytochemistry and biological activities of *Myrtus communis* L. (Myrtaceae)

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Myrtle (*Myrtus communis* L., Myrtaceae) is an evergreen shrub being distributed in the northern parts of Iran and southern parts such as Fars, Kerman, Yazd, and Hormozgan provinces (1). *Myrtus communis* is a medicinal herb being extensively used in traditional medicine. A large number of its components has been studied like polyphenols, myrtucommulone, semimyrtucommulone, 1,8- cineole, α- pinene, myrtenyl acetate, limonene, linalool, and α- terpinolene. All parts of this species have been used extensively in traditional medicine. *M. communis* is used to treat diarrhea, peptic ulcer, hemorrhoid, inflammation, and pulmonary and skin diseases. In addition, clinical studies have shown more pharmacological effects such as anti-oxidative, anti-cancer, anti-diabetic, anti-viral, anti-bacterial, anti-fungal, hepatoprotective, and neuroprotective activities (2,3). Chemical components like volatile substances, terpenoids, triterpene, flavonoids, tannins, and fatty acids have been isolated from different parts of the plant. Although *M. communis* is still less known in developed countries, its anti-oxidant, anti-bacterial, anti-fungal, and analgesic effects have been proven well. Recent studies have indicated that *M. communis* has anti-hyperglycemic, anti-bacterial, anti-viral, anti-fungal, anti-oxidant, anti-mutagenic, anti-hemorrhagic, hepatoprotective, wound healing, and insecticidal properties (1). The present review attempts to give an overview of the phytochemical and pharmacological studies of *M. communis* needing further research to unravel other pharmacological activities of this plant in the long run either for human or animals.

Keywords: *Mirtus communis*, Myrtle, Phytochemistry

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Comparison of chemical composition of *Eucalyptus camaldulensis* essential oil in Ahvaz and Urmia using gas chromatography-mass spectrometry method

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Medicinal plants have always been considered as effective compounds in the treatment of diseases. Research to find novel compounds is essential because of the resistance of microorganisms to antibiotics and surreptitious use of them. Among the compounds extracted from plants, essential oils (EOs) have a unique characteristic because they have anti-fungal, anti-viral and anti-bacterial properties. In this study, the EO of *Eucalyptus camaldulensis* was investigated. In a study conducted by Elia *et al*., (1) samples of *E. camaldulensis* were collected from Ahvaz, Iran, their leaf EO was extracted in a Clevenger apparatus, and the chemical compounds were analyzed using gas chromatography-mass spectrometry (GC-MS). The GC-MS analysis revealed 17 compounds. In another study, the chemical compounds of *E. camaldulensis* cultivated in Urmia, Iran, were determined by GC-MS (2). There were 34 compounds, and 5 similar compounds can be observed between them and Ahvaz compounds. These compounds include 1,8-cineole, α-pinene, γ-terpinene, allo-aromadendrene, and viridiflorol. The 1,8-cineole, which is the most important compound of *E. camaldulensis*, had a higher percentage in the sample grown in Ahvaz (55.20% *vs*.36.62%). Two different habitats are affected by factors, such as climate, and experimental and seasonal conditions (1-3). More research is required to compare the chemical composition of *E. camaldulensis*' EO in different habitats of Iran. Using minimum inhibitory concentration, minimum bactericidal concentration and inhibition zone diameter, the performance of the EO on specific bacteria can be determined and the most effective one can be selected.

Keywords: 1,8-cineole, Essential oil, *Eucalyptus camaldulensis*, GC-MS

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Ethnoveterinary applications of *Curcuma longa*: A review article

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Among various herbal remedies employed in ethnoveterinary medicine, *Curcuma longa* (turmeric) stands out due to its extensive use in traditional medicine systems. Traditional medicine practitioners have utilized turmeric for its anti-inflammatory, anti-microbial, and anti-oxidant properties, making it a valuable resource in treating a range of animal ailments. PubMed and Google Scholar were searched using the keywords *Curcuma longa* and ethnoveterinary. Out of 273 articles, 16 studies were chosen. Pharmacological studies were examined to assess the therapeutic efficacy of *Curcuma longa* and its active constituent, curcumin. *Curcuma longa* is traditionally used in the treatment of over thirty conditions, such as wounds, hepatic disorders, jaundice, and as a blood purifier. It exhibits a wide range of therapeutic activities, including anti-oxidant, anti-inflammatory, anti-bacterial, anti-fungal, anti-viral, anti-diabetic, hepatoprotective, and neuroprotective effects (1,2). Curcumin, the primary active constituent, inhibits leukotriene biosynthesis, reduces prostaglandin formation, and induces apoptosis in cancer cells. The phytochemical composition of *Curcuma longa* includes flavonoids, tannins, anthocyanins, phenolic compounds, and essential oils. This review underscores the versatility and economic significance of *Curcuma longa*, highlighting its potential for broader application in both traditional and modern medicine, particularly in ethnoveterinary contexts. Despite promising pre-clinical evidence, further clinical trials are necessary to validate its therapeutic potential and ensure its efficacy in clinical settings.

Keywords: Curcumin, Ethnomedicine, Herbal medicine, Traditional medicine

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Gallic acid: A review of application, benefits and dosage in veterinary medicine

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Gallic acid is a trihydroxybenzoic acid in which the hydroxy groups are in the 3, 4, and 5 positions. It is a colorless or slightly yellow crystalline compound that is widely used in the food and pharmaceutical industries (1). Gallic acid has been isolated from different plant species such as *Quercus spp.* and *Punica spp.* through different chromatographic methods. However, from an industrial point of view, gallic acid is produced through the hydrolytic decomposition of tannic acid using a glycoprotein esterase, tannase. This acid acts as an astringent, a cyclooxygenase 2 inhibitor, a plant metabolite, an antioxidant, an antineoplastic agent, a human and animal xenometabolite. In recent studies, it has proven that gallic acid can exert its cytotoxic and antitumor effect in livestock by modulating the antioxidant/peroxidative balance (2). In some cases, this compound can control carcinogenesis caused by reactive oxygen species in breast cancer in animals by increasing the activity of superoxide dismutase, catalase, glutathione reductase and glutathione peroxidase. In addition to the research conducted in the field of gallic acid, research on the dose of gallic acid used in the animal body has not yet been determined. Also, the mechanism of action and the ways of the effect of gallic acid and the way of cell death have not yet been determined (3).

Keywords: Cattle, Dosage, Gallic acid, Poultry

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Development of zein nanoparticles using natural surfactants for enhanced drug delivery systems

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Zein proteins, a type of alcohol-soluble storage protein found in corn kernels, are increasingly recognized for their applications in drug delivery and coatings particularly in poultry farming where they assist in nutrient protection and controlled delivery systems (1, 2). However, a major challenge with zein nanoparticles is their structural instability over time which can result in particle aggregation, reduced dispersibility and altered drug delivery properties. To overcome this issue, the nanoprecipitation method is employed. This involves dissolving zein in an organic solvent and then adding it to an aqueous solution with a surfactant which promotes the rapid formation of zein nanoparticles offering more control over their size and surface properties (3). In this study, saponin derived from Acanthophyllum, a natural surfactant, was used to create uniformly distributed nanoparticles. Saponin not only enhances nanoparticle stability but also increases the efficiency of drug delivery particularly for poorly water-soluble drugs such as anti-cancer agents. Approximately 40% of the saponin is absorbed onto the zein ensuring effective encapsulation. The concentration of saponin used was ranged from 1000 ppm to 4000 ppm while, the concentration of zein varied between 0.5% to 2% w/v. These conditions were optimized to produce nanoparticles with better control over their properties. The resulting nanoparticles not only provided enhanced protection for light-sensitive and degradable compounds but also showed improved stability.

Keywords: Drug delivery, Nanoparticles, Poultry, Saponin, Zein

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Are cannabinoids effective on cancers?

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Cannabinoids are naturally compounds found in *Cannabis sativa* or endogenously produced in mammals, birds, reptiles and fish. The production of cannabinoids for different purposes including oil-seed and fiber production had already commenced around 4000 *BC*. At least 113 forms of cannabinoids have been extracted from cannabis plant material. The most well-known compounds are Δ9-tetrahydrocannabinol, cannabidiol and cannabinol (1). There are some reported side effects for cannabinoid consumption including increasing the time of sleep with lower quality, effects on cognition and some neurological disorders. Following search in Pub Med (132 articles) and Google scholar (9 articles) databases between 2010 to 2020, it was found that they are effective in the treatment of lung, colorectal, prostate, breast, pancreas, and hepatic cancers. The effectiveness of natural and synthetic cannabinoids mediated by cannabinoid receptors (type 1 and 2, Vanilloid receptors) has been documented. Another recently recognized receptor for cannabinoid effects is CB3 which is expressed in entire body (2). There are close association between the up-regulation of CB3 and the incidence of melanoma and colorectal cancer (3). There are increasing and convincing data on effectiveness of cannabinoids in treatment of various cancers and still additional well-designed *in vivo* preclinical and clinical trials should be performed. At the same time, the regulatory processes should be also established.

Keywords: Cancer, Cannabinoids, Effectiveness, Receptor, Treatment

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The protective effect of *Achillea millefolium* hydro-alcoholic extract on *in vitro* fertilization in mature male mice treated with fluoxetine

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Fluoxetine (FLX), also known as Prozac, is a widely marketed selective serotonin reuptake inhibitor, used to fight depression and anxiety. *Achillea millefolium* (AM) is a medicinal plant with potential anti-oxidant properties (1-3). The aim of this investigation was to evaluate the effects of different doses of AM extract on *in vitro* fertilization (IVF) in fluoxetine treated mice. Thirty adult male mice (26 ± 3 g) were randomly divided into 5 groups (n = 6). Control groupreceived normal saline (0.2 mL/mice/day) orally for 30 day. In (FLX group mice received FLX at dose of 20 mg/kg daily for 30 days orally. High dose, medium dose and low dose of AM plus FLX groups received AM respectively at doses of 150, 100 and 50 mg/kg and FLX (20 mg/kg) daily for 30 days orally. The percentage of two-cell and blastocyst- stage and arrested embryos was determined on day 5 of fertilization. In this study, FLX caused a significant decrease in fertilization rate and percentage of two-cell and blastocyst embryos and a significant increase in the percentage of arrested embryos. The AM in high and medium doses caused an increase in IVF rate, two-cell and blastocyst embryos percentage and a reduction in percentage of the arrested embryos. Taken together, AM (150 and 100 mg/kg) exhibited repro-protective activities against FLX-induced embryotoxicity in mice.

Keywords: *Achillea millefolium*, Embryo, Fluoxetine, Mice

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Section 2

Immunology

Benefits of combining piperine with prednisolone in an experimental model of rheumatoid arthritis

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Abstract

Anti-inflammatory and immunomodulatory properties of piperine have been documented. This study evaluated the beneficial effects of piperine on clinical symptoms and immune responses in Wistar rats with rheumatoid arthritis (RA) induced by Freund's complete adjuvant (CFA). The RA rats were randomly divided into three groups (n = 10): RA rats treated with PBS (100 mg/kg orally), RA rats treated with piperine (100 mg/kg orally), and RA rats treated with prednisolone (10 mg/kg orally). Treatment commenced on day five post-induction when all rats had a clinical score of ≥1. Disease symptoms were monitored every other day until day 23 post-induction. Treatment with piperine resulted in a significant reduction in disease severity and improved weight gain in RA rats. Biochemical indices of CRP, myeloperoxidase and NO were significantly decreased in the treatment group compared to the non-treatment group. The group treated with piperine exhibited a significant decrease in the expression of T-bet, GATA-3, and RORɣt genes compared to RA rats without treatment. However, there was no statistically significant difference in Foxp3 gene expression in the piperine-treated group compared to the untreated group. Overall, piperine might represent a valuable approach to managing RA.

Keywords: Freund's complete adjuvant, Piperine, Prednisolone Rheumatoid arthritis

Introduction

Rheumatoid arthritis (RA) is among the most common chronic inflammatory joint diseases affecting approximately 1% of the global population (1). Adjuvant induced arthritis is an experimental arthritis model where arthritis is induced in a rat model by injecting Freund's complete adjuvant (FCA). This model of disease induction serves as a reliable approach to studying RA. It is also considered one of the most effective models for assessing the efficacy of various compounds as potential drugs for treating RA and other chronic inflammatory conditions (2).

Long-term management of chronic inflammatory diseases like RA often involves the prescription of anti-inflammatory drugs to regulate the dysfunctional immune system. Consequently, there is a pressing requirement to create secure and efficient medications suitable for extended periods of use. Numerous research teams have investigated non-steroidal anti-inflammatory compounds derived from natural sources aiming to discover novel therapeutic options for clinical application (3). The components and extracts derived from

plants are the subject of increasing research resulting in a rise in their consumption globally for their positive impact on health (4).

Piperine is a nutrient-rich compound in black and long pepper (*Piper nigrum* and *Piper longum*). It is commonly used as a seasoning in various cuisines worldwide and has also been utilized as a traditional medicine in Asia and the Pacific islands particularly in India (5,6). Piper species have demonstrated the ability to block enzyme activity involved in producing leukotrienes and prostaglandins. Furthermore, piperine has been shown to suppress nitric oxide (NO), tumor necrosis factor-α (TNF-α) and the expression of pro-inflammatory genes both *in vitro* and *in vivo* (7).

This study evaluated the beneficial effects of piperine on clinical symptoms and immune responses in Wistar rats with RA induced by CFA.

Material and Methods

Chemicals. Cell culture media and fetal calf serum were obtained from GIBCO/Life Technologies Inc. (Gaithersburg, MD, USA). The ELISA (enzyme-linked immunosorbent assay) kits were purchased by PeproTech EC, Ltd. (London, UK). RNX-Plus solution for RNA isolation was procured from DENAzistAsia (Mashhad, Iran). SYBR Premix Ex TaqII and cDNA reverse transcription kits were purchased from TAKARA (Takara Biomedical Ltd., China). Hematoxylin-eosin staining kits were prepared by Sigma-Aldrich Corporation (St. Louis, MO, USA). Piperine, methotrexate, MTT and other reagents were procured from Sigma-Aldrich Corporation (St. Louis, MO, USA).

Animal. A group of 60 male Wistar rats, aged eight weeks, were acquired from the Faculty of Veterinary Medicine, Urmia University, Urmia, Iran. These rats had an average weight of 150 ± 7 g. They were housed in a controlled environment with a temperature of 23 ± 1 ˚C and a 12-hr light/dark cycle. The rats had unrestricted access to food and water. All experimental procedures were conducted following the ethical standards outlined in the laboratory laws published by the National Institute of Health Guide. The Ethics Committee for Laboratory of our faculty approved the experiment.

Induction of RA and animal groups. The RA was induced in rats through the intradermal injection of 0.1 mL of CFA into the hind paw. The CFA contained 10 mg/mL of killed mycobacterium. To assess the severity of the disease, the volume of the non-injected hind paw was measured every other day using an electronic water plethysmograph. A scoring system was employed where a score of 4 indicated complete swelling of the entire leg with an inability to bend it, a score of 3 represented swelling of the ankle, a score of 2 denoted erythema and swelling of the paws, a score of 1 indicated erythema of the toe and a score of 0 represented a normal paw. Evaluations were conducted every Monday throughout the study with three independent observers assessing each examination. The average of the measurements was reported. The maximum arthritis index possible was 12, and this index was evaluated solely for the non-injected paws. Additionally, the weight changes of each rat were recorded every other day following immunization (8). The rats were placed in groups as follows: Group 1 consisted of 10 healthy rats that did not receive any treatment (Control group). Group 2 consisted of 10 rats in which RA was induced without any treatment (RA group). Group 3 comprised 10 rats in which RA was induced, and 100mg/kg of piperinewas administered daily via

oral gavage (RA+Pip). Group 4 comprised 10 rats in which RA was induced and prednisolone was administered orally at a rate of 10 mg/kg daily (RA+Pred group).

Biochemical assays. On the 28th day, the animals were subjected to deep anesthesia and underwent bleeding to obtain the serum required for the subsequent tests. One of the tests conducted was the myeloperoxidase (MPO) activity test. For this test, a serum sample of 10 µL was mixed with 80 µl of 0.75 mM hydrogen peroxide and 110 µL of the reaction solution containing 2.9 mmol of TMB in 14.5% dimethyl sulfoxide along with 150 mM of sodium phosphate buffer (pH = 4.5). The samples were then incubated at 37 ˚C for 15 min. Afterward, 50 µL of sulfuric acid (2 M solution) was added to halt the reaction. The absorbance of light was measured at 450 nm, with a reference point at 620 nm. The 10 µL of Horseradish Peroxidase (HRP) was used at 2.5 and 25 mU/mL concentrations to establish a positive control. Finally, the MPO activity was determined by comparing the difference in absorbance to the HRP standard curve and the results were reported in mIU/mL (9). To measure NO in serum samples, a simple procedure was followed. Initially, 100 µL of Griess solution which consisted of 0.1% naphthyl ethylene diamide, 3% phosphoric acid and 0.1% sulfanilamide was combined with 100 µl of the serum sample. The mixture was then placed in a dark environment at 25 ˚C for 10 min. Subsequently, the absorbance of light at 540 nm was measured. The NO level was determined by comparing it to the standard curve obtained through the Griess method (8). The total antioxidant capacity (TAC) was determined in serum samples using the ferric reducing antioxidant power assay which evaluated the ability of an antioxidant to reduce a ferric tripyridyltriazine (Fe3+-TPTZ) complex to a colored ferrous tripyridyltriazine (Fe2+-TPTZ). In this experiment, 20 µL of serum sample was mixed with 1 mL of working solution and vortexed. The optical absorption of the sample was measured at 593 nm initially and after 4 min it was compared to that of the control sample (Blank). The absorbance value was plugged into the TAC formula to determine the antioxidant capacity. Finally, the TAC value was calculated using a standard curve (10).

Real-time PCR. To assess GATA3, T-bet, ROR-C, and FOXP3 levels, total mRNA was isolated from rat joints using RNX-Plus solution as per the manufacturer's instructions. The isolated RNA was then used to generate complementary DNA. PCR amplification was carried out in triplicate using a SYBR Green kit following the manufacturer's protocols. The GAPDH gene, serving as a housekeeping gene, was used as a control. Forward and reverse primers for mRNA amplification in the case of GATA3 were 5′-CAA AGC CAG AGT CCT TCA GA-3' and 5′-GAT GGT CTT GGT CCT TAG CC-3', respectively. The forward and reverse sequences for T-bet were 5'-CGG CTG CAT ATC GTT GAG GT-3' and 5'-GTC CCC ATT GGC ATT CCT C-3', for GATA3 were 5'-TCA TTA A GC CCA AGC GAA GG-3' and 5'-GTC CCC ATT GGC ATT CCT C-3', for ROR-ɣT were 5'-GCA GCG CTC CAA CAT CTT CT-3' and 5'-ACG TAC TGA ATG GCC TCG GT-3', and for FOXP3 were 5'-CAC CTG GCT GGG AAA ATG G-3' and 5'-GGA GCC CTT GTC GGA TGA-3', respectively. The outcomes were reported as $2^{-\Delta\Delta Ct}$ (mean fold change).

Statistical Analysis. The data underwent assessment for normal distribution by utilizing the Shapiro-Wilk test. The Kaplan-Meier test was employed to analyze the disease activity index and Survival probability. Subsequently, a one-way analysis of variance and Tukey's post hoc test were conducted to investigate the findings further. The means ± SD was utilized to express the results, with a significance level set at $p < 0.05$.

Results

Inflammatory responses within the joint environment are a crucial clinical indicator in RA and its animal counterparts. Therapeutic regimens commenced on the fifth day following immunization upon observation of an arthritis index on the last day of ≥1 in each rat. The maximum level of paw swelling was documented every alternate day following adjuvant immunization (Table 1). The absence of a statistically significant correlation between the RA+Pip and RA+Pred groups in terms of average RA index can be observed in Figures 1A and B. The Arthritis index results on the final day showed slight variations. The RA+Pred and RA+Pip groups exhibited the lowest values followed by the control group. There were statistically significant differences among all groups (Table 1).

Table 1. The clinical features in RA rats were evaluated by administering piperine and The findings were presented as mean ± S.D. (RA: Rheumatoid arthritis, Pip: Piperine and Pred: Prednisolone).

Groups	Average arthritis index	Arthritis index on the last day	Weight change
RA	6.61 ± 0.73 [a]	7.75 ± 0.72 [a]	-8.94 ± 0.85 [a]
RA + Pip	5.57 ± 0.60 [a]	5.90 ± 0.59 [b]	-4.45 ± 0.73 [b]
RA + Pred	4.06 ± 0.72 [bc]	4.53 ± 0.61 [c]	-4.02 ± 0.61 [b]

Figure 1 illustrates the levels of biochemical factors, including CRP, MPO, and NO in the serum of rats. The control group which received only PBS exhibited the lowest levels of these factors, while the RA group which did not receive any treatment showed the highest levels. Among the treated groups, RA + Pred, and RA+pip groups demonstrated the highest response to treatment for all measured factors. It is worth noting that there were statistically significant differences among all groups in terms of the measured factors. Interestingly, the group treated with prednisolone exhibited a more effective reduction in the inflammation-related factors than those treated with piperine alone (Fig. 1).

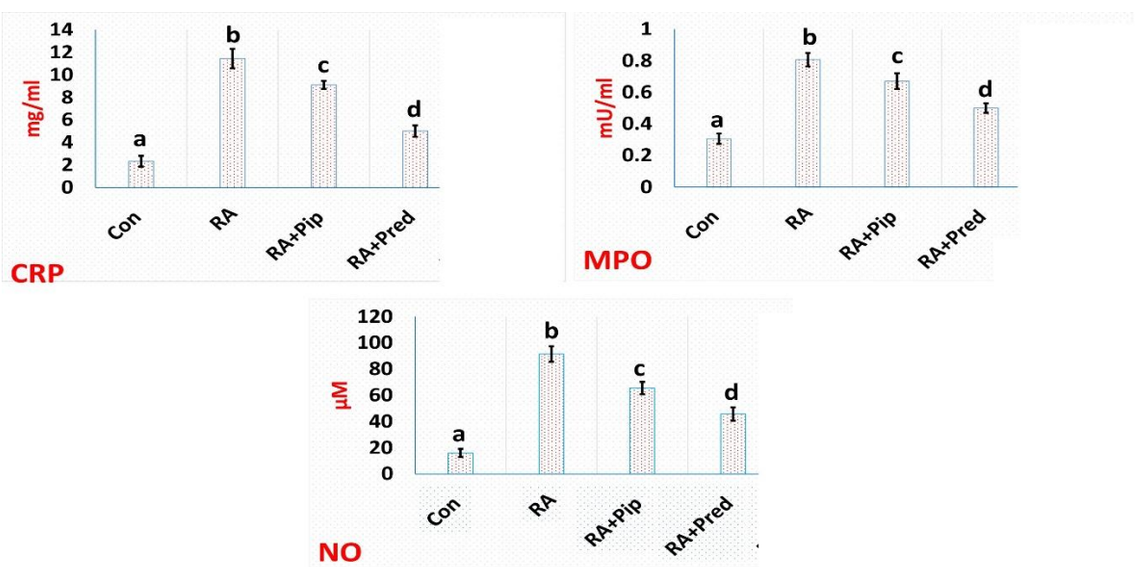

Fig. 1. Biochemical modifications in the sera of rats with rheumatoid arthritis were analyzed. (RA: Rheumatoid arthritis, Pip: Piperine and Pred: Prednisolone).

Based on the results, the expression of transcription factors T-bet, GATA3, RORγT, and FOXP3 in the joints of RA rats showed a significant increase compared to normal rats ($p < 0.05$; Fig. 2). The expression of T-bet and RORγt were significantly decreased in treatment groups compared to RA rats without treatment ($p < 0.05$). The expression of GATA3 was significantly increased in treatment groups compared to RA rats without treatment ($p < 0.05$). Here, the treatment with prednisolone was significantly more effective in reducing the expression of T-bet compared to the RA+Pip group ($p < 0.05$; Fig. 2). Statistically, treatment with piperine was not effective in changing the expression level of FOXP3 ($p > 0.05$).

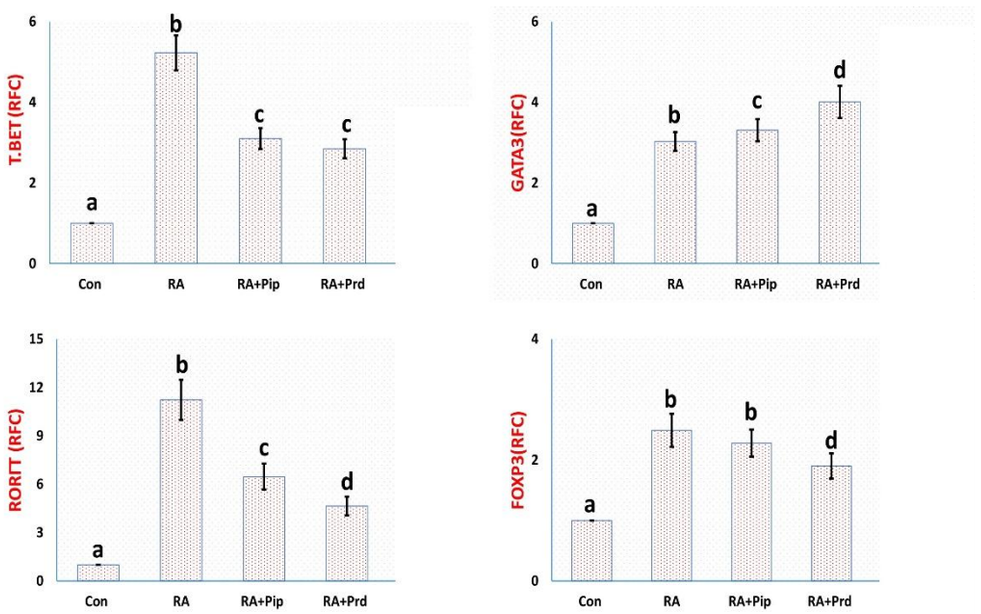

Fig. 2. Within this diagram, one can observe the gene expression of various transcription factors, each responsible for directing the differentiation of Th0 cells into distinct subsets. (RA: Rheumatoid arthritis, Pip: Piperine and Pred: Prednisolone).

Discussion

Numerous therapeutic medications such as sulfasalazine, chloroquine, sodium aurothiomalate, corticosteroids and diclofenac sodium are accessible, however, these treatments have unwanted effects and have restricted effectiveness (11). Hence, alternative therapies are essential for RA. Identifying the bioactive components that show therapeutic advantages against RA is vital. Because of the limitations and side effects of traditional medications, patients and healthcare professionals are eagerly awaiting new treatments such as herbal bioactive substances to manage RA effectively. Given that these patients need long-term medication which raises the risk of side effects a logical combination of current or new drugs could result in improved outcomes while decreasing the side effects of each drug (11,12).

Nitric oxide is a crucial signaling molecule generated during the inflammatory response by activated cells and macrophages (13). Elevated levels of NO have been observed in arthritic rat models mirroring findings in synovial fluids of individuals with RA (14). It is believed that during sterile inflammation, MPO and the oxidants produced by MPO contribute to the escalation of inflammation and tissue injury. Elevated levels of MPO and the inflammatory process are commonly seen in various autoimmune conditions. MPO is known to enhance vascular permeability and trigger inflammatory immune reactions (11). Greater levels of CRP have

been linked to increased disease activity in RA as determined by the core components of the 28-joint Disease Activity Score (13). Attained data in this study showed that treatment with piperine resulted in a significant reduction in disease severity and improved weight gain in RA rats. Biochemical indices of CRP, MPO, and NO were significantly decreased in the treatment group compared to the non-treatment group.

It is anticipated that T-bet played a significant role in guiding Th1 differentiation leading to the expectation of T-bet involvement in autoimmunity mediated by the adaptive immune system. Surprisingly, it was discovered that the central location for T-bet's influence on inflammation regulation was within the DC population (12,14). The GTA-3 play a significant role in guiding Th2 differentiation (14). Furthermore, the phenotype and function of regulatory T cells (Tregs) residing in the bone marrow (BM) of patients with RA and animal models of RA have been successfully characterized by the researchers. The investigations into the *ex vivo* function of Tregs, specifically their suppressive activity on effector T cells have yielded valuable insights. The findings indicated that the reduced number and impaired functional properties of $CD4^{+}FOXP3^{+}$ T cells found in the BM of RA patients might contribute to the inflammatory process observed in RA BM (12). This finding highlighted the significance of the FOXP3 factor in immune modulation and inflammation, thus, offering significant implications for our understanding of RA pathogenesis (12,13). The RORγ isoform specific to T cells, known as RORγt, has also been demonstrated as the crucial transcription factor that defines the lineage and initiates the differentiation process of Th17 cells. Th17 cells produce the inflammatory cytokine IL17, a protein identified in synovial fluid associated with RA. IL17 works in collaboration with IL1β and TNF-α to promote inflammatory conditions in joints and other tissues (12,14). Our results showed that the group treated with piperine exhibited a significant decrease in the expression of T-bet, GATA-3, and RORɣt genes compared to RA rats without treatment. However, there was no statistically significant difference in Foxp3 gene expression in the piperine-treated group compared to the untreated group.

Our study found that piperine effectively alleviated symptoms in an animal model of RA. However, further research is needed to understand the precise mechanism of action of piperine.

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The development and potential of plant-based vaccines in veterinary medicine

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Plant-based vaccine technologies are making significant progress, especially in the field of veterinary medicine. These vaccines offer important solutions for developing countries due to their low cost and easy production processes (1, 2). These vaccines which provide many advantages compared to traditional vaccines thanks to their sustainable production methods, have significant potential in protecting animal health. Innovations in the field of genetic engineering play a role in the development of plant-based vaccines. Methods such as *Agrobacterium*-mediated gene transfer allow genetically modified plants to produce the desired antigens (3). Studies have proven that plant-based vaccines were developed against important animal diseases such as Rabies, *Toxoplasma gondii*, and Newcastle disease to strengthen the immune system of animals (1). These successes have led to an increase in trials on the safety of these vaccines. Thus, the emergence of some regulations for the use of plant-based vaccines has accelerated. These vaccines are shown as an economical and practical solution, especially in the control of epidemic animal diseases. It is expected that such a technology will be widely adopted and its use will increase over time. This review aims to address the development and future potential of plant-based vaccines used in veterinary medicine.

Keywords: Medicinal plants, Plant-Based Vaccines, Veterinary Medicine

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Caffeine as a natural plant compound induces apoptosis in leukemia cacer cells

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The use of natural substances can be an important strategy to prevent malignancy and cancer cells, which have also inhibited cancer in laboratory models (1). Caffeine belongs to the methylxanthine group, being in plant-derived products, such as tea leaves and coffee beans (2). Reports have indicated that caffeine can have different effects, including anti-inflammatory, anti-oxidant, and anti-tumor properties (3). Studies have shown that caffeine has anti-tumor effects through affecting the cell cycle and inducing apoptosis *via* activation of caspases 3 and 9. Furthermore, it has been reported that caffeine could be used as an adjuvant therapy to increase the toxicity of anti-tumor agents. In this study, K562 cells were treated with caffeine for 48 hr. Using an inverted microscope, the morphological changes and growth of cancer cells were examined and images of cancer cells were obtained at 40× magnification. Nucleus morphology was also examined using fluorescent propidium iodide and acridine orange stainings. In addition, caspase 3 activity of cells was measured after K562 cells were exposed to different concentrations of caffeine (100, 200, 400, 600, 800, and 1000 µg/mL) after 48 hr. The results of the present study showed that caffeine as a plant compound can reduce cell proliferation, induce apoptosis, and increase caspase 3 activity in a dose-dependent manner in K562 cells as one of the types of chronic myeloid leukemia cancer cells, and it can be considered an adjuvant treatment for leukemia.

Keywords: Apoptosis, Caffeine, Leukemia

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Encapsulation of hesperidin and evaluation of its anti-proliferative effect on murine breast tumor cells

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Hesperidin ($C_{28}H_{34}O_{15}$), a flavanone glycoside abundantly found in citrus fruits, has garnered significant attention for its anti-inflammatory, anti-oxidant, and anti-cancer properties. However, its therapeutic efficacy is substantially hindered by poor bioavailability. To address this limitation, hesperidin was formulated with poly(lactic-co-glycolic) acid (PLGA) to enhance its stability and bioactive potential, thereby overcoming absorption challenges (1). In this study, hesperidin was encapsulated within PLGA nanoparticles, resulting in favorable physicochemical properties, and the anti-cancer effects were subsequently evaluated on murine breast cancer cells (4T1). To investigate the anti-cancer effects of hesperidin and its nanoparticles, 4T1 cancer cells were treated with various concentrations (20, 40, 60, and 100 µg/mL) 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_{50} concentration of the compounds and incubating them for 48 hr, they were examined at 400× magnification, and images were captured (2). Results from the MTT assay demonstrated a notably high cytotoxic effect of the hesperidin-loaded nanoparticles on 4T1 cells. Further validation through cell morphology evaluation corroborated these findings. Notably, the hesperidin nanoparticles exhibited significantly stronger anti-cancer activity compared to the free hesperidin, while also displaying high biocompatibility with minimal cytotoxic effects on healthy cells.

Keywords: Breast Cancer, Hesperidin, Nanoparticles

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Immunomodulatory effects of medicinal herbs in veterinary medicine

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The immune system has a key role in keeping health and well-being. Any disruption in the function of immune system may lead to multiple lethal diseases like, autoimmune disease, cancer, and other inflammatory conditions. There are three types of immunomodulators including immunosuppressants (inhibit immune cell activation in autoimmune diseases and organ transplantation), immunostimulants (enhance immune responses during microbial infections or tumors), and immunoadjuvants (improve immune system responses to antigens). Artificial immunomodulator agents with a clear mode of action have been produced, but they clinically failed to deliver good therapeutic activity due to their bioavailability and severe adverse effects. Thus, there is a need to develop natural immunomodulators in order to control immune-related diseases with low side effects (1). Medicinal herbs have been used for their several therapeutic properties throughout human history. Several studies have shown that plant-derived compounds have beneficial immunomodulatory effects. But, immunomodulatory role of them has not been widely investigated in veterinary medicine like in human medicine (2). In experimental studies, therapeutic effects of some of these herbs like cardamom, cumin, licorice, turmeric, ginger, marjoram, rosemary, sage, and thyme have been proven in animal diseases. However, the mechanisms of how these herbs work are not clear. Their mode of action, the ability to potentiate the immune system, and their role as growth promoters in animals have attracted research interests in recent decades. It can be considered that plant-based therapeutics represent a promising area, which may prove to be effective, both in terms of animals' therapeutics and industry profits (3).

Keywords: Immunomodulators, Medicinal herbs, Veterinary medicine

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Alteration of macrophage function in mice treated with hydroalcoholic extract of sumac

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Sumac (*Rhus coriaria)* is one of the well-known food spices with medicinal properties (1). Sumac has a wide range of active pharmacological compounds with strong antioxidant activity, including anthocyanins, tannins and flavonoids (2). This survey was set out to evaluate the effects of the hydroalcoholic extract of sumac on the peritoneal macrophage of mice that received it. Male NMRI mice were randomly divided into the following three groups (n=5): Phosphate-buffered saline (PBS)-treated mice, hydroalcoholic extract of sumac -treated mice (40 mg/kg, P.O., 30 consecutive days). At the end of this step, the resident macrophages in the peritoneal cavity of mice were isolated by injecting ice-cold PBS. The macrophages were incubated with 0.1% NBT and 100 ng/mL tetradecanoylphorbol acetate for 20 min to assess the potential for respiratory burst evaluation. The cells were primed with LPS (10 pg/mL) for 24 hr to monitor the potential of nitric oxide production. The collected supernatant was also used to evaluate the levels of cytokines IL-10 and IL-12 via ELISA. The cells were also pulsed with neutral red-stained, heat-stabilized, zymosan suspension at a 1:10 ratio for 30 min to assess the phagocytic ability of macrophages. As a result**,** the administration of hydroalcoholic extract of sumac decreased macrophages respiratory burst, nitric oxide, and IL-12 production and increased their IL-10 production. Macrophages isolated from rats that received an extract of sumac had lower phagocytic potential than those isolated from untreated mice. Finally, it seemed that the use of sumac hydroalcoholic extract inhibited the inflammatory functions of macrophages.

Keywords: Macrophage, Phagocytosis, Respiratory burst, Sumac

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The effect of red algae phlorotannin on lymphocytes and macrophages in NMRI mouse

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Phlorotannins, a category of polyphenolic compounds derived from red seaweed, exhibit a range of advantageous properties including antioxidant, antimicrobial, anti-allergic, anti-diabetic, anti-inflammatory, anti-cancer and neuroprotective effects. The global demand for these biological activities has surged (1, 2). In this study, red algae obtained from the Persian Gulf were employed to extract phlorotannins. Then, lymphocytes and macrophages were isolated from NMRI mice, cultured in specialized media and subjected to different phlorotannin concentrations (0, 20, 60, and 100 mg/mL). The results indicated that the viability of these cells was not adversely affected by the increasing concentrations of the compound. Notably, there was a significant improvement in the survival and proliferation of macrophages. Additionally, a concentration-dependent reduction in the activity of the inflammatory enzyme NO and the release of the pro-inflammatory cytokine IL-1β were observed. In contrast, higher concentrations of phlorotannins led to an increase in the respiratory burst of macrophages and the secretion of immunomodulatory cytokines such as IL-10 and TGF-β. These findings highlighted the strong anti-inflammatory properties of phlorotannins that was considered safe due to their non-cytotoxic nature presenting a promising alternative to traditional chemicals and pharmaceuticals (3).

Keywords: Gene expression, Phlorotannin, Red algae

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Medicinal herbs in veterinary medicine: Unlocking immunomodulatory and anticancer properties for enhanced animal health

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The integration of medicinal herbs into veterinary medicine is gaining attention for their potential immunomodulatory and anticancer properties. This study explores critical medicinal herbs that exhibit immune-enhancing and cancer-fighting effects focusing on their application in animal health. Herbs such as *Echinacea purpurea, Astragalus membranaceus, Withania somnifera* (Ashwagandha), and *Curcuma longa* (Turmeric) have demonstrated significant benefits in improving immune function and supporting cancer management in animals (1). These herbs are known for their ability to stimulate the immune system, induce apoptosis, inhibit tumor growth and prevent metastasis. Echinacea, widely recognized for its immune-stimulating properties enhances natural killer cells and boosts phagocytic activity particularly in managing chronic viral and bacterial infections (2). On the other hand, Astragalus serves as a preventive measure against immune deficiencies during disease outbreaks, enhancing cytokine production and T-cell response. *Withania somnifera*, an adaptogen, strengthens the body ability to cope with stress while exhibiting anticancer properties through tumor growth inhibition and apoptosis induction (3). Regarding anticancer mechanisms, *Curcuma longa* inhibits angiogenesis, local invasion and metastasis while possessing strong anti-inflammatory and antioxidant effects. These herbs offer a holistic approach to cancer management, focusing on treating the disease and enhancing overall animal well-being. These herbs produce synergistic effects, maximizing therapeutic potential and reducing side effects. Despite the promising results, further clinical research specific to veterinary use is necessary to fully establish the efficacy and safety of these herbs in animal health. As veterinary medicine evolves, integrating medicinal herbs offers a complementary approach to conventional therapies for enhancing animal health.

Keywords: Animal health, Anti-cancer properties, Immune response, Medicinal herbs

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Potent antiprolifrative effects of combination of ATRA with gallic acid on human MCF-7 breast cancer cells

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Breast cancer is the most common malignancy in women worldwide (1) and is one of the main causes of cancer-dependent death among women in developed and developing countries (2). In recent decades, plants, fruits and natural food have been recognized as a valuable resource in developing new and advanced cancer treatments (3). Recent studies have demonstrated the pro-oxidant and anticancer activities of two natural compounds, gallic acid and All-Trans-Retinoic acid (ATRA) in human cancer cells. In the present study, we evaluated the effects of combined gallic acid and ATRA treatment in MCF-7 cells by MTT assay after 48 hr. Our data showed an additive cytotoxic effect in the concurrent treatment of 200 μM ATRA and 80 μM gallic acid in MCF-7 cells. However, it was found that the sequential treatment of cells with gallic acid (80 μM) for 1 hr followed by ATRA (200 μM), exhibited increased effectiveness with a combined index below 1. In conclusion, the findings of our study proposed that combined ATRA and gallic acid treatment could have a significant impact in clinical settings as a chemotherapeutic strategy for breast cancer.

Keywords: Anticancer, Antiprolifrative, Breast cancer, Citotoxicity, Pro-oxidant

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Section 3

Microbiology

Exploring plant-based anti-viral medicines in veterinary practice: Efficacy, challenges, and future prospects

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Abstract

Plant-based anti-viral medicines have garnered significant attention in veterinary medicine because they can provide effective, eco-friendly, and sustainable alternatives to synthetic drugs. The rise in anti-viral resistance and adverse side effects associated with conventional treatments has driven the exploration of phytochemicals for managing viral infections in animals. Plant-derived compounds, including flavonoids, alkaloids, saponins, and tannins exhibit potent anti-viral activities. These bioactive compounds can inhibit viral replication, disrupt viral entry and assembly, and modulate host immune responses, offering a multi-faceted approach to combating viral pathogens. Studies have demonstrated the efficacy of plant extracts and isolated compounds against various veterinary viral infections, such as those caused by avian influenza virus, foot-and-mouth disease virus, and canine parvovirus. For instance, extracts from plants like *Echinacea purpurea*, *Andrographis paniculata*, and *Glycyrrhiza glabra* have shown promising results in reducing viral load and improving clinical outcomes in affected animals. Integrating plant-based anti-virals into veterinary practice enhances the therapeutic arsenal and supports the One Health approach by minimizing environmental contamination and promoting biodiversity. Despite the promising potential, challenges remain in standardizing formulations, ensuring consistent efficacy, and understanding these phytochemicals' pharmacokinetics and safety profiles in different animal species. Continued research and development and rigorous clinical trials are essential to validate the efficacy and safety of plant-derived anti-virals. Embracing plant-based anti-viral medicines in veterinary practice could revolutionize the management of viral diseases, ensuring better animal health outcomes and contributing to sustainable veterinary practices.

Keywords: Phytochemicals, Plant-based anti-virals, Sustainable treatments, Veterinary medicine, Viral infections

Introduction

The increasing prevalence of viral infections in animals, along with the rising concern over the efficacy and safety of conventional anti-viral drugs, has spurred interest in alternative therapeutic strategies. Among these, plant-based anti-viral medicines have emerged as a promising avenue. These phytochemicals offer a range of benefits, including their potential for reduced side effects, lower risk of resistance development, and environmental sustainability (1). This article delves into the efficacy, challenges, and prospects of plant-based anti-viral medicines in veterinary practice, highlighting their role in addressing viral infections in animals.

The need for alternative anti-viral medicines. Conventional anti-viral drugs have been the cornerstone of managing viral infections in both human and veterinary medicine. However, the extensive use of these synthetic drugs has led to several significant challenges (2).

Anti-viral resistance. The overuse and misuse of anti-viral drugs have contributed to the development of drug-resistant viral strains. This resistance diminishes the efficacy of standard treatments, making it harder to control viral outbreaks in animal populations. For instance, resistance to anti-viral drugs in managing canine parvovirus and avian influenza has been documented, necessitating the search for alternative therapies.

Adverse side effects. Synthetic anti-viral drugs can cause various side effects, ranging from mild gastrointestinal disturbances to severe organ toxicity. These adverse effects can limit the usability of these drugs, especially in sensitive animal species or those with pre-existing health conditions (3).

Environmental impact. The production and disposal of synthetic drugs contribute to environmental pollution, which can have detrimental effects on ecosystems. Plant-based anti-viral medicines, on the other hand, are generally considered more environmentally friendly due to their biodegradable nature.

Plant-based anti-viral medicines: mechanisms and benefits. Plant-derived compounds have been used in traditional medicine for centuries and are now being explored for their anti-viral properties in veterinary medicine. These compounds include flavonoids, alkaloids, saponins, and tannins, each with unique mechanisms of action against viral pathogens (4).

Mechanisms of action. 1. Inhibition of viral replication: Several plant-derived compounds can inhibit viral replication by interfering with viral enzymes or disrupting viral genome synthesis. For example, flavonoids, such as quercetin and kaempferol have been shown to inhibit the replication of various viruses by targeting viral polymerases. 2. Disruption of viral entry and assembly: Certain phytochemicals prevent viruses from entering host cells or assembling new virions. Alkaloids like berberine can block viral entry by binding to viral surface proteins, while saponins can disrupt viral envelopes, hindering the assembly of viral particles. 3. Modulation of host immune responses: Some plant compounds enhance the host's immune response, providing an indirect anti-viral effect. Polysaccharides from plants like *Echinacea purpurea* can stimulate the production of interferons and other immune mediators, boosting the host's ability to fight off viral infections (4).

Benefits of plant-based anti-viral medicines. 1. Reduced risk of resistance: Due to their complex and multi-target mechanisms, plant-based anti-virals are less likely to induce resistance compared to synthetic drugs. 2. Fewer side effects: Phytochemicals typically have a lower incidence of adverse effects, making them safer for use in a wide range of animal species. 3. Environmental sustainability: Plant-derived medicines are biodegradable and often sourced from renewable resources, reducing their environmental footprint.

Efficacy. Numerous studies have demonstrated the efficacy of plant-based anti-viral medicines against a variety of veterinary viral infections (5).

Echinacea purpurea. *Echinacea purpurea*, commonly known as purple coneflower, has been extensively studied for its immunomodulatory and anti-viral properties. Extracts from this plant have shown efficacy against respiratory viruses in horses and dogs. In a study involving horses with equine herpesvirus, *Echinacea* extract reduced viral load and improved clinical symptoms, highlighting its potential as a supportive treatment in veterinary practice.

Andrographis paniculata. *Andrographis paniculata*, also known as the king of bitters, contains andrographolide, a compound with potent anti-viral activity. Research has demonstrated its effectiveness against a range of viruses, including avian influenza and Newcastle disease virus in poultry. Andrographolide inhibits viral replication and enhances immune responses, providing a dual approach to combat infections (6).

Glycyrrhiza glabra. *Glycyrrhiza glabra*, or licorice root, is rich in glycyrrhizin, a compound with broad-spectrum anti-viral activity. Studies have shown that glycyrrhizin can inhibit the replication of viruses, like canine parvovirus and feline calicivirus. Additionally, it modulates the host's immune response, further aiding in viral clearance.

Scutellaria baicalensis. *Scutellaria baicalensis*, also known as Chinese skullcap, contains baicalin, which has demonstrated anti-viral activity against a variety of viruses. In veterinary applications, baicalin has been shown to inhibit the replication of porcine reproductive and respiratory syndrome virus and bovine viral diarrhea virus, offering a potential alternative to traditional anti-viral drugs in livestock management.

Sambucus nigra. *Sambucus nigra*, commonly known as elderberry, has long been used in traditional medicine for its anti-viral properties. Recent studies have shown that elderberry extracts can inhibit the replication of influenza viruses in poultry and swine, reducing the severity and duration of the infection. The polyphenols in elderberry are believed to be responsible for these effects, providing a natural means of controlling viral outbreaks in farm animals (7).

Challenges in implementing plant-based anti-viral medicines. Despite the promising potential of plant-based anti-virals, several challenges must be addressed to ensure their effective integration into veterinary practice.

Standardization and quality control. One of the major challenges is the standardization of plant extracts. The concentration of active compounds can vary significantly depending on the plant's growing conditions, harvest time, and extraction methods. Establishing standardized protocols for the cultivation and processing of medicinal plants is crucial to ensure consistent efficacy.

Pharmacokinetics and bioavailability. Understanding the pharmacokinetics and bioavailability of plant-based compounds in different animal species is essential. Factors such as absorption, distribution, metabolism, and excretion can vary widely between species, affecting the therapeutic efficacy of phytochemicals. Comprehensive studies are needed to determine the optimal dosing regimens for various animals.

Safety and toxicity. While plant-based anti-virals are generally considered safer than synthetic drugs, it is important to assess their safety profiles thoroughly. Some plant compounds may have toxic effects at high doses or when used over extended periods. Rigorous safety evaluations, including toxicity studies and long-term assessments, are necessary to ensure their safe use in veterinary medicine (7).

Regulatory approval. The regulatory approval process for plant-based anti-virals can be complex and time-consuming. In many regions, these products must meet stringent safety and efficacy standards before they can be approved for use in animals. Navigating the regulatory landscape and ensuring compliance with relevant guidelines is essential for bringing these products to market.

Future prospects and directions. The future of plant-based anti-viral medicines in veterinary practice is promising, with several key areas of research and development poised to advance the field (8).

Advanced extraction and formulation techniques. Innovations in extraction and formulation techniques can enhance the efficacy and bioavailability of plant-based anti-virals. Techniques such as supercritical fluid

extraction and nanotechnology-based delivery systems can improve the stability and absorption of phytochemicals, maximizing their therapeutic potential.

Genomic and metabolomic approaches. Genomic and metabolomic approaches can provide deeper insights into the mechanisms of action of plant-based anti-virals. By identifying the specific genes and metabolic pathways targeted by phytochemicals, researchers can develop more targeted and effective treatments. These approaches can also aid in the discovery of new anti-viral compounds from a broader range of plant species (9).

Synergistic combinations. Combining plant-based anti-virals with conventional anti-viral drugs or other natural compounds can enhance their efficacy and reduce the risk of resistance development. Synergistic combinations can provide a multi-faceted approach to managing viral infections and improving animal clinical outcomes (10).

Integration into one health approach. The One Health approach, which recognizes the interconnectedness of human, animal, and environmental health, provides a framework for the holistic integration of plant-based anti-virals into veterinary practice. By reducing the reliance on synthetic drugs and promoting sustainable practices, plant-based anti-virals can contribute to the overall health and well-being of animals, humans, and ecosystems.

Education and awareness. Raising awareness among veterinarians, farmers, and pet owners about the benefits and potential of plant-based anti-virals is crucial for their widespread adoption. Educational initiatives and training programs can help stakeholders understand the proper use and potential benefits of these natural medicines, fostering greater acceptance and utilization in veterinary practice (11).

Plant-based anti-viral medicines hold significant promise for revolutionizing the management of viral infections in veterinary practice. Their diverse mechanisms of action, reduced risk of resistance, and lower incidence of side effects make them attractive alternatives to conventional anti-viral drugs. However, several challenges, including standardization, pharmacokinetics, safety, and regulatory approval must be addressed to fully realize their potential. Continued research and development, coupled with advanced extraction techniques and a deeper understanding of their mechanisms, will pave the way for integrating plant-based anti-virals into veterinary medicine. By embracing these natural alternatives, the veterinary field can move towards more sustainable and effective approaches to manage viral diseases, benefiting animal health, and contributing to the broader goals of the One Health initiative.

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Anti-fungal activity of *Teucrium polium* and *Artemisia aucheri* Boiss. essential oils

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Abstract

Nowadays, utilization of natural compounds such as plant essential oils (EOs) instead of anti-fungal drugs against fungal pathogens has enhanced. Anti-microbial, anti-inflammatory and anti-oxidant activities of EOs, alone and in combination with commercial agents are well known. So, in this study we aimed to evaluate the inhibitory activities of two Eos, including *Teucrium polium* and *Artemisia aucheri* Boiss. on three yeasts, including *Candida albicans,*. *Candida tropicalis* and *Candida dubliniensis* using minimal inhibitory concentrations (MICs) and minimum fungicidal concentration (MFC) methods. Both EOs showed fungistatic and fungicidal activities, with MICs ranging from 0.019-0.15 µL/mL and MFCs ranging from 0.039-0.6 µL/mL. *T. polium* EO was significantly more effective than *A. aucheri* Boiss. EO against tested micro-organisms especially *C. tropicalis* which was the most sensitive fungus. The MICs of *T. polium* and *A. aucheri* Boiss. EOs were respectively 0.019 and 0.039 µL/mL against *C. tropicalis*. Evaluation of anti-fungal activities of *T. polium* and *A. aucheri* Boiss. EOs as natural herbal compounds showed that these EOs are convenient against *Candida* species.

Keywords: Anti-fungal activity, *Artemisia aucheri* Boiss., *Candida* species, *Teucrium* polium

Introduction

Candida spp. are the most common cause of fungal infections in humans (1). These are yeast fungi which the more reported species, *Candida albicans*, is usually associated with pathological conditions; however, other species, such as *C. tropicalis*, *C. glabrata* and *C. krusei* are frequently identified (2). *Candida* spp. have been the 4th most prevalent nosocomial pathogen in intensive care units and the infections by *Candida* species have grown in an alarming speed. These species have the potential to develop anti-fungal resistance either intrinsically or during treatment (3).

Nowadays, utilization of natural compounds such as plant essential oils (EOs) instead of anti-fungal drugs against fungal pathogens has enhanced (4). The EOs are natural products, volatile compounds and secondary metabolites produced by aromatic plants. They contain numerous aromatic compounds giving plants a distinctive odor, flavor and aroma. These aromas are complex mixtures of a large number of constituents, but mainly include monoterpenes, sesquiterpenes and their oxygenated derivatives (alcohols, aldehydes, esters, ethers, ketones, phenols and oxides) in variable ratios (5). Being lipophilic, these oils typically integrate into membrane structures causing increased cell permeability, leaching of intra-cellular components and

inactivation of enzymes (6). Anti-microbial, anti- inflammatory and anti-oxidant activities of EOs, alone and in combination with commercial agents are well known (7).

The EOs have diverse anti-fungal activity and can act against *Candida* by inhibiting ergosterol synthesis, altering cell wall morphology, inhibiting enzymes involved in cell wall synthesis, changing cell membrane permeability and producing oxygen reactive species. Furthermore, EOs can also interact with the mitochondrial membrane, leading to cidal effects.

Teucerium polium L. (family: *Lamiaceae*) is a hairy perennial herb commonly known as Kalpoureh in Iran and is the most famous species of *Teucrium* belonging to the subfamily *Lamioideae* and among the largest genera of the entire *Lamiaceae* family (8).

Artemisia aucheri Boiss. is a shrub from *Asteraceae* family being widespread in Iran. In traditional medicine, *A. aucheri* is used for its astringent, disinfectant, anti-microbial and anti-parasitic properties (9).

In this study, we aimed to evaluate the inhibitory activities of two Eos, including *T. polium* and *A. aucheri* Boiss. on *Candida* spp., including *C. albicans, C. tropicalis* and *Candida dubliniensis* using minimal inhibitory concentrations (MICs) and minimum fungicidal concentration (MFC) methods.

Material and Methods

Preparation of *T. polium* and *A. aucheri* Boiss. EOs. The *T. polium* and *A. aucheri* Boiss. were purchased from the local spice store in Ardabil province, Iran. Then, they were extracted *via* distillation by water for 3 hr using a Clevenger unit. After that, EOs were dried using anhydrous sodium sulfate (Na_2SO_4) and stored at 4 ˚C until further evaluations (10).

Microbial strains. Anti-fungal activity of EOs was investigated against three yeasts, including *C. albicans* ATCC 10231, *C. tropicalis* ATCC 750 and *C. dubliniensis* CD36.

Determination of MIC and MFC. For determination of MIC, micro-dilution broth method was used based on the Clinical Laboratory Standards Institute guidelines (11). Stocks and dilutions of EOs were prepared in 10% dimethyl sulfoxide. Final concentrations in the micro-dilution plates ranged from 5-0.009 µL/mL. The micro-dilution plates were prepared using RPMI 1640 broth medium (Sigma) with l-glutamine and without sodium bicarbonate and buffered at pH of 7.0 with 0.165 mol/L of morpholine propane sulfonic acid (Sigma). Fungal suspensions were prepared after vortexing and adjusting to a 0.50 McFarland standard transmittance at a wavelength of 530 nm. The final inoculum yielded was 0.50×10^{3} - 2.50×10^{3} cells/mL. Two wells were served as the growth control and sterility check. The MICs were visually determined, and observed for the presence or absence of cells growth. After reading the MIC, 20 µL of culture wells with no growth of fungal cells and also a positive control were sub-cultured onto Sabouraud dextrose agar and incubated. The lowest concentration without a fungal colony was considered MFC.

Results

The results of anti-fungal activities of *T. polium* and *A. aucheri* Boiss. EOs against tested yeasts are shown in Table 1.

Table 1. Anti-fungal activities of *Teucrium polium* and *Artemisia aucheri* Boiss. essential oils against *Candida* species.

Microorganisms	*Teucrium polium*		*Artemisia aucheri* Boiss.	
	MIC (µL/mL)	MFC (µL/mL)	MIC (µL/mL)	MFC (µL/mL)
C. albicans	0.078	0.156	0.156	0.625
C. dubliniensis	0.039	0.156	0.039	0.312
C. tropicalis	0.019	0.039	0.039	0.156

MIC: Minimal inhibitory concentration; MFC: Minimum fungicidal concentration.

Discussion

In the last few years, there has been a targeted interest towards biologically active compounds isolated from aromatic plant species for the elimination of pathogenic microorganisms. The main reason for this is the resistance that microorganisms have built against antibiotics. In this study, the inhibitory activities of two Eos, including *T. polium* and *A. aucheri* Boiss. EOs were tested on *Candida* spp., using MIC and MFC methods. Both EOs showed fungistatic and fungicidal activities, with MICs ranging from 0.019-0.15 µL/mL and MFCs ranging from 0.039-0.6 µL/mL. *T. polium* EO was significantly more effective than *A. aucheri* Boiss. EO against tested microorganisms, especially *C. tropicalis* which was the most sensitive fungus. The MICs of *T. polium* and *A. aucheri* Boiss. EOs were respectively 0.019 and 0.039 µL/mL against *C. tropicalis*.

In a similar study, Sabz *et al*., investigated anti-fungal activity of *T. polium* EO on clinical *Candida* and *Aspergillus* isolates. The broth micro-dilution method was used to determine the anti-fungal activity. Generally, the range of MIC was varied from 0.009 to 0.312 µL/mL among different *Candida* species, being close to our results (12).

In another study, anti-fungal activity of *A. aucheri* EO was detected against two yeasts, including *C. albicans* and *Saccharomyces cereviciae*, and two mold species, including *Streptomyces natalensis* and *Aspergillus niger*. Among the yeasts and fungal species, *C. albicans* was the most sensitive, while *A. niger* was resistant, being in agreement with our findings (13).

It is important to scientifically investigate those plants which have been used in traditional medicines as potential sources of novel anti-microbial compounds. Evaluation of anti-fungal activities of *T. polium* and *A. aucheri* Boiss. EOs as natural herbal compounds showed that these EOs are convenient especially against *Candida* species.

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Anti-fungal activity of barberry aqueous extract

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Abstract

Fungal infections have increased over the last two decades. Pathogenic molds have been reported as casual agents of food spoilage and foodborne diseases and can contaminate foods from cultivation to harvest, transportation and storage. It is important to scientifically investigate the plants which have been used in traditional medicines as potential sources of novel anti-microbial compounds. For this reason, in this study the anti-fungal activity of barberry aqueous extract against fungi including *Aspergillus niger*, *Aspergillus flavus*, *Penicillium chrysogenum*, *Candida albicans* and *Candida glabrata* was evaluated using minimal inhibitory concentration (MIC) method. The results showed that *A. niger* and *A. flavus* are the most resistant species to extract with MIC of 50 mg/mL. *Candida* species were more sensitive and MIC was 12.50 mg/mL against *C. albicans* and *C. glabrata*. Evaluation of anti-fungal activity of barberry aqueous extract as a natural herbal compound showed that this extract has anti-fungal activity especially against *Candida* species.

Keywords: Anti-fungal activity, Aqueous, Barberry, Extract

Introduction

Spices have been applied as food additives since ancient times. In addition to improvement of organoleptic properties of food (color, flavor, and taste), they can increase the shelf life of food by decreasing microbial count and retarding lipid oxidation (1). Barberry (*Berberis vulgaris* L.) is extensively cultivated in Southern Khorasan province (northeast of Iran) and is a popular condiment between Iranians (2). Barberry fruit is a popular spice in Iran being often added to stew and soup and is consumed in different forms, including dried fruit, fruit concentrate, juice, jam and marmalade in Iran (3). It is also used as an additive in meat dishes in Georgia (4). Fungal infections have increased over the last two decades. Pathogenic molds have been reported as casual agents of food spoilage and foodborne diseases and can contaminate foods from cultivation to harvest, transportation and storage. Nowadays, utilization of natural compounds instead of anti-fungal drugs against fungal pathogens has enhanced. In this study we aimed to evaluate the anti-fungal activity of barberry aqueous extract as a natural herbal compound.

Materials and Methods

Preparation of barberry aqueous extract. Barberry (*B. vulgaris L.)* fruit was purchased from a local market in Tabriz, Iran. Ground fruits (100 g) were added to distilled water (1 L) and heated at 100 ˚C for 60

min (5). The obtained extract was filtered through Whatman filter paper (Sigma-Aldrich, St. Louis, MO), and the filtrate was concentrated on a rotary evaporator (Heidolph, Laborata 4003, Schwabach, Germany) and then lyophilized. The lyophilized extract was placed in sealed bottles and stored at 4 ˚C.

Microbial strains. Anti-fungal activity of barberry extract was investigated against *Aspergillus niger* (PTCC 5012), *Aspergillus flavus* (PTCC 5018*), Penicillium chrysogenum* PTCC 5037, *Candida albicans* (ATCC 10231) and *Candida glabrata* (ATCC 90030)**.**

Minimal inhibitory concentration (MIC) determination. For determination of MIC, micro-dilution broth method was used based on the Clinical Laboratory Standards Institute guidelines (6). Stocks and dilutions were prepared in water. Final concentrations in the micro-dilution plates ranged from 6.25-200 mg/mL. The micro-dilution plates were prepared using RPMI 1640 broth medium (Sigma) with l-glutamine and without sodium bicarbonate and buffered at pH of 7.0 with 0.165 mol/L of morpholine propane sulfonic acid (Sigma). Fungal suspensions were prepared after vortexing and adjusting to a 0.50 Mc Farland standard transmittance at a wavelength of 530 nm. The final inoculum yielded was 0.50×10^{3} - 2.50×10^{3} cells/mL. Two wells served as the growth control and sterility check. The MICs were visually determined, and observed for the presence or absence of cells growth.

Results

Micro-dilution broth method was used to determine anti-fungal activity of barberry extract. The results of anti-fungal activity of barberry aqueous extract against tested microorganisms are shown in Table 1.

Table 1. Anti-fungal activity of barberry aqueous extract.

Fungi	Minimal inhibitory concentration (mg/mL)
Aspergillus niger	50.00
Aspergillus flavus	50.00
Penicillium chrysogenum	25.00
Candida albicans	12.50
Candida glabrata	12.50

Discussion

Over the past decades, numerous research studies have focused on developing an efficient and eco-friendly method for managing phytopathogens (7). *Candida* spp. are the most common cause of fungal infections in humans. These are yeast fungi which more reported species, *C. albicans*, is usually associated with pathological conditions (8).

The phytochemical analysis of the *Berberis* fruit revealed the presence of alkaloids, tannins, carotenoid, vitamin, protein, lipid, anthocyanin, and phenolic compounds (9). Berberine alkaloid, a bioactive compound found in barberry fruit, has been found to have anti-fungal properties due to its ability to inhibit sterol and cell wall biosynthesis and induce cell damage by increasing reactive oxygen species production (10). In a similar study, anti-fungal activity of methanolic extract of barberry was evaluated against *Fusarium* spp (11). The MIC values of *B. vulgaris* fruit extract against *Fusarium graminearum* and *Fusarium solani* were 150 and 75 mg/mL, being similar to our results. This study explored the potential of *B. vulgaris* aqueous extract as an anti-fungal agent especially against *Candida* species.

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Evaluation of antibacterial and antifungal activity of aqueous extract of saffron

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Abstract

In recent years the public interest to use natural preservatives derived from plant, animal and microbial strains have enhanced. In addition to positive impact on increasing the shelf life of foods, these compounds do not have harmful effects of chemical preservatives. Accordingly, in this study we evaluated antimicrobial effect of aqueous extract of saffron on important food pathogen bacteria and fungi. For this reason, the aqueous extract of saffron was prepared and kept in dry form. Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC) and minimum fungicidal concentration (MFC) of extract were determined by broth microdilution method against *Salmonella Typhimurium, Listeria monocytogenese, Escherichia coli, Aspergillus niger and Penicillium notatom*. MICs against *S. typhimurium, L. monocytogenese, E. coli, A. niger and P. notatom* was 100 mg/mL. The MBC and MFC were upper than 200 mg/mL. The results of this study represented eligible effect of aqueous extract of saffron to control foodborne pathogens.

Keywords: Antibacterial, Antifungal, Aqueous extract, Broth microdilution, Saffron

Introduction

Microorganisms, including both gram-positive and gram-negative bacteria, as well as fungi, are responsible for a wide range of infections in humans. Over time, effective antimicrobial substances have been developed to combat pathogenic microorganisms. However, in recent years, microorganisms have developed resistance to common antimicrobial drugs, necessitating the discovery of novel antimicrobials (1). From ancient times, plant extracts have been used for various purposes such as enhancing food and beverage flavors and treating various diseases. Given the growing public concern about the side effects of chemical preservatives in recent years, the use of plant extracts has been recognized as a promising method to extend food shelf life due to their natural origin and increased safety compared to chemical preservatives (2).

Saffron (*Crocus sativus L.*), a perennial plant from the Iridaceae family, is traditionally used to enhance the taste and flavor of food (3). The dried stigma of this plant is commonly used in the food industry as an aromatic spice and coloring agent (4). Previous studies have found that the extract of this plant possesses antimicrobial, antioxidant and anticancer properties (5-7). Saffron contains over 150 different volatile compounds. The main constituents of this plant are crocin, picrocrocin, and safranal which are responsible for the color, taste and smell of saffron, respectively. Each of these compounds plays a crucial role in the antioxidant and antimicrobial properties of saffron (8).

Due to the functional properties of saffron in food and its widespread availability across various regions of Iran as well as the transmission of significant foodborne bacteria such as *Salmonella typhimurium, Listeria*

monocytogenes, and *Escherichia coli* and because of the growth and toxin production of *Aspergillus niger* and *Penicillium notatum* in food this study aimed to evaluate the antimicrobial effects of aqueous saffron stigma extract.

Materials and Methods

Microbial strains. The *S. typhimurium* (ATCC 14028), *L. monocytogenes* (ATCC 19115), *E. coli* (ATCC 10536), *A. niger* (PTCC 5162) and *P. notatum* (PTCC 5304) were used in this study.

Preparation of bacterial and fungal inoculums. The lyophilized bacteria were prepared from the collection center of industrial microorganisms (Iranian Research Organization for Science and Technology, Iran). The bacterium was cultured consequently in the nutrient broth (Merck, Germany) at 37 ˚C for 24 h. The second culture was then mixed with sterile glycerin in a ratio of 5: 1 and stored at – 20 ˚C to be used during the study (9). Briefly, 100 µL of the bacterial suspension was transferred to 10 mL of the nutrient broth and incubated at 37 ˚C for 24 h. This culture was repeated under the same condition. To prepare fresh fungal cultures, fungi were cultured in potato dextrose agar containing chloramphenicol and kept in 28 ˚C for 5 days.

Extraction of aqueous extract. The saffron was collected from a field in Gonabad city. To prepare the aqueous extract, 13.8 g of clean and crushed saffron stigmas were mixed with 50 mL of distilled water and boiled for 20 min. The resulting mixture was passed three times through filters with large to small porosity degrees. The filtered solution was placed in a water bath at 50 ˚C until the water evaporated completely. The residual dry matter was distributed in sterile microtubes and stored in a refrigerator for the next experiments (10).

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC). A broth microdilution method was used to determine the MIC according to the NCCLS (11). The inocula of the bacterial strains were prepared from overnight broth cultures and suspensions were adjusted to 0.5 Mc Farland standard turbidity. The water was used to dissolve the extract and then diluted to the highest concentration (200 mg/mL). A serial doubling dilution of the was prepared in microtubes, then introduced to each well and the final concentration of bacteria in each well was adjusted to 2×10^6 CFU/mL. The plate was incubated for 24 h at 37 ˚C. A sterile swab was impregnated with the content of each well which bacterial growth was inhibited and then cultured on the surface of nutrient agar and incubated for 24 - 48 h at 37 °C, and the count of bacteria was counted. The minimum concentration of extract that inhibited the growth of 99.9% of bacteria was considered as the MBC.

Determination of MIC and MFC. For determination of MIC, microdilution broth method was used based on the Clinical Laboratory Standards Institute (12). Stocks and dilutions of aqueous extract of saffron were prepared in water. Final concentrations in the microdilution plates ranged from 6.25 - 200 mg/mL. The microdilution plates were prepared by using RPMI 1640 broth medium (Sigma) with l-glutamine and without sodium bicarbonate and buffered at pH 7.0 with 0.165 mol/L of morpholine propane sulfonic acid (Sigma). Fungal suspensions were prepared after vortexing and adjusting to a 0.5 Mc Farland standard transmittance at a wavelength of 530 nm. The final inoculum yielded was of 0.5×10^3 - 2.5×10^3 cells/mL. Two wells served as the growth control and sterility check. MICs were visually determined and were observed for the presence or absence of cells growth. After reading the MIC, 20 µL of culture wells with no growth of fungal cells and

also a positive control was subcultured onto SDA and incubated. The lowest concentration without a fungal colony was considered MFC.

Results

The results of antibacterial and antifungal activity of saffron aqueous extract is shown in Table 1.

Table 1. Antibacterial and antifungal activity of saffron aqueous extract

Microorganisms	pH	temperature	MIC (mg/mL)	MBC/MFC (mg/mL)
Salmonella typhimurium	7	37	100	200≥
Listeria monocytogenes	7	37	100	200≥
Escherichia coli	7	37	100	200≥
Aspergillus niger	7	25	100	200≥
Penicillium notatum	7	25	100	200≥

Discussion

Studies have been conducted regarding the antimicrobial effects of saffron and its compounds. The antibacterial effect of aqueous extract and isolated compounds from saffron, including crocin, crocetin, picrocrocin and safranal, using three microbial strains including *E. coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were investigated by diffusion method in agar. The results showed that the aqueous extract of saffron did not have inhibitory effect on these microorganisms and among the special compounds of saffron, safranal inhibited the growth of *E. coli* and *S. aureus* strains. Other compounds of saffron did not have an inhibitory effect on the mentioned microorganisms(13). In another study, The anti-helicobacter effect of saffron was evaluated (14). In this study, the MIC of the methanolic extract was measured as 677 mg/mL. The results showed that saffron has moderate anti-helicobacter effects. The results of our study showed that MIC of aqueous extract of saffron stigma against food pathogenic bacteria including *Listeria monocytogenese*, *E. coli* and *S. typhimurium* was 100 mg/mL. These results were consistent with the results of the effect of methanol extract of saffron against *Helicobacter pylori* and indicated the moderate effect of water extract of saffron against the studied microorganisms. The results of another study stated that the alcoholic extract of saffron and safranal was effective in preventing the growth of *Candida albicans* and *Doublenensis* fungi in laboratory conditions (15). The results of the antifungal effect of saffron aqueous extract against *A. niger* and *P. notatum* in the present study showed that saffron aqueous extract, like the alcoholic extract, had moderate antifungal properties and inhibited the growth of the studied fungi.

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Antimicrobial and antioxidant properties of *Satureja hortensis* and *Satureja macrantha*

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The genus *Satureja*, belonging to the Lamiaceae family, comprises approximately 200 species of aromatic herbs and shrubs. They are commonly found in the Middle East, Mediterranean region, Europe, Western Asia, North Africa, Canary Islands, and South America. More than 30 species of this genus are distributed in the eastern regions of the Mediterranean (1). In Iran, it is known as Marze and in Türkiye, T it is called Kayakekiği. Due to their sweetness and simple cultivation characteristics, they are used as flavoring agents in the food, pharmaceutical, and cosmetic industries. Traditionally, *Satureja* species have been used to treat gastrointestinal disorders, such as cramps, nausea, indigestion, and diarrhea, as well as to relieve muscle pain, and as tonic and carminative agents (1). Several studies have been conducted on the growth-promoting potential of *Satureja* species in animals, which have been proven to contain phenolic compounds, such as thymol and carvacrol. These compounds possess a broad spectrum of anti-microbial activity, making them a potential alternative to antibiotics used as anti-microbial growth promoters (2). In our research, the compositions, and anti-microbial and anti-oxidant effects of methanol and aqueous extracts, as well as essential oils obtained from *Satureja hortensis* collected from the flora of Erzurum, Türkiye, and *Satureja macrantha* collected from the flora of Urmia, Iran, were comparatively studied.

Keywords: Anti-microbial, Anti-oxidant, *Satureja hortensis*, *Satureja macrantha*

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Anti-bacterial effect of hydroalcoholic extract of radish leaf and fruit on *Salmonella typhimurium* and *Bacillus cereus* in laboratory conditions

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The use of plant extracts as natural alternatives to chemical or synthetic anti-microbials for combatting foodborne pathogens and extending shelf life is a growing trend in the food industry. The medicinal properties of plants are derived from secondary metabolites, such as tannins, terpenoids, coumarins, alkaloids, and flavonoids, having specific anti-microbial and anti-oxidant activities (1). Radish is commonly used in traditional medicine to treat several infectious diseases (2). This has made the study of its anti-microbial activity a great interest for researchers. In this study, the anti-microbial effects of hydroalcoholic extract being prepared using the percolation method were tested on *Bacillus cereus* and *Salmonella typhimurium*. Concentrations of 250, 125, 62.50, and 31.25 were prepared from the hydroalcoholic extract of the plant and radish tuber using dimethyl sulfoxide solvent. The anti-microbial effects were investigated using well-diffusion methods. In this study, the hydroalcoholic extracts of radish leaves and fruits had the greatest effect on *B. cereus* and *S. typhimurium*, and their anti-bacterial effect increased with increasing concentrations. In summary, this study confirmed that extracts from radishes possess *in vitro* anti-bacterial activity. However, if plant oils and extracts are to be used for food preservation or medicinal purposes, safety and toxicity issues must be addressed.

Keywords: *Bacillus cereus*, Radish, *Salmonella Typhimurium*

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A review of anti-microbial effects and chemical composition of *Thymus fedtschenkoi*

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The emergence of drug resistance against antibiotics, which makes it difficult to treat some infections, has propelled researchers look for new compounds with anti-microbial properties. Medicinal plants are among the sources being used to produce new anti-microbial compounds. Anti-bacterial, anti-fungal and anti-viral properties have been identified in natural compounds isolated from different species of Thymus such as essential oils. *Thymus fedtschenkoi* grows in Türkiye, Caucasus and Iran, and is one of the native species of Thymus in Iran. Thymol and carvacrol have been reported as major components of the essential oil of *T. fedtschenkoi*, and sometimes linalool and terpinyl acetate are also mentioned as main components (1). *Thymus fedtschenkoi* as a medicinal plant is traditionally used in the treatment of common colds and respiratory infections as an additive and flavor in several traditional Iranian foods and herbal teas. According to the studies, although there is a substantial difference in the chemical composition of the extract from different segments of *T. fedtschenkoi*, they exhibit similar, yet significant anti-bacterial activity against Gram-positive and Gram-negative bacteria. This highlights the importance of *T. fedtschenkoi* and its essential oil in traditional medicine for the treatment of various bacterial infections and justifies its major application as an additive and flavor in numerous traditional Iranian foods and herbal teas (2). In conclusion, natural compounds isolated from *T. fedtschenkoi* such as essential oils have been shown to have significant anti-microbial effects on various types of microorganisms.

Keywords: Anti-microbial effect, Essential oil, *Thymus fedtschenkoi*

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A review of anti-microbial activity and chemical composition of essential oil of *Nigella sativa*

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Herbs and spices contain medicinal properties making them useful to prevent or treat diseases. *Nigella sativa* is an annual flowering plant growing in countries bordering Mediterranean sea, Pakistan, India, and Iran. It has been identified as anti-malarial, anti-asthmatic, anti-tumor, anti-viral, anti-microbial, anti-inflammatory, gastro-protective, anti-hypertensive, anti-diabetic, anti-atherosclerotic, anti-oxidant, and anti-cholesterol agent. According to the studies, *N. sativa* oil is identified to produce anti-microbial activity against various types of microorganisms, especially multiple-antibiotic resistant bacteria. Based on the studies, compounds such as phellandrene, α-pinene, β-pinene, p-cymene, cis-carveol, trans-anethole, thymoquinone, thymol, α-longipinene and longifolene are identified in the composition of *N. sativa* oil (1). Thymoquinone, one the most active components of *N. sativa* oil, has different pharmacological properties as well as a broad anti-microbial activity against various types of microorganisms, such as Gram-positive and Gram-negative bacteria, viruses, parasites, and fungi (2). In conclusion, *N. sativa* essential oil has been shown to have anti-microbial activity against a broad range of microbes and it can be used as an anti-microbial supplement for the development of new therapeutic agents.

Keywords: Anti-microbial activity, Essential oil, *Nigella sativa*, Thymoquinone

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Investigating the chemical composition of rosemary essential oil of ten populations from Iran and its antibacterial activity on two types of Gram positive and negative bacteria

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Antibiotics, as one of the most important medicines against infectious diseases, have an important role in curing diseases (1). Despite researches to produce new antibiotics, with the increase of antibiotic resistance, efforts to find alternatives have continued. Among these alternatives, plant essential oils (EOs) have been particularly important (2). Rosemary EO is beneficial due to its antibacterial and antifungal contents (1). In this study, the chemical compositions of ten populations of rosemary in different regions of Iran including Tehran, Kerman, Khorram Abad, Kermanshah, Tabriz, Durood, Borujerd, Malayer, Jowkar and Bisotun were investigated. Among the five main compounds of each population that had the highest percentage (gas chromatography mass spectrometry method), α-Pinene was present in all populations. Also, 1,8-cineole was present in all the regions except for Borujerd. Therefore, 1,8-cineole and α-Pinene were the main compounds of rosemary EO (1, 2, 3). It is suggested to conduct more research on the effects of rosemary EO from different regions on the common bacteria and to identify the most efficient EO. According to the results of the studies, rosemary EO is more effective on Gram-positive bacteria. The main reason for this effect is the absence of an outer membrane made of lipopolysaccharide which makes Gram-positive bacteria vulnerable to the penetration of EOs. Although the outer membrane of Gram-negative bacteria is also permeable to some hydrophobic compounds with low molecular weight, it can explain the difference between EOs performance on Gram-positive and negative bacteria.

Keywords: Antibacterial effect, Essential oil, Populations, Rosemary

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Investigating the antifungal effect of cinnamon extract and curcumin compared to two standard drugs against *Aspergillus flavus* and *Penicillium citrinum*

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The development of strategies to control fungal infections may be an effective mean for therapeutic interventions. Plant fungicides based on synthetic chemicals cause severe and long-term environmental pollution and are highly and acutely toxic causing cancer in humans and animals (1). In the current study, we aimed to investigate the antifungal effects of two natural substances namely cinnamon and curcumin as fungi statistic and/or fungicide on *Aspergillus flavus* and *Penicillium citrinum*. Our results showed that curcumin at 500 µg/mL resulted in minimal inhibition of both tested fungi and consequently at 1 mg/mL was able to act as a fungicide agent. Moreover, cinnamon extract resulted in a weaker MIC and MFC on tested fungi (MIC: 12.5 mg/mL, MFC: 50 mg/mL, *A. flavus*; MIC:12.5 mg/mL, MFC:25 mg/mL, *P. citrinum*), when compared to currently used chemical agents such as natamycin and amphotericin B. The cinnamon-induced antifungal effect was weaker than the used antifungal agents. Our results suggested that screening the antifungal effects of natural substances would be a novel approach in encountering with fungal infections and most likely they could be useful substances for preventing of fungal growth in packaging of food materials.

Keywords: Antifungal, *Aspergillus flavus*, Cinnamon, Curcumin, *Penicillium Citrinum*

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Section 4

Parasitology

In vitro anthelmintic of glucose-based carbon quantum dots against strongyle eggs: A morphological Study

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Abstract

Strongyle infections pose a significant threat to animal health and the development of novel therapeutic agents is crucial for effective control. In this study, we investigated the efficacy of copper-doped carbon quantum dots (Cu@CQDs) against strongyle infections. Our results showed that Cu@CQDs at a concentration of 12.5 µg/mL exhibited notable efficacy in inducing structural changes in strongyle eggs, leading to deformities. The observed efficacy was likely due to the accumulation of Cu@CQDs around the surface of the eggs which disrupted their normal functioning. The results showed that the negative control group had a hatching rate of 92%, with 8 out of 100 eggs failing to hatch, while the positive control group had a significantly lower hatching rate of only 3%. In the CQDs treatment group, the percentage of unhatched eggs was 89%, 72%, 57% and 44% at concentrations of 12.5, 6.25, 3.125 and 1.5625 µg/mL, respectively. Similarly, in the Cu@CQDs treatment group, the percentage of unhatched eggs was 95%, 79%, 69% and 48% at the corresponding concentrations. Our findings suggested that Cu@CQDs might be a promising therapeutic agent for combating strongyle infections and further studies are necessary to fully elucidate their mechanism of action and explore their potential applications in veterinary medicine.

Keywords: Anthelmintic, Carbon quantum dot, Glucose, Strongyle

Introduction

Gastrointestinal parasitic nematodes pose a significant threat to the animal husbandry sector resulting in substantial economic burdens globally (1). The estimated annual economic losses attributed to these diseases are staggering, reaching tens of billions of dollars. Strongyles, prevalent internal parasites in equines can manifest in various clinical symptoms including weight loss, compromised coat condition and impaired athletic performance (2). The emergence of anthelmintic resistance in strongyles has become a pressing global issue, prompting the creation of novel drugs (3). However, these new drugs have yielded limited efficacy and incurred substantial costs (4). Carbon quantum dots (CQDs) have garnered attention due to their multifaceted applications including their potential as antimicrobial agents (5). The CQDs have been found to exhibit photoelectric properties making them suitable as electron donors and acceptors (6).

Research has explored the effects of CQDs on microorganisms revealing their potential as antimicrobial agents. Carbon quantum dots have been shown to exert a lethal effect on both Gram-negative and Gram-

positive bacteria, primarily through cell wall damage and the production of free radicals (7). Additionally, CQDs have been found to exhibit broad-spectrum antifungal activity against various fungal species (8).

The application of CQDs in parasitology is a relatively novel approach and their anthelmintic activity remains unexplored. Given the high prevalence of strongyle infections and the emergence of drug resistance, the unique properties of CQDs present a promising avenue for investigation. The primary objective of this study was to synthesize CQDs and evaluate their potential anthelmintic effects against strongyle eggs.

Materials and Methods

Carbon quantum dots synthesis. The synthesis of CQDs and copper-doped CQDs (Cu@CQDs) was achieved through a hydrothermal method utilizing glucose as the carbon source and copper (II) chloride as the modifying agent. The synthesis process involved dissolving 1.2 g of glucose in 150 mL of deionized water to produce CQDs and adding 0.25 g of copper (II) chloride to the glucose solution to produce Cu@CQDs. The resulting solutions were then transferred to a steel autoclave equipped with a Teflon chamber and heated at 200 ˚C for 6 hr. After cooling to room temperature, the synthesized products were purified using Whatman No. 2 filter paper and centrifuged at 15,000 rpm for 20 min. The purified products were then dried using a freeze-drying machine and subjected to characterization and specialized tests as described by Zhu et al. (9).

Fecal samples. The collection of fresh fecal samples from horses was conducted in various geographical locations within Tabriz city with the horses ranging in age from 1.5 to 5 years. Sampling took place between April and August 2023 and the samples were obtained from farms that had not received any anti-parasitic treatment for at least 8 weeks prior to the commencement of the experiment. The samples were transported to the parasitology laboratory at the Veterinary Faculty, Urmia University under anaerobic conditions using plastic gloves. In the laboratory, 10-15 g of feces were suspended in 0.5 L of water and passed through 300 and 100 sieves. The resulting liquid was transferred to laboratory tubes and centrifuged at 200 g for 2 min, after which the supernatant was discarded. The precipitate was then mixed with a sugar-saturated solution and the strongyle eggs were extracted from the supernatant following centrifugation (Fig. 1). The eggs were collected, counted and the eggs per gram (EPG) was determined using the McMaster method. Fecal samples that tested positive were selected for fecal culture and L3 larvae were isolated after incubating the samples for 8 days at 28 ˚C. The experiment was confirmed to contain only strongyles as verified by Braga et al. (10).

Egg hatch inhibition (EHI) test. The experimental procedure involved selecting fresh fecal samples with a minimum of 100 parasite EPG of feces and adjusting the concentration to 100 eggs per µL by suspending them in deionized water. The eggs were examined under a light microscope (Olympus, model CH40, Japan) to differentiate between hatched and unhatched eggs and to determine their counts. For the experiment, 100 µLof egg suspension were dispensed into each well of an 84-well plate chamber followed by the addition of 100 µLof carbon quantum dots (CQDs) and copper-doped CQDs (Cu@CQDs). Each type of CQD was tested at four different concentrations: 12.5 µg/mL, 6.25 µg/mL, 3.125 µg/mL, and 1.5625 µg/mL. Negative control samples containing PBS and egg suspension and positive control samples containing ivermectin 1% and egg suspension were also included in the experiment (11). The plates were incubated for 48 hr at 27 ˚C. The egg hatching process was interrupted by the addition of 10 μL of Lugol solution. After the incubation period, at least 100 eggs and first-stage larvae (L1) were counted using a light microscope in each plate. The total number

of eggs or larvae in each well was counted and the percentage of inhibition of egg hatching (%EHI) was calculated using the formula: % EHI = [(number of eggs)/(number of larvae (L1+L2) + number of eggs)] ∗ 100 (11, 12). The experiments were repeated three times for each concentration as well as for the negative and positive control samples.

Ultrastructural analysis of eggs using scanning electron microscope (SEM). The ultrastructural analysis of strongyle eggs was conducted using scanning electron microscopy (SEM) after exposure to CQDs and Cu@CQDs. The eggs from both the treatment and control groups were fixed in a mixture of 1% (v/v) glutaraldehyde and 4% (v/v) formaldehyde in a 0.15 M sodium phosphate buffer with a pH of 7.2 for 24 hr at 4 ˚C. To prepare the samples for SEM, they underwent dehydration through a series of increasing concentrations of ethanol (30, 50, 70, 90, 100%). The egg samples were then fixed onto a slide with gelatin and observed under SEM (MIRA3 FEG-SEM, DLS, Nanotorac Wave, Microtrac Co., USA), (13).

Statistical analysis. The collected data was analyzed using the Statistical Package for the Social Sciences (SPSS) software, version 24.0 (SPSS Inc, Chicago, IL, USA). Statistical analysis was performed using a one-way analysis of variance followed by a Tukey post hoc test to determine significant differences between groups. The data were also subjected to Levene's test to assess the homogeneity of variances. A significance level of $p < 0.05$ was used to determine statistically significant results.

Results

Egg hatch inhibition. The treatment and control groups were incubated with the eggs for 48 hours at 27 ˚C to assess egg hatching. The results showed that the negative control group had a hatching rate of 92%, with 8 out of 100 eggs failing to hatch, while the positive control group had a significantly lower hatching rate of only 3%. In the CQDs treatment group, the percentage of unhatched eggs was 89%, 72%, 57% and 44% at concentrations of 12.5, 6.25, 3.125 and 1.5625 µg/mL, respectively. Similarly, in Cu@CQDs treatment group, the percentage of unhatched eggs was 95, 79, 69 and 48% at the corresponding concentrations (Table 1).

Fig. 1. Strongyle eggs after exposure to negative control group **(A)**. (Converted to L1), positive control group **(B)**. Copper-doped carbon quantum dots **(C)** and carbon quantum dots **(D)**. Images were captured at 40× magnification.

Table 1. Percentage of Egg hatch inhibition (EHI) in control and treatment groups.

Treatment	Concentration (µg/mL)	EHI (%)
Copper-doped CQD	12.5	95
	6.25	79
	3.125	69
	1.5625	48
Pure CQD	12.5	89
	6.25	72
	3.125	57
	1.5625	44
Negative control group	-	8
Positive control group	-	97

Ultrastructural analysis. In this study, SEM was employed to investigate the effects of CQDs and Cu@CQDs on the morphology of strongyle eggs. The concentrations tested were 12.5, 6.25, 3.125, and 1.5625 µg/mL. Furthermore, SEM imaging was also performed on eggs from the positive and negative control groups for comparative purposes. Our results indicated that exposure to CQDs induced significant structural alterations in the eggs. Specifically, we observed the accumulation of CQDs around the surface of the eggs which was associated with deformities in the eggs (Fig. 2).

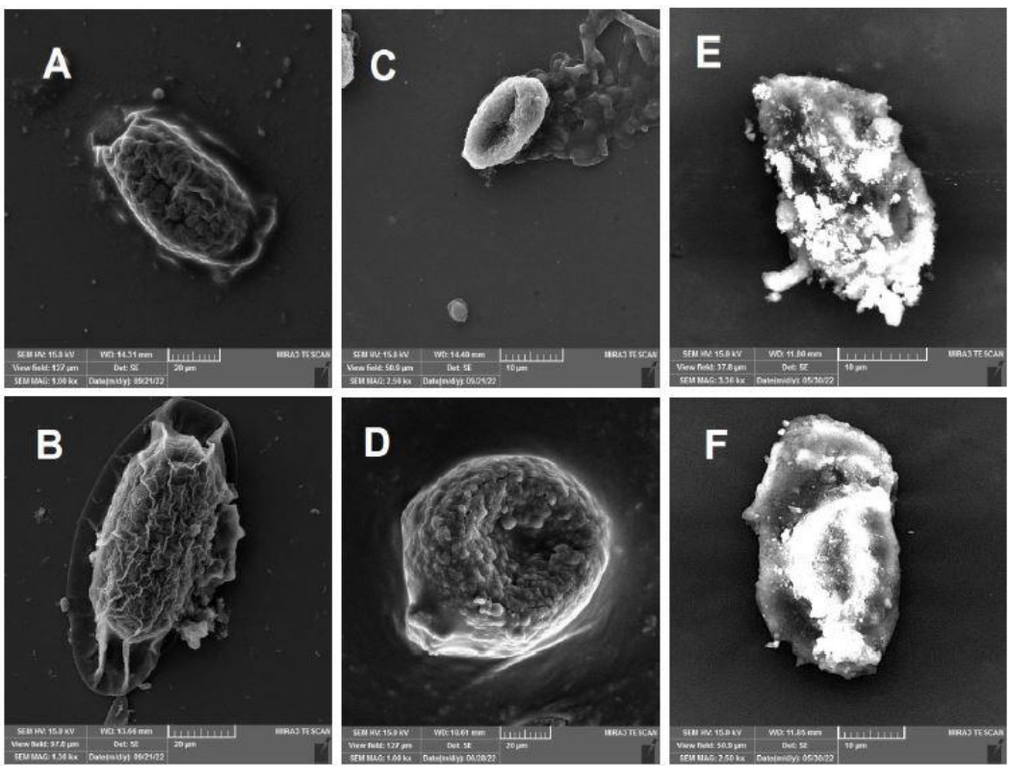

Fig. 2. Scanning electron microscopy image of strongyle egg after exposure to negative control group **(A, B)**, Carbon quantum dots **(C, D)**, Copper-doped carbon quantum dots **(E, F)**.

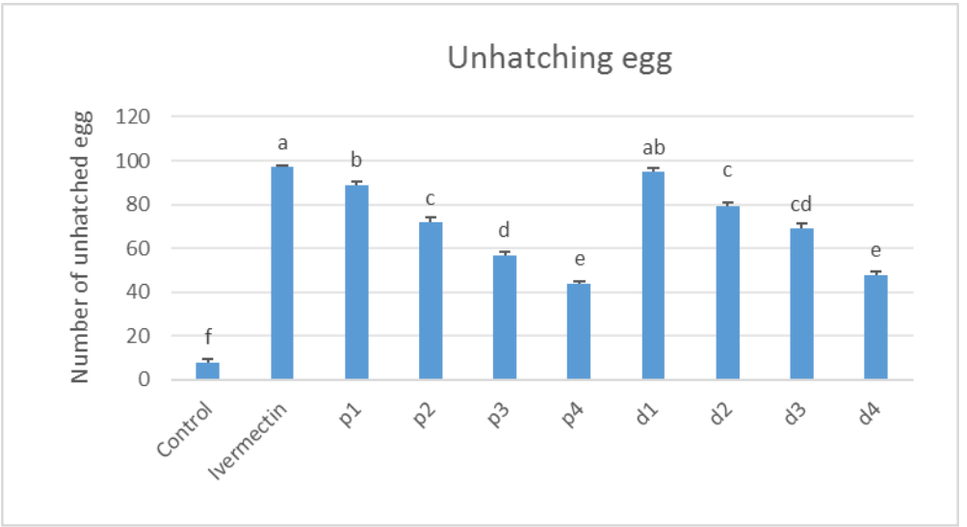

Fig. 3. Number of unhatched strongyle eggs after exposure to negative control group, positive control group and treatment groups. (P1= carbon quantum dots (CQDs) at a concentration of 12.5 µg/mL, P2= CQDs at a concentration of 6.25 µg/mL, P3= CQDs at a concentration of 3.125 µg/mL, P4= CQDs at a concentration of 1.5625 µg/mL, D1= copper-doped carbon quantum dots (Cu@CQDs) at a concentration of 12.5 µg/mL, D2= Cu@CQDs at a concentration of 6.25 µg/mL, D3= Cu@CQDs at a concentration of 3.125 µg/mL, D4= Cu@CQDs at a concentration of 1.5625 µg/mL. p-value < 0.05).

Discussion

The percentage of EHI in the Cu@CQDs treatment group was higher than that of the CQDs treatment group with the highest EHI observed at a concentration of 12.5 µg/mL. The EHI values in the negative and positive control groups were 8% and 97%, respectively. These results suggested that Cu@CQDs have a significant inhibitory effect on the hatching of strongyle eggs.

A comparison of the positive control group (Ivermectin) and the Cu@CQDs treatment group at a concentration of 12.5 µg/mL (d1 group) revealed that both exhibited similar effects in preventing the hatching of strongyle eggs indicating that both could demonstrate significant inhibitory effects (Fig. 3). Furthermore, it was evident that the Cu@CQDs treatment group (d) performed better compared to the CQDs treatment group (p) as they prevented a greater number of eggs from hatching.

Observations made through light microscopy and SEM (Figs. 1 and 2) suggested that the inhibition of egg hatching in the experimental groups was likely due to the accumulation of CQDs around the eggs which was associated with deformities in the eggs. The eggs treated with CQDs exhibited a predominantly liquid composition accompanied by small, irregularly shaped morula cells. Moreover, the larvae developing within these eggs displayed aberrant morphology, characterized by compacted and wrinkled structures. In contrast, the control group depicted a typical developmental progression with larvae undergoing normal growth stages and successfully hatching from the egg (Fig. 1A).

The mechanism of action of CQDs in combating nematodes like strongyle is not fully understood, however, it is believed to involve multiple pathways. One possible mechanism is the alteration of the surface charges of nematode cells which can disrupt their normal functioning and ultimately lead to their death.

The results of the present study suggested that Cu@CQDs at a concentration of 12.5 µg/mL demonstrated significant efficacy in inducing structural changes in strongyle eggs. This finding was consistent with the observed accumulation of Cu@CQDs around the surface of the eggs which was associated with deformities in the eggs. The efficacy of Cu@CQDs at this concentration highlighted their potential as a novel therapeutic agent for combating strongyle infections.

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Acaricidal and cytotoxic activities of *Artemisia absinthium* against *Haemaphysalis* spp. ticks and determination of its toxicity by MTT assay

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The *Haemaphysalis* species of ticks are responsible for transmission of pathogens in vertebrate animals and humans (1). Recently, the use of natural compounds has been proposed as a useful and inexpensive approach to fight against ticks (2). The aim of this study was to evaluate *in vitro* arcaricidal activity of hydroalcoholic extract of *Artemisia absinthium* against the *Haemaphysalis* spp. and determine its toxicity using MTT assay. The acaricidal activity of *A. absinthium* in concentrations of 50, 100, 150, 200 and 250 mg/mL was investigated after 10, 30 and 60 min. In this experiment, the spraying and contact methods were used. The main compounds of *A. absinthium* were identified using gas chromatography-mass spectrometry (GC–MS). Then, the toxicity of each concentration was evaluated through MTT assay. Mortality percentages and LC_{50} values were also calculated. Data were analyzed by GraphPad Prism 5 software. The results of this study showed that the concentration of 250 mg/mL of *A. absinthium* had the highest acaricidal effect (90.00%) in the exposure time of 60 min, and spraying method was more effective than contact method. The results of the MTT test showed that toxicity increases with increasing concentration, and low concentrations of *A. absinthium* have very little toxicity. The GC-MS showed that n-Docosane (9.72%) is the main ingredient of *A. absinthium*. It is concluded that the hydroalcoholic extract of *A. absinthium* contains potent acaricidal compounds and it may be used as a natural acaricide against *Haemaphysalis* spp.

Keywords: Acaricide, *Artemisia absinthium*, *Haemaphysalis* spp., Toxicity

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Anti-leishmania effect of *Ferula asafoetida* extract on *Leishmanaia major* and determined it cytotoxicity on mammalian cells

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Cutaneous leishmaniasis is one of the zoonotic diseases being considered as a health problem in Iran and several countries around the world (1). At present, glucantime is used to treat cutaneous leishmaniasis. Due to its high side effects and resistance, the use of alternative therapies, especially the use of plants and natural compounds, has been considered by researchers (2). The aim of this study was to investigate the *in vitro* anti-leishmania activity of hydroalcoholic extract of *Ferula asafoetida* on *Leishmania major*, and to determine its toxicity using MTT assay. In this experimental study, the effect of hydroalcoholic extract of *F. asafoetida* on *L. major* parasite at concentrations of 300-1200 µg/mL was evaluated and its cytotoxicity on mammalian cells was determined. Mortality percentages and LC_{50} values were also calculated. The main compounds of extract were identified using gas chromatography-mass spectrometry. Data were analyzed by GraphPad Prism 6 software. The IC_{50} of *F. asafoetida* extract after 24 hr on *L. major* was calculated to be 37.00±2.00 mg/mL. The highest mortality (100%) was observed at a concentration of 1200 µg/mL after 72 hr of exposure. However, *F. asafoetida* was significantly cytotoxic against mammalian cells. The major chemical components of *F. asafoetida* were identified as 2-methoxy-3-methyl-butyric acid and methyl ester. The extract of *F. asafoetida* has an inhibitory effect on the growth of *L. major* and it is suggested that more complete studies be performed on the components of this plant and *in vivo* lethal effect of the parasite.

Keywords: Anti-leishmania, *Ferula asafetida*, *In vitro*, *Leishmania major*, Toxicity

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A review on the use of natural and herbal medicines against protoscolices of hydatid cysts

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Hydatidosis is the most important global parasitic infectious diseases, both in humans and animals. *Echinococcus granulosus* is the causative agent of hydatid cysts, which can be lodge at different organs of host such as liver, lung and even heart and brain which may lead to death (1). Presently, numerous scolicidal chemical agents have been administrated for inactivation of the hydatid cyst contents. Because of increasing resistance and adverse effects of medications, there is a need to find alternative therapies either with the least or without side effects (2). Till now, several efforts have been conducted on herbal extracts against protoscolices of hydatid cysts throughout the world. Therefore, the current review searched the following electronic databases: PubMed, Science Direct, Scopus, and Google Scholar (published papers according to the keywords). The results of this review study showed that the extracts of plants such as *Allium sativum*, *Ferula assa-foetida*, *Trachyspermum ammi*, *Rhus coriaria*, *Berberis vulgaris* and *Punica granatum* are among the most effective plants used against protoscolices of hydatid cysts. In addition, medicinal plants showed the best efficacy and promising results are discussed elaborately.

Keywords: *Echinococcus granulosus*, Hydatid cysts, Natural products, Scolicidal agent

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Comparison of Anti-*Ichthyophthirius multifiliis* effects of some medicinal plants with malachite green

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The most common treatment for the parasite *Ichthyophthirius multifiliis* is malachite green, which has carcinogenic effects and causes environmental pollution. Therefore, using alternative treatments, especially medicinal plants, seems logical (1). In this study, the anti-parasitic effects of five herbal extracts of *Lawsonia inermis*, *Satureja khuzestanica*, *Citrullus colocynthis*, garlic, and thyme on *I. multifiliis*, the causative organism of white spot syndrome, were investigated. First, hydroalcoholic extracts of all plants were prepared using the maceration method (2). Next, the theront and tomont stages of the parasite, being isolated from heavily infected rainbow trout, were exposed to serial two-fold dilutions of each extract. Immobilized (dead) parasites were recorded every 3 hr for 12 hr at each dilution, and the 12-hr LD_{50} of each extract was calculated using the Probit method. Malachite green was used as a positive control. The results showed that the 12 hr LD_{50} values of *L. inermis*, *S. khuzestanica*, *C. colocynthis*, garlic, thyme, and malachite green on parasite tomonts were 4.68, 2.32, 5.21, 1.00, 2.50, and 0.24 mg/L, respectively. Meanwhile, the 12 hr LD_{50} values on theronts were 0.57, 0.45, 2.37, 1.66, 0.57 mg/L, and 0.121 mg/L, respectively. Among the five medicinal plant extracts used in this study, garlic and thyme extracts demonstrated the highest anti-parasitic effects. However, the anti-parasitic effects of these extracts were negligible compared to the malachite green, a commonly used conventional chemical treatment for ichthyophthiriasis (3). These extracts can be used as complementary treatments alongside chemical treatment.

Keywords: Anti-parasitic effect, *Ichthyophthirius multifiliis*, Medicinal plants, Theront, Tomont

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Effect of ajowan (*Trachyspermum ammi*) on hydatid cyst protoscoleces: An experimental study

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Hydatid cyst disease poses a serious health issue worldwide. Protoscoleces, essential stages in the parasite's life cycle, are critical targets for therapeutic research (1). This study assessed the impact of ajowan (*Trachyspermum ammi*), a plant-based compound, on the mortality of hydatid cyst protoscoleces (2, 3). Protoscoleces were extracted from hydatid cysts and kept in phosphate-buffered saline with a pH of approximately 7.40. They were treated with ajowan at concentrations of 10, 1, 0.10, and 0.01 µg/mL for durations of 1, 30, 60, and 120 min. After that, the protoscoleces were stained with 1% eosin and analyzed under a light microscope to determine mortality rates. The study results revealed a significant increase in protoscolex mortality with higher ajowan concentrations and longer exposure times. At 1 µg/mL, mortality reached 100% after 30 min. Lower concentrations required more extended treatment; 80% mortality was achieved at 0.10 µg/mL after 60 min, and 50% at 0.01 µg/mL after 120 min. Statistical analysis confirmed that these differences in mortality rates across various concentrations and durations were statistically significant. These results underscore the potent effect of ajowan on hydatid cyst protoscoleces, even at low concentrations and brief treatment times. The study concludes that *T. ammi* shows considerable promise as a therapeutic agent for managing hydatid cyst disease. This research lays the groundwork for further studies and development of new treatments for this parasitic infection.

Keywords: Ajowan, Hydatid cyst, Protoscolece, *Trachyspermum ammi*

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Ovicidal effects of *Trachyspermum ammi* alcoholic extract on *Fasciola hepatica* eggs: A comparative study with closantel

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Trachyspermum ammi, commonly known as ajwain or carom seeds, is a traditional herbal remedy used in various cultures for its medicinal properties. Recent studies have highlighted its potential as an anthelmintic agent, demonstrating efficacy against a range of parasitic infections (1-3). This study investigated the impact of *T. ammi* alcoholic extract on the eggs of *Fasciola hepatica*, a liver fluke causing significant health and economic issues in both humans and animals globally. With the increasing resistance and adverse effects associated with conventional chemical anthelmintics, there is a growing interest in exploring natural alternatives. Various concentrations of ajwain extract, including 0.01, 0.10, 1, and 10 µg/mL wereapplied to *F. hepatica* eggs for 1, 30, 60, and 120 min. Closantel was used as a positive control at the same concentrations. The results demonstrated that the efficacy of ajwain extract on *F. hepatica* eggs increased significantly with higher concentrations and longer exposure times. The eggs exposed to higher concentrations and longer durations became noticeably non-viable. Microscopic observations revealed structural damage to the eggs and a reduction in their metabolic activity. These findings suggest that the alcoholic extract of *T. ammi* can serve as a natural and effective agent in controlling fascioliasis. However, further research isneeded to determine the precise mechanisms of action and assess any potential side effect.

Keywords: Ajwain, *Fasciola hepatica*, Ovicidal activity, *Trachyspermum ammi*

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Comparative efficacy of fennel (*Foeniculum vulgare*) and ajwain (*Trachyspermum ammi*) essential oils on the mortality of *Linguatula serrata* nymphs

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Linguatula serrata is a significant parasite affecting livestock and humans. The use of essential oils as alternative methods for controlling parasites has gained attention (1,2). This study aimed to investigate the impact of fennel (*Foeniculum vulgare*) and ajwain (*Trachyspermum ammi*) essential oils on the mortality of *L. serrata* nymphs. Nymphs of *L. serrata* were isolated from the mesenteric lymph nodes of goats and washed in a phosphate buffered solution (3). They were then treated with different concentrations (10, 1, 0.10, and 0.01 µg/mL) of fennel and ajwain essential oils at various time intervals (1, 6, 12, 24, and 48 hr). Mortality of the nymphs was assessed based on their mobility. The results showed that all nymphs died within 24 hr at concentrations of 10, 1, and 0.10 µg/mL of ajwain essential oil. In contrast, only 40% of the nymphs died at the same concentrations of fennel essential oil within 24 hr. However, after 48 hr, all nymphs in both essential oils were completely dead. It isconcluded that ajwain essential oil exhibited a more potent effect on the reduction of nymph viability compared to the fennel essential oil. Ajwain essential oil was effective in killing all nymphs at lower concentrations and shorter exposure times. These findings suggest that essential oils can be used as natural and safe methods for controlling *L. serrata* parasites in livestock and potentially in humans. Further research is recommended to elucidate the precise mechanisms of these essential oils effects and to explore their effects under various conditions.

Keywords: *Foeniculum vulgare*, *Linguatula serrata*, *Trachyspermum ammi*

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Scolicidal activity of *Capparis spinosa* hydroalcoholic extract on *Echinococcus granulosus* protoscolices

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Hydatidosis (cystic echinococcosis) is one of the most important helminthic and major zoonotic diseases caused by *Echinococcus granulosus* (1). There is no safe and suitable remedy for Cystic echinococcosis, so the discovery of new compounds with promising scolicidal effects, particularly from herbal sources, is of great importance for therapeutic uses in its treatment and prevention (2). Considering the anti-parasitic properties of *Capparis spinosa*, this study was conducted to compare the *in vitro* effect of *C. Spinosa* hydroalcoholic extract with albendazole on protoscolices of hydatid cyst (3). In this study, at the flowering stage of *C. spinosa* plant was collected from Firouzkouh city, Mazandaran province, Iran. Protoscolices were extracted from the livers infected with hydatid cyst and exposed to different concentrations of *C. spinosa* (10, 25, 50 and 100 µg/mL) for 10, 30, 60 and 120 min. The viability of protoscolices was measured by 0.10% eosin staining. All concentrations of *C. spinosa* led to significant mortality compared to the control group. After 10 min, the highest mortality was occurred in the *C. spinosa* extract (72.33%). The difference between *C. spinosa* extract concentrations at 10 min was significant. At thirty min, the highest mortality was observed in the *C. spinosa* extract 100 group (92%). The highest mortality rate was in the treatment group with *C. spinosa* extract 100 µg/mL (100%) after 1 hr. At 2 hr, the highest mortality was observed in the *C. spinosa* extract 100 µg/mL (100%). All concentrations of *C. spinosa* in comparison with albendazole control group caused significant mortality in protoscolices. This study showed that *C. spinosa* hydroalcoholic extract has an acceptable scolicidal effect compared to the albendazole.

Keywords: Albendazole, Capparis spinosa, Hydatid cyst, Mortality, Protoscolex

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In vitro scolicidal activity of the *Syzygium aromaticum* hydroalcoholic extract on *Echinococcus granulosus* protoscoleces

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Hydatid cyst is a parasitic disease caused by *Echinococcus granulosus* and rarely *Echinococcus multilocularis*. Liver and then lungs are the most common site of infection (1). Currently, the therapeutic procedures for cystic echinococcosis are quite limited. Surgery is still the only effective treatment for this disease (2). So far, many chemical protoscoleces killers have been used to prevent protoscoleces leakage during surgery, but it is necessary to consider the adverse effects of chemicals and use of medicinal herbs. Considering the anti-parasitic properties of cloves, this study was conducted to evaluate and compare the effect of cloves and albendazole drug on protoscolices of hydatid cyst under *in vitro* conditions (3). The flowering stage of *Syzygium aromaticum* plant was collected from Firouzkouh city, Mazandaran province, Iran. Protoscoleces were aseptically aspirated from infected sheep's livers. To assess the scolicidal effects of these compounds, protoscoleces were exposed to 10, 25, 50, µg/mL concentrations for 10, 30, 60, and 120 min. The standard drug used in this study was albendazole. The viability of protoscoleces was evaluated using 0.10% eosin. Hydroalcoholic extract of *S. aromaticum* at all tested concentrations led to significant mortality in protoscolices which showed a direct relation with increase in concentration. After 60 and 120 min, at a dose of 100 ug/mL, the highest mortality occurred in the cloves group (100%), while at a concentration of 100 µg/mL, the difference between the time of 60 and 120 min was not significant. The results of this study showed that *S. aromaticum* hydroalcoholic extract has an acceptable scolicidal effect compared to albendazole and can be suggested as a natural protoscolicidal agent.

Keywords: Albendazole, Cloves, Hydatid cyst, Protoscoleces, *Syzygium aromaticum*

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A review of anti-parasitic effects of *Cichorium intybus*

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Common chicory (Cichorium intybus) is a somewhat woody, perennial herbaceous plant of the family Asteraceae, usually with bright blue flowers, rarely white or pink. Many varieties are cultivated for salad leaves, chicons (blanched buds), or roots (var. sativum) which are baked, ground and used as a coffee substitute and food additive. In the 21st century, inulin, an extract from chicory root, has been used in food manufacturing as a sweetener and source of dietary fiber. Chicory is also grown as a forage crop for livestock. In a study a group of cattles were infected experimentally with *Ostertagia ostertagi*. Then the chicory plant was added to the cattle diet. Then it was observed that the number of eggs of this parasite in the feces was significantly reduced and chicory was mentioned as an anti-ostratagia (3). Also, in another study that was conducted on a group of horses that were naturally infected with cyathostomin, it was observed that in those horses which diet contained chicory plant, the rate of egg excretion in feces and also the larval development were decreased significantly. Therefore, it could be concluded that chicory had inhibitory effects on larval development and excretion of cyathostomin eggs in feces (1). In a research that used chicory root and leaves in two separate groups, it was observed that chicory pulp root had more anti-parasitic effects than leaves (2). Adding chicory to livestock diet showed anti-parasitic effects.

Keywords: Anti-parasitic, Chicory, Cyathostomin, *Ostertagia ostertagi*

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Investigation of effect of green tea (*Camellia sinensis*) and Jujube (*Ziziphus vulgaris*) extracts on *Varroa destructor* (Acari: Varroidae)

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Varroa destructor, an ectoparasitic mite, is responsible for causing varroasis which is the main pathologies affecting both brood and adult honeybees (*Apis mellifera*) (1). Natural substances have the potential to effectively control honeybee diseases and can be used as a natural alternative to reduce contamination of beehive products (2). The present study was carried out to evaluate the effect of green tea and Jujube extracts on *V. destructor* in laboratory conditions and at the apiary. This investigation was carried out to evaluate effects of three concentration of the green tea and Jujube extracts in three replications on the *V. destructor* under laboratory condition. In control group, mites were dipped in distillate water. Twenty adult mites were used for each treatment. In an infested apiary, three groups (three hives with average of 3 mites per 20 hony bees for each hive) were chosen to evaluate basic concentration of green tea and Jujube extracts (2.5%) in comparison with Apistan and control groups. The adult mite mortality for the tree concentration of 1, 2, 3% was respectively recorded as 14.35%, 65.77%, and 98.84 for green tea and 8.48%, 49.81%, and 90.43%, for Jujube exract. There was significant effect between green tea and Jujube extracts in treatment and control group. The highest effect of green tea and Jujube extracts and Apistan on *V. destructor* was found in 36 (36.41%), 48(34.73%) and 24 (47.54%) hr, respectively. It was concluded that green tea and Jujube extracts had lethal effect on *V. destructor* infestaion in honey bees.

Keywords: Apistan, *Camellia sinensis*, Honey bee, *Ziziphus vulgaris*, *Varroa destructor*

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Medicinal plants as sustainable control agents against helminth parasite infection

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Helminth infections are a significant cause of economic losses in animal husbandry. These diseases threaten livestock worldwide in various temperature zones, including tropical, subtropical and temperate regions. Gastrointestinal nematodes (GINs) can lead to substantial economic losses in livestock globally (1). Treatment of GIN infections have become challenging due to increasing anthelmintic resistance in many nematode species. Overuse and frequent treatments have led to this problem. Unfortunately, commonly used anthelmintic drug families, benzimidazoles and macrocyclic lactones are no longer as effective putting modern livestock at risk (2). This highlights the urgent need for new approaches such as genetic control techniques, pasture management, nutritional modification, biological regulation, vaccine development and the use of herbal products. There is a steady increase in studies aimed at confirming the anthelmintic potential of plants. Many conventional and new medicines have been derived from plants and some of their active ingredients have been tested for their ability to treat parasites both *in vitro* and *in vivo*. Natural herbal products offer great hope because they contain a large reservoir of ingredients with medicinal properties. These natural products have been used effectively against a variety of parasitic diseases by boosting viability and egg production reducing worm burden altering antioxidant enzyme levels and additionally inducing worm apoptosis (3). In conclusion, plants have the potential to benefit helminth control, however, further research is needed to determine safe and effective dosages for practical use in animal husbandry.

Keywords: Helminth, Parasite, Plants

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Medicinal plants as sustainable control agents against *Varroa destructor* (Acari, Varroidae), an ectoparasitic mite of honeybees (*Apis mellifera)*

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Varroa destructor, an ectoparasitic mite, causes varroasis, a major disease affecting both brood and adult honeybees (*Apis mellifera*). This poses a significant threat to beekeepers (1). Synthetic acaricides such as pyrethroids, organophosphates, and formamidines are commonly used to control the mite in commercial beekeeping. However, their overuse has led to resistance in many countries and contamination of hive products. Researchers are seeking safer control methods with minimal impact on the environment which should be incorporated into Integrated Pest Management programs to reduce reliance on synthetic acaricides (2). To address this issue, there is a growing trend to use natural products as part of an integrated control strategy. One advantage of organic alternatives is their lower toxicity levels in mammalian species and minimal environmental damage. Natural substances have the potential to effectively control honeybee diseases and reduce contamination of beehive products. Plants are the primary source of such chemical compounds serving as natural laboratories producing a variety of chemicals. Alkaloids, steroids, tannins, flavonoids, terpenoids, saponins, phenols and resins are examples of secondary metabolites found in plants which have insecticidal properties. These substances can poison arthropods through ingestion, contact or inhalation. Recent studies have shown that plant extracts can be as effective as or more effective than conventional acaricides against varroa (3). In conclusion, plants have the potential to benefit varroasis control, however, further research is needed to determine safe and effective dosages for practical use in honeybee management.

Keywords: Honey bee, Plants, Varroa

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A review on plant extracts for the control of ticks

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Due to resistance to chemical pesticides in livestock products, herbal medicines from plant extracts against pests are preferred. The present study carried out in databases from 2000 to 2024. Many studies reviewed including: Ethno veterinary plants against *Rhipicephalus microplus*, oil of *Melaleuca alternifolia Cheel* on *Ixodes ricinus* nymphs, *Metarhizium anisopliae* on *H. punctata* and *H. anatolicum*, extracts of *Murraya koenigii* on *Boophilus microplus* which showed significant impact in killing ticks. The killing effect of extract of *Consolida* on the egg and larval stages of *H. anatolicum* and *Rhipicephalus Bursa*, Cymbopogon winterianus, Vitex *negundo and Withania somnifera* extracts against deltamethrin resistant *H. anatolicum*, aqueous extracts of neem leaves against *Rhipicephalus sanguineous* were dose–dependent. The highest toxicity level of bitter almond alcoholic extract was revealed for the adult than nymph of *Argas* and *Haemaphysalis* (1). In another study, using garlic, the best role in controlling of Nymph stage of *Ixodes scapularis* in short time was observed (2). The acaricidal activity of *Cymbopogon winterianus*, *Vitex negundo* and *Withania somnifera* against pyrethroid resistant *Rhipicephalus* had a low effect on adult mortality (3). The extract of Myrrh from *Commiphora molmol* tree has effect on the adult form of *Argas persicus*. The efficacy of the extract of *Melia Azedarach* on the tick *Boophilus Microplus* showed that it did not kill the adult female but it killed the larval stage and feeding blood females. Each plant extract may be effective on different stage of tick life cycle. Therefore, combination using of these extracts can be more effective.

Key words: Control, Extract, Plant, Ticks

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A review on use of medicinal plants against intestinal worms

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Due to drug resistance and ineffectiveness of vaccine in some parasites, alternative sources of anti-parasitic drugs are required. *Chenopodium ambrosioides* (Amaranthaceae) and ascaridole which has been isolated from this plant are useful for parasitic intestinal helminth. Ascaridole is efficient against hookworm infection, however, poisonous and mutagenic (1). Another anthelmintic plant is the fern *Dryopteris filix-mas* (Dryopteridaceae) which is active against intestinal cestodes and its performance is the worm muscle paralysis (2). Also, this drug has significant side effects for humans and filixic acid is utilized as an anthelmintic in veterinary praxis. Other paralyzing agents are anthelmintic alkaloids arecoline from *Areca catechu* (Arecaceae) and the pelletierine from *Punica granatum* (Lythraceae) which target acetylcholine receptors (1). Other traditional herbs include *Artemisia maritima* (with santonin), *Teloxys graveolens* (Amaranthaceae), *Artemisia abrotanum* (Asteraceae) *Zanthoxylum liebmannianum* (Rutaceae), *Embelia schimperi* (Myrsinaceae), *Thymus vulgaris* (Lamiaceae), *Albizzia anthelmintica*, *Millettia thonningii*, *Butea frondosa* (Fabaceae) and several others (3). The identified plants and compounds offer a chance to develop new drugs against parasitic infections. Most of them need to be tested in more detail particularly in animal models and if successful, in clinical trials. Most of the antiparasitic properties of extracts have been tested *in vitro* only. Therefore, interpretation of the *in vitro* research results into *in vivo* trials is crucially needed. Moreover, more studies are needed to use new compounds alone or in combination with anthelmintic drugs to demonstrate their safety and efficacy.

Keywords: Herbal, Medicinal plants, Worms

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Anti-leishmanial effects of herbal monoterpenes

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Leishmaniasis, the third most important disease transmitted by vectors, is caused by protozoan parasites of the genus *Leishmania* (1). Chemotherapy of leishmaniasis has several limitations. Medicinal plants are rich resources containing valuable and accessible materials. As a subset of volatile plant compounds, monoterpenes have been attributed to many biological and pharmacological actions of plant products in recent years (2). One of the main members of monoterpenes is herbal monoterpenes (MT) (3). This study investigated the biological effects of MT on *Leishmania*, antileishmanial effect, cytotoxicity, apoptosis induction and gene expression changes. Herbal monoterpene was probed for its antileishmanial potentials using the MTT biochemical colorimetric assay and a model macrophage cell. Annexin-V/PI staining by flow cytometry was used for evaluation of apoptosis. Its immunomodulatory properties were assessed by analyzing their effect on the transcription of cytokines related to Th1 and Th2 responses. The MT showed significant inhibitory effects against *L. major* promastigotes and amastigotes. The apoptotic effect of herbal monoterpenes against *L. major* promastigotes was increased concentration-dependently. Herbal monoterpenes ability to attenuate the transcriptional expression of IL-12P40, JAK1, iNOS, IL-10, and TGF-β genes and to enhance the metacaspase gene at mRNA level was evidenced. The present study confirmed that MT was a suitable candidate compound to interfere with the growth of *L. major* in a eukaryotic cell model by moderate scavenging activities. This study was the first to investigate the performance of MT as a pure compound against *L. major* stages, whereas, previous studies have elaborated using terpenes obtained from plants.

Keywords: Gamma-terpinene, Gene expression, *Leishmania major*

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In-vitro assessment of Alliacin (alcoholic extracts of garlic, onion and leek) effect on red mite (*Dermanyssus gallinae*)

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Red mite control was usually based on Organophosphates, Carbamates and Pyrethroids (1). Due to the increased resistance to these toxins, the number of toxins currently used is limited. The use of plant compounds is an alternative approach for controlling mites (2,3). In the present study, the effect of Alliacin (alcoholic extracts of garlic, onion and leek) and alcoholic extract of garlic, onion, leek and two-component compounds of garlic and onion, garlic and leek, onion and leek alcoholic extracts with concentration 1.5, 3, 7.5, 15 and 30% and Cypermethrin with concentrations of 1, 2 and 4 mL per L for 36 hr at different time intervals (2,4, 6, 8, 10, 12, 24 and 36 hr) was examined on the chickens red mite. The results showed that Cypermethrin only at a concentration of 4 mL per L after 24 and 36 hr of exposure was able to kill 20.66 and 29.66% of mites, respectively. Alliacin at a concentration of 30% was able to kill 86.66 and 96.66% of mites in 24 and 36 hr, respectively. The best results were related to the two-component compounds of garlic and leek alcoholic extract and garlic and onion alcoholic extract at a concentration of 30%, in 12 hr could kill 95% and 86.66%, and eliminate 98.33 and 100% of red mites in 24 hr, respectively. Due to resistance of red mite to chemical toxins, Alliacin and the two-component compounds of garlic, onion and leek extract could be used as alternatives to eliminate red mite.

Keywords: Alliacin, *Dermanyssus gallinae*, Garlic, Leek, Onion

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Investigation of the scolicidal effects of oak gall extract and its nanoparticles on protoscolices of hydatid cysts under *in vitro* conditions

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Hydatid cyst disease, caused by the parasite *Echinococcus granulosus*, is highly prevalent in livestock-breeding areas (1). The primary treatment for this disease involves surgery, often accompanied by the use of protoscolecidal agents to reduce the risk of recurrence (2). Due to concerns about side effects and drug residues, the use of medicinal plants and novel methods has gained increasing attention (3). In this study, hydatid cyst samples were collected from a slaughterhouse in Sanandaj and prepared for protoscoleces extraction and experimentation in the parasitology laboratory. In this study, the scolicidal effects of five different concentrations (5, 10, 25, 50, and 100%) of crude and nano-sized sumac extracts were evaluated against live protoscoleces at 10, 30, 60, and 120 min. The results showed that the viability of protoscoleces was decreased with increasing both concentration and exposure time. The highest number of live protoscoleces was observed at 10 min, while the lowest number was observed at 60 min. Moreover, the number of protoscoleces was decreased significantly with increasing concentration ($p < 0.001$). Based on these findings, nano-Oak gall could be considered an effective compound in eliminating viable protoscoleces and could be used as a therapeutic option alongside surgery to reduce the risk of hydatid cyst recurrence.

Keywords: Echinococcus Granulosus, Hydatid cyst, Nanoparticle, Oak gall, Protoscoleces

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Protoscolicidal effects of crude extract of *Nigella sativa* and its *nanoparticles* on hydatic cyst protoscoleses *in vitro*

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Hydatic cyst disease, caused by the parasite *Echinococcus granulosus*, is highly prevalent in livestock-breeding areas (1). The primary treatment for this disease involves surgery often accompanied by the use of protoscolecidal agents to reduce the risk of recurrence (2). Due to concerns about side effects and drug residues, the use of medicinal plants and novel methods has gained increasing attention (3). The aim of this study was to investigate the scolicidal effects of *Nigella sativae* extract and its nanoparticles on protoscolices of hydatic cysts under *in vitro* conditions. Hydatic cyst-infected livers and lungs were obtained from a slaughterhouse in Sanandaj. These were then transferred to the parasitology laboratory for the extraction of protoscolices, processing and subsequent experimental procedures. In this study, the scolicidal effects of five different concentrations (5%, 10%, 25%, 50%, and 100%) of *N. sativa* extract and its nanoparticles were evaluated at time intervals of 10, 30, 60, and 120 min when exposed to protoscolices. The results showed that as both concentration and time were increased, the viability of protoscolices was decreased. The highest number of live protoscolices was observed at 10 min, while the lowest was at 60 min. With increasing concentration, the number of protoscolices showed a decreasing trend which was statistically significant ($p > 0.001$). Based on the findings of this study, *N. sativa nanoparticles* could be used as a protoscolicidal agent in hydatic cyst surgery.

Keywords: Hydatic cyst, Nanoparticles, *Nigella sativa*, Protoscolices, Scolicidal

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Hiridotherapy in the field of medicine and veterinary medicine

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Leech therapy represents a time-honored practice that traces its origins to ancient Egypt and the dawn of civilization. The popularity of *Hirudo Medicinalis* has fluctuated throughout the annals of history reaching its zenith in Europe during the early 19th century when resources became scarce (1). Hirudotherapy is utilized in both human healthcare and veterinary practices. In the realm of veterinary medicine, particularly in instances where conventional treatments have proven ineffective, it is employed post-surgery and in scenarios where tissues are at risk due to venous congestion. The most prevalent species of medicinal leech include: *H. medicinalis*, *Hirudo asiatica*, *Hirudomanellensis*, *Hirudo Orientalis* (Iranian species), *Hirudo michaelseni*, *Hirudo nipponia*, *Hirudo granulosa*, and *Macrobdella decorata*. The saliva of medicinal leeches is composed of over 100 bioactive compounds that exhibit therapeutic properties including anticoagulant, vasodilator, thrombolytic, anti-inflammatory, analgesic and anesthetic activities. Notable bioactive constituents encompass hirudin, hyaluronidase, choline, destabilase, apyrase, eglin, bedlin, decorsin, hirostatin, tryptase inhibitors, histamine-like substances, complement inhibitors, carboxypeptidase A inhibitors and acetylcholine (2). The saliva of leeches comprises an array of neurotransmitters including serotonin, dopamine, acetylcholine and enkephalin which facilitate analgesia and promote relaxation within the physiological systems of patients (3). The efficacy of leech therapy has been substantiated through numerous investigations within the realm of medical science and a plethora of studies are presently underway in this domain. Among its applications and associated advantages for human health, one can cite the treatment of conditions linked to inflammation, hip and elbow dysplasia and ailments affecting tendons, ligaments and fascia, vertebral disorders as well as wound management. Several studies have demonstrated the beneficial effects of this therapeutic approach in conditions such as laminitis, tendinitis, ataxia, spinal osteoarthritis in equines, discopathies, eczema, neuritis and mastitis in canines and felines within the context of veterinary science.

Keywords: Hirudin, Hirudotherapy, Laminitis, Leech

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In vitro investigation of the comparative effects of hydroalcoholic extract of valerian root and its nanoparticles on hydatic cyst protoscoleces

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Hydaticosis is one of the most dangerous zoonotic diseases in the world caused by the larval stage of the cestode *Echinococcus granulosus* (1). Numerous studies have evaluated the antiparasitic properties of metallic nanoparticles on hydatic cyst protoscoleces in recent years. Given the unknown effects of wormwood and its nanoparticles on hydatic cyst protoscoleces, this study experimentally investigated the effects of valerian and wormwood extract and its nanoparticles on hydatic cyst protoscoleces. In this experimental study, hydatic-infected livers and lungs were obtained from a slaughterhouse in Sanandaj. Protoscolex viability was determined using the 0.1% eosin test under a light microscope. Different concentrations of wormwood extract (100 and 200 mg/mL) and its nanoparticles were used for durations of 1, 6, and 12 hr. All stages were repeated three times and the results were analyzed using statistical software. This experiment revealed that both valerian and its nano form had a lethal effect on hydatic cysts and protoscoleces. The mortality rate of the hydroalcoholic extract at a concentration of 100 mg/mL for hydatic cysts after 1, 6, and 12 hr was 9.91%, 25%, and 29%, respectively. The effect of the hydroalcoholic nanoparticles was 11%, 16%, and 22.2%, respectively. At a concentration of 200 mg/mL, the hydroalcoholic extract showed mortality rates of 13.5%, 30% and 35%, respectively, and the nanoparticles showed 13%, 28% and 34%, respectively. The protoscolex survival rate was decreased with increasing time and concentration. Although both substances caused the death of protoscoleces under laboratory conditions, the wormwood extract was more effective than its nanoparticles.

Keywords: Hydatic cyst, Hydroalcoholic extract, Nanoparticles, Protoscoleces, Valerian root

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Section 5

Pathology

Effects of gallic acid on KIM-1, IFN-γ and PPAR-γ levels on acrylamide-induced kidney damage in rats

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Abstract

Acrylamide (ACR) is a chemical that is widely used in many areas and can cause damage to many tissues and organs. It occurs as a result of heat treatment of foods containing mainly carbohydrates and proteins. In this study, the effects of gallic acid (GA) on kidney damage induced by ACR were investigated. A total of 40 male Sprague Dawley rats were used in this study and the rats were divided into five experimental groups including control, ACR, ACR+GA50, ACR+GA100 and GA100. In the experiment, ACR was applied to the ACR groups at a dose of 50 mg/kg, while GA was applied to the GA groups at doses of 50 and 100 mg/kg. At the end of the 14-day experiment, the rats were euthanized and their kidney tissues were removed. The level of kidney injury molecule-1 (KIM-1), a marker of damage in kidney tissue, was determined by ELISA method. Also, in order to determine whether inflammation developed in kidney tissues taken from rats, the interferon-γ (IFN-γ) and peroxisome proliferator-activated receptor gamma (PPAR-γ) levels were determined using the ELISA method. While both KIM-1 and IFN-γ levels increased significantly in the groups treated with ACR, there was a decrease in the kidney tissues of the treated rats with GA. The PPAR-γ levels also indicated that ACR induced marked nephrotoxicity. As a result, in this study it was determined that GA had a protective effect against ACR-induced renal inflammation and this protective effect was achieved especially with high doses of GA.

Keywords: Acrylamide, Gallic acid, Interferon-γ, Kidney injury molecule-1, Peroxisome proliferator-activated receptor gamma

Introduction

Acrylamide (ACR) is a substance known for its markedly side effects in many tissues and kidney. Because of this feature and its widespread use in the industrial field, research on it has increased. The ACR occurs especially with the heat treatment of carbohydrate-rich foods. Exposure to these foods causes damage to many organs and tissues (1).

After ACR exposure, it rapidly dissolves in body tissues and reaches the liver and kidney tissues. After ACR enters the body, its metabolites are formed. These metabolites also cause damage to many tissues and organs. One of the tissues damaged is the kidney. The damage that occurs in the kidneys is caused by reactive oxygen species and oxidative damage happens (2). Kidney injury molecule-1 (KIM-1), a transmembrane glycoprotein, is expressed by proximal tubule cells and is an early, sensitive, and specific marker of renal injury (3). Peroxisome proliferator-activated receptor gamma (PPAR-γ) is expressed in different parts of the

nephron, such as the Bowman capsule, podocytes, proximal convoluted tubules, distal convoluted tubules, and collecting ducts. The PPAR-γ has a role in renal inflammation, lipid metabolism, hypertension, fibrosis, and apoptosis (4). Mesenchymal stem cells used for therapeutic purposes can exhibit anti-inflammatory effects by being activated by interferon-γ (IFN-γ) secreted from natural killer cells in damaged tissues (5).

Gallic acid (GA) is a phenolic molecule found naturally in a wide range of fruits as well as medicinal plants. There are many polyphenols that may have protective effects against the damage caused by ACR. One of these polyphenols is GA and GA is a powerful anti-oxidant and anti-inflammatory substance. The GA also has anti-bacterial roles. It has protective effects against damage to tissues and cells caused by many chemical agents (6).

In this study, the roles of GA against inflammatory damage occurring in the kidney tissues of ACR-administered rats were investigated.

Materials and Methods

The ACR (≥ 99.00%; Cas No: 10236-47-2) and GA (Cas No: 149-91-7) were provided fromSigma Chemical Co. (St. Louis, MO). The KIM-1, IFN-γ and PPAR-γ ELISA kits were purchased commercially (BT Laboratory, China). A total of 40 rats with an average weight of 200-250 g were used. The rats were obtained from Atatürk University Experimental Research and Application Center, Erzurum, Türkiye and were housed in ventilated rooms with a room temperature of 25 ˚C, a humidity rate of 60-65%, and a 12-hour light/dark cycle. All rats were supplied with unlimited food and water intake. The rats were weighed before the experiment and divided into 5 separate groups, with 8 rats in each group. Experimental groups were as follows: Control: 1 mL physiological saline was given intra-gastric (IG) for 14 days; ACR: ACR was given IG at a dose of 50 mg/kg for 14 days; ACR+GA50: ACR at a dose of 50 mg/kg and GA at a dose of 50 mg/kg were given IG for 14 days; ACR+GA100: ACR at a dose of 50 mg/kg and GA at a dose of 100 mg/kg were given IG for 14 days; GA100: GA was given IG at a dose of 100 mg/kg for 14 days. On the 15^{th} day, all rats were weighed and euthanized under sevoflurane anesthesia. The kidneys of all rats were washed with cold phosphate buffer and then, placed in a -20 deep freezer until analysis. Equally small pieces of kidney tissues were taken and placed in Eppendorf tubes and then, phosphate buffer solution adjusted to pH of 7.40 was added and homogenized in the MagNA Lyser device. Then, it was centrifuged at 4000 rpm for 10 minutes and the resulting sera were transferred to different Eppendorf tubes. The KIM-1, IFN-γ, and PPAR-γ activities were measured in the obtained supernatants in accordance with the protocol of commercially available ELISA kits. After the studies were completed, one-way ANOVA was used in the statistical analysis of more than two independent groups in the SPSS 20.00 statistical data program and then, quantitative values were obtained and evaluated by applying the Tukey test. Obtained values were expressed as mean ± standard error, and $p < 0.05$ was considered significant.

Results

The PPAR-γ, IFN-γ and KIM-1 levels in kidney tissues of rats treated with ACR. The effects of GA on PPAR-γ, IFN-γ and KIM-1 levels in the kidney tissues of rats in the experimental groups are shown in Figures 1, 2, and 3, respectively. The PPAR-γ level was low in the kidney tissues of the rats in the ACR group,

and there was a statistical difference between them and the rats in the control group ($p < 0.01$). The PPAR-γ levels obtained from the ACR+GA50 group were quite low compared to the control group, and there was a statistical difference between them ($p < 0.05$). There was no statistical difference between the ACR+GA100, GA100 and control groups ($p > 0.05$; Fig. 1). When the IFN-γ levels obtained from the ACR-applied group were compared with the control group, there was a significant difference between them ($p < 0.01$). The IFN-γ levels obtained from the ACR+GA50 group were lower than the ACR group, but there was still a difference between them and the control group ($p < 0.05$). When the IFN-γ levels obtained from the ACR+GA100 and GA100 groups were compared with the control group, there was no significant difference between them and they were statistically similar ($p > 0.05$; Fig. 2).

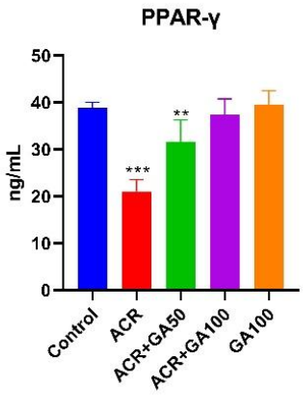

Fig. 1. The effects of acrylamide (ACR) and gallic acid (GA) administrations on peroxisome proliferator-activated receptor gamma (PPAR-γ) level in the experimental groups. There are statistically significant differences between the values expressed with different symbols and the control group. *** $p < 0.01$; ** $p < 0.05$.

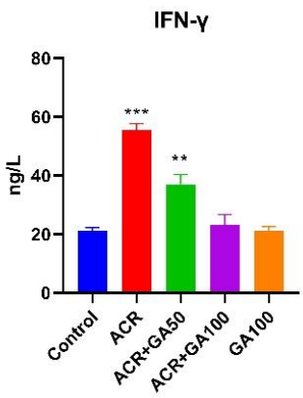

Fig. 2. The effects of acrylamide (ACR) and gallic acid (GA) administrations on interferon-γ (IFN-γ) level in the experimental groups. There are statistically significant differences between the values expressed with different symbols and the control group. ***$p < 0.01$; ** $p < 0.05$.

It was determined that KIM-1 levels increased significantly in the ACR group and there was a statistically significant difference between them and the control group ($p < 0.01$). In the ACR+GA50 group, it was determined that the data obtained were lower than the ACR group, but there was still a difference between them and the control group ($p < 0.05$). The KIM-1 levels obtained from the ACR+GA100 and GA100 groups were very close to each other and there was no statistically significant difference between them ($p > 0.05$; Fig. 3).

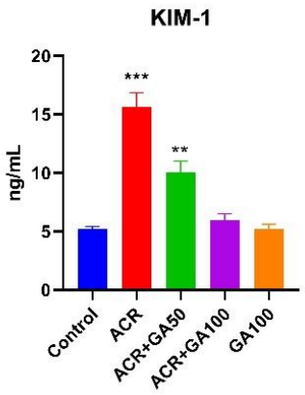

Fig. 3. The effects of acrylamide (ACR) and gallic acid (GA) administrations on kidney injury molecule-1 (KIM-1) level in the experimental groups. There are statistically significant differences between the values expressed with different symbols and the control group. ***$p < 0.01$; ** $p < 0.05$.

Discussion

In this study, the effects of GA on KIM-1, IFN-γ, and PPAR-γ levels in relation to inflammation in kidney tissue due to ACR administration were determined. The KIM-1 and IFN-γ levels were especially effective at high doses of GA. It was observed that these levels were very essential for kidney tissue cells and suppressed due to ACR application. It was observed that GA application together with ACR played an important role in protecting cells by significantly increasing KIM-1, IFN-γ, and PPAR-γ levels.

Kidney injury molecule-1is a promising biomarker of kidney injury. Its expression in proximal renal tubular epithelial cells (mostly S3 segment) is highly elevated in the early stages of acute kidney injury (7), and increased urinary KIM-1 levels are associated with more advanced kidney injury (8). The KIM-1 is a transmembrane glycoprotein with a molecular mass of 104 kDa, a member of the transmembrane immunoglobulin and mucin domain protein family and the immunoglobulin superfamily (9). Normal kidney tissues show trace amounts of KIM-1 expression, whereas increased KIM-1 expression is observed in acute kidney injury resulting from ischemia, hypoxia, or toxicity (8). Increased expression has also been reported in tubulointerstitial nephropathies and polycystic kidney disease (2). The ectodomain of KIM-1 (90 kDa) is cleaved by matrix metalloproteinases and released into the urine, and this mechanism is also up-regulated in proximal tubule injury (10). In a meta-analysis of 11 studies including approximately 3000 patients, Shao *et al.*, estimated the diagnostic sensitivity of KIM-1 in acute kidney injury as 74.00% and specificity as 86.00% (9). However, they also reported that urinary KIM-1 concentrations may be significantly affected by concomitant diseases, such as diabetes, hypertension, and atherosclerosis (9). In our study, the KIM-1 level was significantly higher in the ACR group, indicating that kidney damage had occurred. In the ACR+GA100 group, it was observed that the treatment was well-formed and effective.

Macrophages resident in the kidney have important roles in renal homeostasis and pathology. Macrophages (M1and M2) play a role in acute and chronic kidney injuries by recognizing different types of phenotypes. While M1 macrophages stimulate damage in the kidneys, M2 macrophages provide repair responses to kidney damage. Deletion of PPAR-γ in renal hematopoietic cells enhances the inflammatory response (11). It has been reported that glomerulonephritis similar to autoimmune glomerulonephritis occurs

in mice lacking macrophage expression of PPAR-γ (12). It has also been reported that pioglitazone, a PPAR-γ agonist, suppresses the formation of renal calcium oxalate crystals by suppressing M1 macrophage polarization (13). It has also been reported that it increases differentiation into M2 macrophages (14). Deletion of PPAR-γ from macrophages results in impaired phagocytosis and macrophage polarization and altered lipid utilization (15). Moreover, PPAR-γ agonists suppress the expression of inflammatory factors in monocytes and macrophages by inhibiting NF-γB, transcription activators and AP-1 pathways. The PPAR-γ agonist also blocks the pro-inflammatory effects of IFN-γ by inhibiting JAK/STAT pathways (16). With these effects, PPAR-γ helps to create protective effects, such as anti-inflammatory, anti-oxidant, anti-apoptotic and anti-fibrotic effects in kidney tissue (17). It has been determined that PPAR-γ agonists have a very beneficial effect in a wide variety of kidney diseases, including ischemia/reperfusion injury, diabetic nephropathy, hypertensive nephropathy, experimental glomerulonephritis and chemically induced renal failure (18). In a study, it was observed that GA binds to PPAR-γ receptors and activates them, contributing to the formation of an anti-inflammatory response (19). The IFNs are cytokines that were first identified due to their ability to interfere with viral replication in hosts. They have many functions ranging from the regulation of immune responses associated with autoimmunity to oncogenesis. There are 3 different types. The first of these is type 1 IFNs, including IFN-α and IFN-β, being expressed by innate immune cells. The second, type 2, also called IFN-gamma, is expressed by natural killer cells and T lymphocytes. The third, also known as type 3, IFN-λ, is found distributed in tissues. The IFN-γ binds to IFN-γ receptors 1 and 2, which activate the JAK1 and JAK2 pathways, respectively (20). In one study, it was observed that IFN-γ increased the occurrence of renal damage by inhibiting the development of mesangial cells in the kidneys. In another study, it was determined that GA caused a suppression in IFN-producing cells and exhibited an anti-inflammatory property. In our study, it was determined that GA application had an important role in preventing ACR-induced kidney damage by suppressing IFN-γ expression while supporting PPAR-γ expression in the kidneys.

As a result, ACR application increased the renal inflammatory process and damage. It was observed that the level of KIM-1, an inflammation marker, increased due to ACR application. While the level of IFN-γ increased in the ACR group, the level of PPAR-γ decreased. We determined that GA application is effective in controlling these levels and returning them to normal levels, especially at high doses.

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Investigation of the efficacy of β-caryophyllene against cadmium-induced lung damage in rats

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Abstract

In recent years, increasing industrialization has introduced many different toxic chemicals into our lives. One of the most common and toxic of these chemicals in nature is Cadmium (Cd). Since Cd causes various toxications in many living things, especially humans, many natural compounds are used to reduce its effect. In this study, the protective activity of β-caryophyllene (BCP) against the toxic effect of Cd in rat lung tissues was investigated. In the study, 50 wistar albiona rats were divided into 5 groups; Control, Cd (6.5 mg/kg), BCP200+Cd, BCP400+Cd and BCP400. At the end of the 7-day study, lung tissues of the rats were taken and examined by ELISA, histopathological and immunohistochemical methods. It was observed that Cd caused increases in MDA, 8-OHdG and TNF-α levels and decreases in superoxide dismutase, glutathione and catalase levels in lung tissues, while these levels approached normal values with BCP administration. In the study, it was observed that BCP suppressed the oxidative stress and inflammation caused by Cd in lung tissues. In the light of these results, it was concluded that BCP might be a strong protective agent in Cd-induced lung toxicity.
Keywords: β-caryophyllene, Cadmium, Inflammation, Lung, Oxidative stress

Introduction

Increasing industrialization and the resulting environmental pollution adversely affect public health. In particular, heavy toxic substances emitted into the environment are important in the formation of this danger. One of these metals is Cadmium (Cd). The Cd is a heavy toxic agent widely found in nature (1). Although Cd usually enters the body through the food chain, it also causes serious complications through the respiratory tract (2). Since the half-life of Cd in the body is quite long, it causes various severe complications in tissues (3). It causes oxidative stress and inflammation in tissues by causing ROS increases in various tissues and organs, especially in lung tissue (4). Studies have reported that Cd causes oxidative DNA damage (5) and inflammation (6) in lung tissues, leading to respiratory problems.

The β-caryophyllene (BCP) is a powerful antioxidant substance found in many natural products especially clove oil (7). The BCP, a sesquiterpene, is known to have antioxidant and anti-inflammatory activity against various toxications in lung tissues (8). The BCP, which has wide therapeutic effects, is a natural CB II (Cannabinoid receptor II) antagonist. It has also been found to exhibit antimicrobial, antioxidant, anti-carcinogenic and anti-arthritic activity (9-11). It was observed that BCP showed organo-protective effects

against the harmful effects of drugs, xenobiotics or other chemical toxic substances on liver, kidney, pancreas, intestine and brain (11-13).

In this study, it was aimed to investigate the effects of BCP on some parameters related to oxidative stress and inflammation in Cd-induced lung toxicity in rats and to contribute to the literature.

Materials and Methods

Animals. Fifty adult male Wistar rats (200 ± 20 g) were housed individually in plastic cages on wood chip-type bedding, fed on chow pellet and had free access to water. Randomly, the rats were divided into five groups of Control, Cd (6.5 mg/kg), BCP200+Cd, BCP400+Cd, BCP400 (n=10). Rats were given Cd intraperitoneally for 7 days and BCP intragastrically for 7 days. At the end of the study, all rats were sacrificed under general anesthesia and lung tissues were removed. Some of the lung tissues were placed in 10% buffered formalin solution and some in -800C for histopathologic examinations. Ethical approval was obtained from Atatürk University Animal Experiments Local Ethics Committee (2024/07).

Homogenization of lung tissue. Equal weights of lung tissue samples were placed into screw-capped tubes. The tissues were then homogenized with 1500 µl of phosphate-buffered saline (PBS) using a Magna Lyser homogenizer set at 5000 rpm for around 10 min. After the homogenization process, the samples were centrifuged at 5000 rpm for 10 min. The supernatants were then gently transferred into clean Eppendorf tubes for subsequent analyses.

Oxidative parameters and antioxidant enzymes analysis. Oxidative stress markers and antioxidant enzyme activities were assessed using an ELISA plate reader (Bio-Tek, Winooski, USA) at a wavelength of 450 nm. The parameters measured included malondialdehyde (MDA), superoxide dismutase (SOD), glutathione (GSH), and catalase (CAT) (Sunred, China). These measurements were conducted in accordance with the instructions provided by the respective ELISA kits, using the supernatants previously collected.

Histopathological evaluation. The tissue samples taken at the end of the evaluation were fixed in 10% formaldehyde solution for 48 hr and embedded in paraffin blocks after routine tissue follow-up procedures. Four µm thick sections were taken from each block and the preparations prepared for histopathologic examination were stained with hematoxylin-eosin (HE) and examined by light microscopy (Olympus BX 51, Japan). The sections were evaluated as absent (-), mild (+), moderate (++) and severe (+++) according to histopathologic features.

İmmunohistochemical evaluation. For immunoperoxidase examination, tissue sections taken on adhesive (poly-L-Lysin) slides were deparaffinized and dehydrated. Then primary antibody (8-OHdG Cat. No: sc-66036, Reconstitution Ratio: 1/100, US; TNF-α Cat No: sc-52746, Reconstitution: 1/100, US) were added and incubated according to the instructions for use. 3-3' Diaminobenzidine chromogen was used as chromogen in the tissues. The stained sections were examined by light microscopy (Olympus BX 51, Japan). Sections were evaluated as absent (-), mild (+), moderate (++) and severe (+++) according to their immunopositivity.

Statistical analysis. GraphPad Prism 8.0.2 program was used for statistical analysis in histopathologic examinations and data were evaluated by accepting $p<0.05$ as significant. Nonparametric Kruskal-Wallis test was used to determine group interaction and Mann Whitney U test was used to determine the differences between groups. One-way ANOVA followed by Tukey test was used for the evaluation of ELISA tests.

Results

ELISA findings. The activities of SOD, GSH and CAT were significantly reduced in the Cd group compared to the Control, BCP200+ Cd, BCP400+ Cd and BCP400 groups. Treatment with BCP resulted in a dose-dependent increase in these enzyme activities with a particularly notable effect observed in the high-dose BCP group. Evaluation of MDA levels revealed a significant increase in the Cd group compared to other groups, however, BCP treatment led to a dose-dependent reduction in MDA levels (Fig. 1).

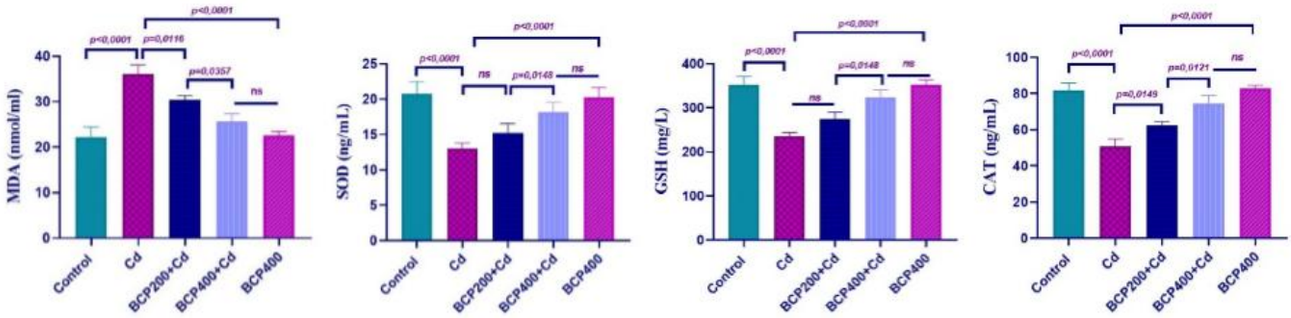

Fig. 1. Bar graphs Illustrate the MDA levels, SOD activity, GSH levels and CAT activity in lung tissues (n = 10). Results are expressed as mean ± SEM.

Histopathological findings. When rat lung tissues were examined histopathologically, normal histologic structure was observed in control (Fig. 2A) and BCP 400 (Fig. 2E) groups. In the Cd group (Fig. 2B), severe mononuclear cell infiltration around the bronchi and bronchioles and severe degeneration and necrosis of bronchial and bronchiolar epithelial cells were observed. In BCP200+Cd (Fig. 2C) and BCP400+Cd (Fig. 2D) groups, these indices were decreased in a dose-dependent manner. Scoring of histopathologic findings and statistical analysis data are presented in Fig. 3.

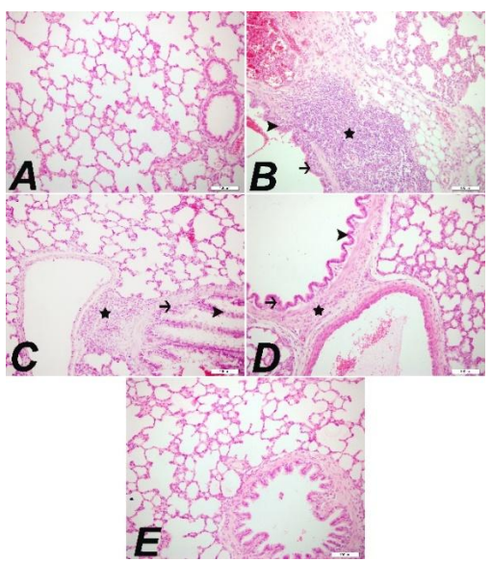

Fig. 2. Lung tissue, Control (A), Cd (B), BCP200+Cd (C), BCP400+Cd (D) and BCP400 (E). Mononuclear cell infiltrates (star), degeneration (arrow) and necrosis (arrowhead) of epithelial cells, H&E, Scale Bar: 70 µm.

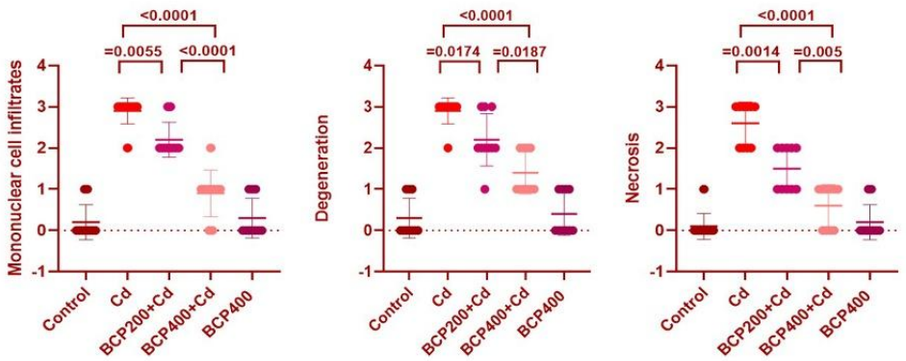

Fig. 3. Scoring of histopathological examinations in lung tissues and statistical analysis data (n=10).

Immunohistochemical findings. When the lung tissues were examined immunohistochemically, 8-OHdG (Fig. 4A and 4E) and TNF-α (Fig. 5A and 5E) expressions were not observed in control and BCP 400 groups. In the Cd group, severe expression of 8-OHdG (Fig. 4B) in the bronchiole and bronchiole epithelial cells and TNF-α (Fig. 5B) in inflammatory cells were detected. In BCP200+Cd (Fig. 4C and 5C) and BCP400+Cd (Fig. 4D and 5D) groups, these expression levels were decreased dose-dependently. Scoring of immuno-histochemical findings and statistical analysis data are presented in Figure 6.

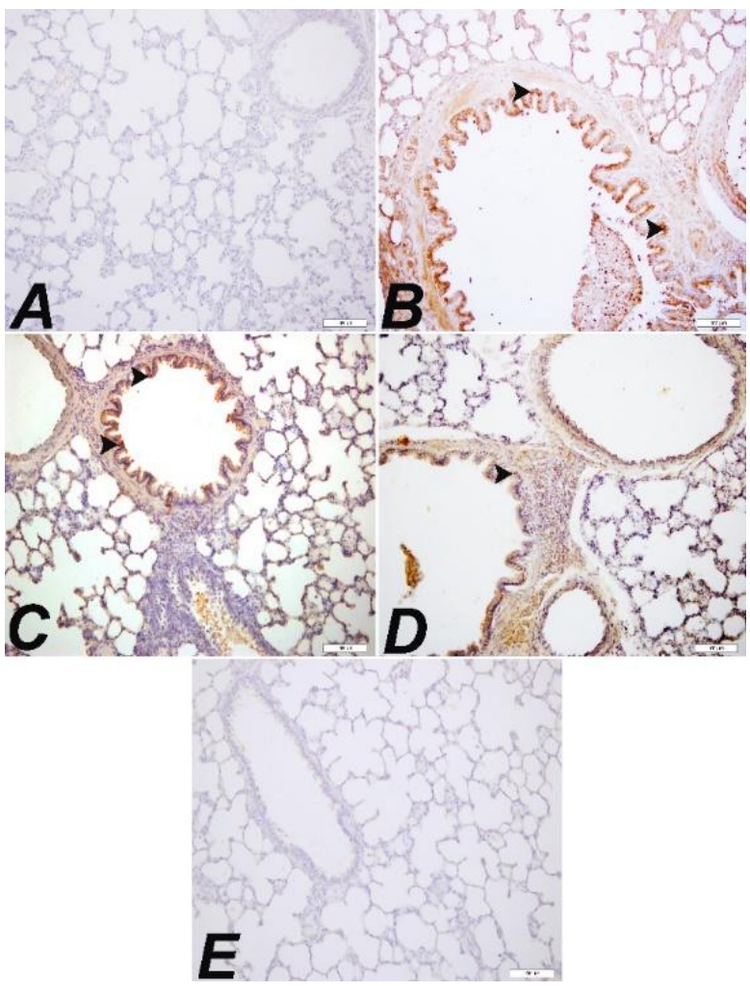

Fig. 4. Lung tissue. Control (A), Cd (B), BCP200+Cd (C), BCP400+Cd (D) and BCP400 (E). 8-OHdG expressions in epithelial cells (arrowhead), IHC-P, Scale Bar: 70 µm.

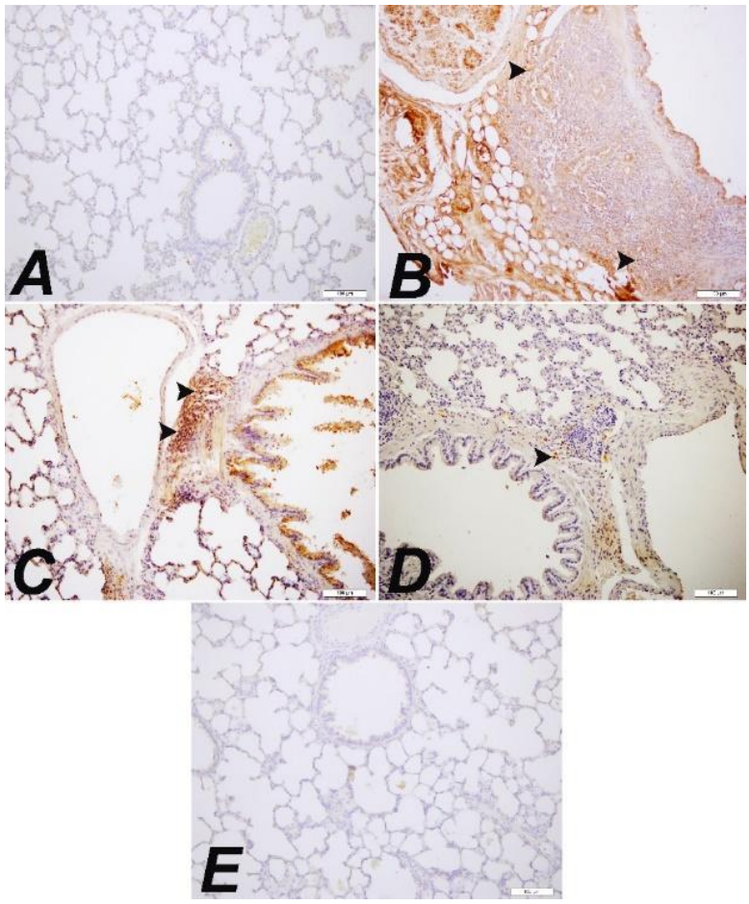

Fig. 5. Lung tissue. Control (A), Cd (B), BCP200+Cd (C), BCP400+Cd (D) and BCP400 (E). TNF-α expressions in infiltrates cells (arrowhead), IHC-P, Scale Bar: 70 µm.

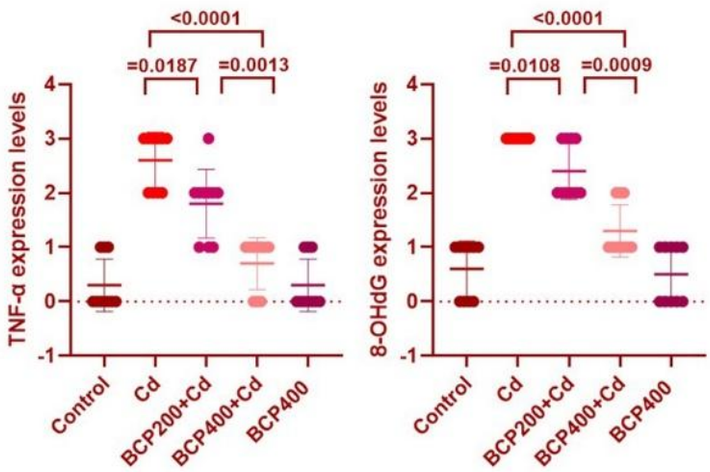

Fig. 6. Scoring of immunohistochemical examinations in lung tissues and statistical analysis data (n=10).

Discussion

Cadmium (Cd) is a heavy metal widely found in nature. This metal causes various toxic effects in many living organisms, especially humans. Cd causes various toxications on many systems in the body, especially the respiratory system (14). The β-caryophyllene (BCP), known as cannaboid 2 receptor, is a powerful antioxidant substance found in many natural compounds, especially ginger. For this purpose, it was used in many studies to reduce the harmful toxic effects of various heavy metals (15). In this study, the efficacy of BCP in Cd-induced respiratory toxicity was investigated for the first time.

In previous studies, it was reported that Cd triggered oxidative stress by causing ROS increases in the respiratory system and accordingly caused increase in MDA and 8-OHdG levels and decrease in SOD, GSH and CAT levels (16). Again, it was revealed that BCP decreased MDA and 8-OHdG levels by suppressing the increase in ROS thanks to its antioxidant activity against some heavy metals and also upregulated SOD, GSH and CAT levels (6). In the present study, it was determined that BCP showed antioxidant activity against Cd-induced oxidative stress in lung tissue.

It has been reported that Cd triggers inflammatory response in lung tissue and accordingly causes the release of proinflammatory cytokines such as TNF-α (7). BCP, on the other hand, has been shown to have anti-inflammatory activity in lung tissue (17). In the present study, it was observed that TNF-α expression levels were increased significantly in the lung tissues of rats due to Cd administration, while BCP administration decreased TNF-α levels and suppressed inflammation.

In conclusion, in this study, it was determined that BCP reduced Cd-induced lung damage by showing antioxidant and anti-inflammatory activity.

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Investigation of the effects of rutin against diabetic nephropathy in rats

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Abstract

Diabetes Mellitus is a disease characterized by excessively high blood sugar levels causing complications in many systems. Diabetes mellitus can lead to disorders in carbohydrate, fat and protein metabolism as well as serious health problems such as neuropathy, retinopathy, nephropathy, peripheral vascular disease, coronary artery disease and atherosclerosis. Diabetic hepatopathies are a common chronic complication affecting approximately 30% of patients with diabetes. Rutin (quercetin rutinoside) is a glycoside of the flavonoid quercetin. Rutin prevents platelet aggregation has anti-inflammatory effects and reduces circulating fat and cholesterol. In this study, we aimed to determine the effects of rutin against nephropathy due to dibetes mellitus. For this purpose, 40 female Sprague-Dawley rats were randomly divided into 4 groups as Control, Diabetes, Diabetes+Metformin and Diabetes+Rutin50 Groups. At the end of the study, rats were sacrificed and kidney tissue samples were taken. In the diabetes group, degeneration, necrosis, glomerular atrophy and hyperemia were observed in the renal tubule epithelium due to hyperglycemia while these pathological conditions were significantly reduced in the routine treatment group. In addition, immunohistochemical and immunofluorescence analyses revealed that the expression levels of TIM and 8-OHdG which were at very high levels due to diabetes,were decreased significantly. As a result of the examinations, it was determined that rutin treatment reduced apoptosis and DNA damage by reducing oxidative damage in kidney tissues and increased tissue resistance by strengthening immunity. This suggested that Rutin had alternative or complementary effects in the treatment of diabetes. We believe that it will shed light on future studies.

Key words: Diabetes mellitus, Oxidative stress, Rutin, TIM

Introduction

Diabetes mellitus (DM), popularly known as diabetes mellitus, is a metabolic disease characterized by chronic hyperglycemia which is caused by insufficient secretion of the hormone insulin secreted by the β cells of the pancreas or decreased effect of insulin in peripheral tissues as a result of many factors caused by genetic and immune structure, leading to disorders in carbohydrate, protein and fat metabolism causing complications in almost all systems. Chronic hyperglycemia can lead to disorders in carbohydrate, fat and protein metabolism as well as serious health problems such as neuropathy, retinopathy, hepatopathy, nephropathy, peripheral vascular disease, coronary artery disease and atherosclerosis. Diabetic neuropathies are one of the most common chronic complications affecting approximately 50% of patients with diabetes (1).

Rutin (quercetin rutinoside) is a glycoside of the flavonoid quercetin. Rutin is widely available in the diet and is used empirically in the treatment of various diseases. Rutin (vitamin P) is a type of flavonoid and is found as a natural glycoside-based flavonoid in the leaves, flowers, seeds and stems of the buckwheat plant. Under certain conditions, rutin and its metabolite quercetin can also act as pro-oxidants. Rutin prevents platelet aggregation, bears anti-inflammatory effects and reduces circulating fat and cholesterol. Many other beneficial effects have also been identified. It has been found to be neuroprotective, alleviate renal apoptosis and inflammation, reduce cancer risk, reduce the incidence of coronary heart disease and increase life expectancy (2).

It is estimated that there are 529 million diabetics in the world according to 2021 data. It is predicted that this number, which was 326 million in 2010, will exceed 1.3 billion in 2050. We aimed to determine the effects of rutin, a strong anti-oxidant against nephropathy, which is among the most important complications of dibetes mellitus by histopathological, immunohistochemical, immunofluorescence methods.

Materials and Methods

Experimental animals will be obtained from Atatürk University Medical Experimental Research Center. In the study, 40 female adult Sprague-Dawley rats weighing 220-250 g were used. The rats were randomly divided into 4 groups as Control, Diabetes, Diabetes+Metformin, Diabetes+Metformin and Diabetes+Rutin50 Groups by weighing and equalizing their average body weights. Diabetes were induced in the experimental groups by applying streptozotocin (STZ) experimentally. The STZ (BioVisionCat No: 1930-1000) 50 mg/kg, single dose (0.5 mL) dissolved in cold citrate buffer (0.1 M, pH 4.5) and administered intra peritoneally. On the 8th day of the administration, after a fasting period of 12 hr, blood taken from the tail veins was measured with a glucometer and rats with fasting blood glucose levels higher than 250 mg/dL was considered as diabetic.

The experimental groups are shown in detail below: Group 1: (Control Group) : Rats in this group (n=10) were injected intraperitoneally (i.p.) with 45 mg/kg single dose of saline. Group 2: Diabetic group: Rats in this group (n=10) were injected with 50 mg/kg single dose of STZ (Sigma, USA) in cold citrate solution with pH: 4.5 in cold citrate buffer and administered i.p. Group 3: Diabetic group + Metformin: Rats in this group (n=10) received a single dose of 50 mg/kg STZ (Sigma, USA) in cold citrate buffer with pH: 4.5 in cold citrate buffer and administered i.p. In addition, 100 mg/kg Metformin (Glifor 1000mg) was administered by gavage at the same time every day. Group 4: Diabetic group + Rutin50: The rats in this group (n=10) received a single dose of 50 mg/kg STZ (Sigma, USA) at pH: 4.5 in cold citrate buffer and administered i.p. In addition, 50 mg/kg freshly prepared Rutin was diluted in saline and administered by gastric gavage every day.

The rats were fed ad-libitum at room temperature of approximately 250C until the time of the study, kept in an environment with a 12-hr light-dark cycle and ventilation. On the 21st day after the induction of diabetes, all rats were sacrificed by cervical dislocation under general anesthesia and kidney tissues were removed for histopathological and immunohistochemical examinations.

On the 28th day of the experimental groups, the animals were sacrificed and kidney tissue samples were taken and determined by histopathologic, immunohistochemical and immunofluorescence methods whether the routine had effects against diabetes-induced nephropathy.

Histolopathologic investigations. Kidney tissue samples were fixed in 10% buffered formaldehyde solution. After fixation, these tissues were passed through graded alcohol and xylene series and embedded in

paraffin blocks. Sections of 5 µm thickness were taken serially from the paraffin blocks at 50-100 micrometer intervals. The sections were stained with Hematoxylin and Eosin and histopathologic changes were evaluated.

Immunohistochemical investigations. All sections taken on adhesive (poly-L-Lysin) slides for immunoperoxidase examination were deparaffinized and dehydrated by passing through xylol and alcohol series. They were then were washed in distilled water for 5 min. Endogenous peroxidase were inactivated by washing with phosphate buffer solution (PBS, pH 7.2) for 5 min and then kept in 3% H2O2 for 10 min. After washing in PBS for 5-10 min, they were incubated for 5 min with Protein block, which was compatible with all primary and secondary antibodies to prevent nonspecific background staining. At the end of the incubation, after the excess of the block solution remaining on the tissue sections was poured, the primary antibody (TIM, Cat. No: sc166785) and PBS in the control group was dripped without washing. In accordance with the primary antibody, they were kept at room temperature for 1 hr or at +4 ˚C for 1 night. Wash with PBS 2 times for 5 min each and incubated with biotinized secondary antibody for 10-30 min at room temperature. The sections were washed again with PBS, incubated in streptavidin-peroxidase for 10-30 min and washed with PBS in the same way. After washing, 3-3' Diaminobenzidine (DAB) chromogen was added to the sections and the sections were kept for 5-10 min. depending on the uptake of chromogen. For ground staining, they were kept in Mayer's hematoxylin for 1-2 min and then washed in tap water. It was passed through alcohol and xylol series, covered with a coverslip and examined with a light microscope (Leica DM 1000).

Statistical analysis. In order to determine the intensity of positive staining, 5 random areas were selected from each image and evaluated in the ZEISS Zen Imaging Software program. Data were statistically described as mean and standard deviation (mean±SD) for % area. Mann-Whitney U test was performed to compare positive immunoreactive cells and immunopositive stained areas with healthy controls. An AP value of <0.05 was considered significant and data were presented as mean ± SD.

Results

Histopathologic findings. Control Group: Normal histologic structure in histopathologic examination of kidney tissues was observed (Fig. 1). Diabetic group: Histopathologic examination of renal tissues revealed that renal tubules epithelium showed severe degeneration and moderate necrosis, interstitial severe hyperemia was observed in the vessels and glomerular membranes in the intervals (Fig. 1). Diabetic + Metformin group: In histopathologic examination of kidney tissues, kidney mild degeneration and necrosis of tubule epithelium, moderate hyperemia of vessels was observed (Fig. 1). A statistically significant difference was detected when compared with the diabetic group were performed. Diabetic + Routine 50 group: Histopathologic examination of renal tissues revealed that the kidney mild degeneration of the epithelium and hyperemia of the vessels (Fig. 1). Diabetic statistically significant difference was found when compared to the Control group.

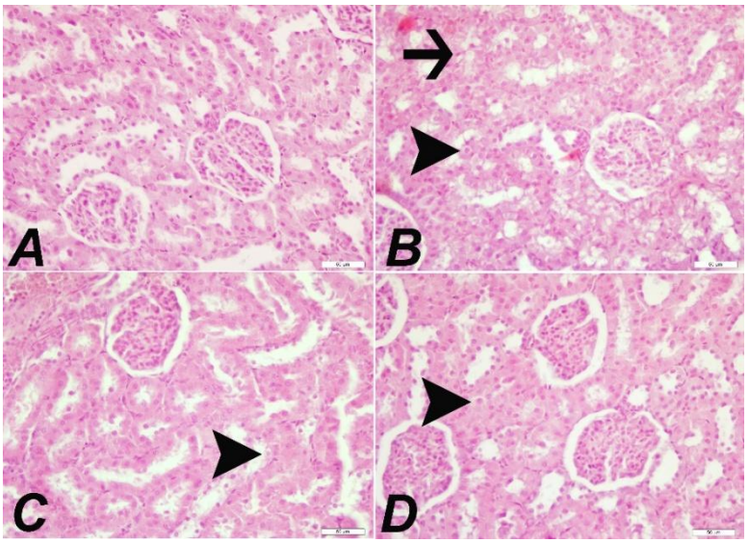

Fig. 1. Kidney tissue, Control (A), Diabetes (B), DM+metformin (C), and DM+Rutin (D). Degeneration (arrowhead) and necrosis (arrow) of tubular epithelial cells, Hematoxylin and Eosin, Scale Bar: 50µm.

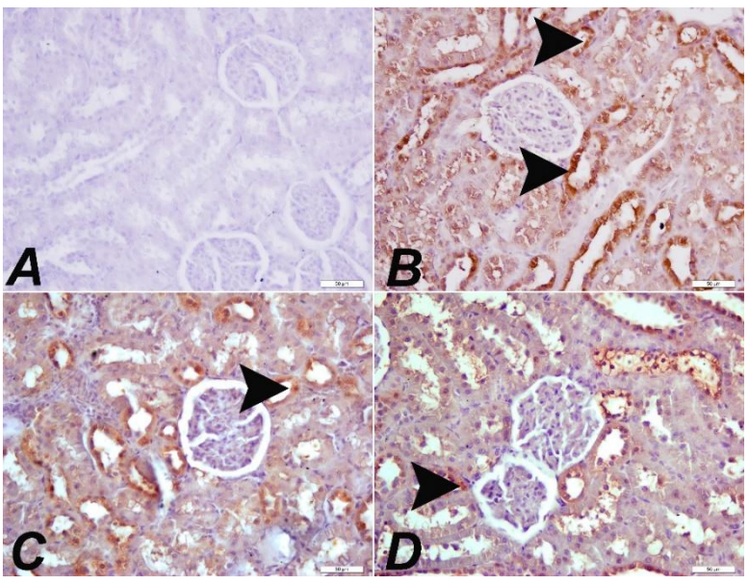

Fig. 2. Kidney tissue, Control (A), Diabetes (B), DM+metformin (C), and DM+Rutin (D). TIM expressions in tubule epithelial cells (arrowheads), IHC-P, Scale Bar: 50µm.

Discussion

Diabetes mellitus is a common widespread disease that has increased in prevalence over the past few decades to become a major public health problem of the twenty-first century. Complications traditionally associated with DM include macrovascular conditions such as coronary heart disease, stroke and peripheral arterial disease and microvascular conditions such as diabetic kidney disease, retinopathy and peripheral neuropathy (1). It has been shown that flavonoids or extracts rich in flavonoids can prevent and treat DM and also improve diabetic complications (2). In the present study, it was observed in histopathological examination that rutin, a flavonoid, minimized kidney damage in diabetic nephropathy.

Diabetic nephropathy, a chronic complication of DM, is directly related to the inflammatory process in the body. Studies show that Tim exhibits various expressions and functions in different diseases (2). However, the effects of Tim in DM progression are still not fully understood. Recent studies suggest that innate rather than adaptive immunity plays an important role in diabetic kidney disease (3). In our study, we observed that

the expression of Tim, an immune system checkpoint molecule, was decreased in diabetic nephropathy in rutin treated animals.

It has been reported that prolonged hyperglycemia leads to overproduction of reactive oxygen species (ROS) and oxidative stress in tissues (4). Overproduction of ROS and oxidative stress can cause cell damage (5). However, ROS overproduction and oxidative stress are widely accepted theories to explain DM (6). These studies highlight the importance of 8-hydroxy-2′-deoxyguanosine (8-OHdG) as a biomarker for oxidative stress and DNA damage in patients with diabetes and the fact that the same biomarker has a very good discriminatory power for the identification of diabetic nephropathy (7). Rutin also helps maintain the level of the biological antioxidant reduced glutathione. Under certain conditions rutin and its metabolite Quercetin may also act as a pro-oxidant (8). In our study, the elevated 8-OHdG expression and levels were observed in rutin treated animals. The increase in oxidative stress in diabetes and its complications were consistent with other studies.

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Effects of zingerone against diabetic hepatopathy in rats

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Abstract

According to estimates by the World Health Organization, there were 422 million people living with diabetes worldwide in 2014, and in 2019, it was reported that 1.50 million people died directly due to diabetes. Diabetes mellitus (DM) can lead to disturbances in carbohydrate, fat, and protein metabolism, along with serious health problems, such as neuropathy, retinopathy, nephropathy, peripheral vascular disease, coronary artery disease, and atherosclerosis. Diabetic hepatopathies are one of the common chronic complications affecting approximately 30.00% of diabetic patients. Zingerone is reported to have strong anti-inflammatory, anti-diabetic, anti-lipophilic, anti-diarrheal, and anti-spasmodic properties, as well as the ability to suppress oxidative stress and aging, with anti-apoptotic and radio-protective effects. In this study, 60 adult female Sprague-Dawley rats (220-250 g) were used and divided into six groups. The experimental groups were formed as control, DM, DM + metformin, DM + Zingerone 25, DM + Zingerone 50, and Zingerone 50. On the 28th day of the study, all groups were sacrificed, and liver tissues were used for histopathological, immunohistochemical, and immunofluorescence analyses. To determine the oxidative damage caused by diabetes in liver tissue, the expression level of 8-hydroxy-2'-deoxyguanosine (8-OHdG) was measured, and the expression level of caspase-3 was measured to assess apoptosis. While the levels of 8-OHdG and caspase-3 increased in the DM group, it was observed that tissue damage decreased in a dose-dependent manner in the Zingerone treatment groups. In this study, it was determined that Zingerone has protective effects against oxidative stress and apoptosis in diabetes-induced hepatopathy in rats.

Keywords: Diabetes, Hepatopathy, Histopathology, Zingerone

Introduction

Diabetes mellitus (DM) is a type of metabolic disease characterized by irregular energy metabolism resulting from deficiencies in insulin secretion, insulin action, or both, leading to hyperglycemia and dyslipidemia (1). The main symptoms of DM are polyuria (frequent urination), polydipsia (dehydration), polyphagia (increased hunger), and weight loss (2). According to the World Health Organization's 2016 report, while 108 million people were diabetic in 1980, it is estimated that 422 million people were diagnosed with DM worldwide by 2014. The DM is also one of the four most common non-communicable diseases and is currently the leading seventh cause of global morbidity and mortality. This high mortality rate is associated with complications, such as progressive diabetic liver disease (DKH) developing in these patients. Examples of different spectra of DKH seen in patients with type 2 DM with persistent hyperglycemia include liver cirrhosis, non-alcoholic fatty liver disease, and hepatic carcinomas (3). The DKHs primarily develop because

of hyperglycemia, hypertriglyceridemia, and insulin resistance. In the absence of sufficient cyto-protective molecules, these co-factors induce inflammation, oxidative damage, and ultimately necrosis or apoptosis.

In diabetes, chronic hyperglycemia creates an environment facilitating the formation of free radicals while depleting endogenous anti-oxidant reserves, ultimately leading to oxidative tissue damage (4). Additionally, increased free radical levels activate the transcription of pro-inflammatory cytokines and pro-apoptotic genes, mediating chronic inflammatory responses and hepatocyte death, respectively (5). Abnormally high glucose levels can induce apoptosis by activating Bax-caspase proteases altering mitochondrial function (6).

Therapeutic strategies that can effectively prevent or reverse the negative effects of prolonged oxidative stress and inflammation in DM may be highly beneficial in preventing diabetic complications, such as DKH. Over the past decades, significant efforts have been made to manage diabetes. These efforts have led to the discovery of various orthodox medications currently used to achieve better glycemic control and thus, prevent DM complications. The major disadvantage of oral anti-diabetic agents is their inability to effectively treat diabetes; moreover, they are associated with increasing side effects (7).

In our country, it is known that various regions have resorted to herbal treatments for diabetes management, and scientific research is being conducted on the hypoglycemic effects of medicinal plants. Some of these herbal applications help control blood glucose levels, while others lead to positive changes in blood lipid profiles. Herbal products may contain active components that can act through various mechanisms to reduce diabetic symptoms, providing multi-faceted benefits (8) and thus, appearing as promising therapeutic pathways.

Zingerone [4-(4-hydroxy-3-methylphenyl) butan-2-one], also known as vanillylacetone, is considered a significant component of ginger and contributes to the sweet flavor of cooked ginger. It is recognized as a botanical dietary supplement in North America and Europe. Zingerone is a crystalline compound being poorly soluble in water and soluble in ether. It lacks any spiciness or sharpness typically associated with ginger, suggesting it may be produced from the degradation of ginger rather than directly sourced from it. Chemically, zingerone resembles other flavoring compounds, like vanillin and eugenol (9). Previous studies have reported that zingerone possesses various pharmacological potentials, including anti-cancer, anti-oxidant, anti-microbial, anti-inflammatory, and anti-apoptotic properties (10). Treatments involving zingerone have shown to suppress oxidative stress and aging (11).

Materials and Methods

Animals. Sixty adult female Sprague-Dawley rats (220-250 g) were housed individually in plastic cages on wood chip-type bedding, fed with chow pellet, and had free access to water. The rats were weighed and randomly divided into six groups, including control, diabetes, diabetes + metformin, diabetes + zingerone 25, diabetes + zingerone 50, and zingerone 50, ensuring that the average body weights are equal. Diabetes was induced experimentally in the diabetic groups by administering streptozotocin. For this purpose, streptozotocin (BioVision Cat No: 1930-1000) was dissolved in cold citrate buffer (0.10 M; pH: 4.50) and administered at a dose of 50 mg/kg as a single dose (0.50 mL) *via* intra-peritoneal injection. On the 8th day following treatment, after a 12-hr fasting period, blood was collected from the tail veins and analyzed using a glucometer. Rats with fasting blood glucose levels exceeding 250 mg/dL were considered diabetic. Ethical

approval was obtained from Atatürk University Animal Experiments Local Ethics Committee, Erzurum, Türkiye (2024/02).

Sampling. On the 21st day after diabetes induction, all rats were euthanized under general anesthesia *via* cervical dislocation. Liver tissues were collected for histopathological, immunohistochemical, and immunofluorescence examinations.

Histopathological evaluation. Liver tissue samples were fixed in a 10.00% buffered formaldehyde solution. After fixation, the tissues were passed through graded alcohol and xylene series, and embedded in paraffin blocks. Sections of 5 µm in thickness were obtained serially at intervals of 50-100 µm. The sections were stained with hematoxylin and eosin to evaluate histopathological changes.

Immunohistochemical evaluation. For immunoperoxidase staining, all sections taken onto adhesive (poly-L-lysine) slides were deparaffinized and dehydrated through xylene and alcohol series. The slides were then washed in distilled water for 5 min. Following this, they were rinsed with phosphate-buffered saline (PBS; pH: 7.20) for 5 min and treated with 3.00% H_2O_2 for 10 min to inactivate endogenous peroxidase. After washing with PBS for 5-10 min, the sections were incubated with a protein block compatible with all primary and secondary antibodies for 5 min to prevent non-specific background staining. After the incubation, excess blocking solution was removed from the tissue sections without washing, and primary antibody (8-hydroxy-2'-deoxyguanosine [8-OHdG]) was applied, with PBS used for the control group. The primary antibody was incubated at room temperature for 1 hr or overnight at +4 ˚C. Following this, the sections were washed twice with PBS for 5 min each and incubated with a biotinylated secondary antibody at room temperature for 10-30 min. After another wash with PBS, the sections were treated with streptavidin-peroxidase for 10-30 min and subsequently washed with PBS again. After washing, the sections were treated with 3,3'-diaminobenzidine chromogen, waiting for 5-10 min according to the color development. For counterstaining, the sections were held in Mayer's hematoxylin for 1-2 min and then, rinsed with tap water. Finally, the sections were processed through alcohol and xylene series, mounted with a coverslip, and examined under a light microscope (Leica DM 1000). The sections were evaluated for immunopositivity as negative (-), mild (+), moderate (++), and strong (+++).

Immunofluoresence evaluation. For immunofluorescent examination, tissue sections taken onto adhesive (poly-L-lysine) slides were deparaffinized and dehydrated. Endogenous peroxidase was inactivated by treating the sections with 3.00% H_2O_2 for 10 min. The tissues were then boiled in a 1.00% antigen retrieval solution (citrate buffer; pH: 6.10) and allowed to cool to room temperature. To prevent non-specific background staining, the sections were incubated with a protein block for 5 min. Following this, the primary antibody (caspase-3, Cat No: ab32351; Dilution ratio: 1/100; US) was applied and incubated according to the manufacturer's instructions. An immunofluorescent secondary antibody (FITC, Cat No: ab6785; Dilution ratio: 1/1000) was then applied and incubated in a dark environment for 45 min, followed by washing with distilled water. Next, DAPI mounting medium (Cat No: D1306; Dilution ratio: 1/200; UK) was added to the sections and allowed to incubate in the dark for 5 min before covering with a coverslip. The stained tissues were examined under a fluorescence microscope (Zeiss AXIO, Germany) and evaluated for immunopositivity as negative (-), mild (+), moderate (++), and strong (+++).

Statistical analysis. To determine the intensity of positive staining from the images obtained, five random areas were selected from each image and evaluated using the ZEISS Zen Imaging Software. The data were statistically described in terms of mean and standard deviation (mean ± SD) for the area percentage. To compare the positively stained immunoreactive cells and immunopositive areas with healthy controls, the Mann-Whitney U test was performed. A *p*-value of < 0.05 was considered statistically significant, and the data were presented as mean ± SD.

Results

Histopathological findings. Control: When the liver tissues were examined histopathologically, normal histological appearance was observed (Fig. 1). Diabetes: When liver tissues were examined histopathologically, severe degeneration and necrosis in hepatocytes and severe hyperemia in vessels were observed (Fig. 1). Diabetes + metformin: When liver tissues were examined histopathologically, mild degeneration in hepatocytes and hyperemia in vessels were observed (Fig. 1). Diabetes + zingerone 25: When liver tissues were examined histopathologically, moderate degeneration in hepatocytes, mild necrosis, and hyperemia in vessels were determined (Fig. 1). Diabetes + zingerone 50: When liver tissues were examined histopathologically, mild degeneration in hepatocytes and hyperemia in vessels were observed (Fig. 1). Zingerone 50: When the liver tissues were examined histopathologically, it was determined that they had a normal histological appearance (Fig. 1). Scoring of histopathologic findings and statistical analysis data are presented in Figure 2.

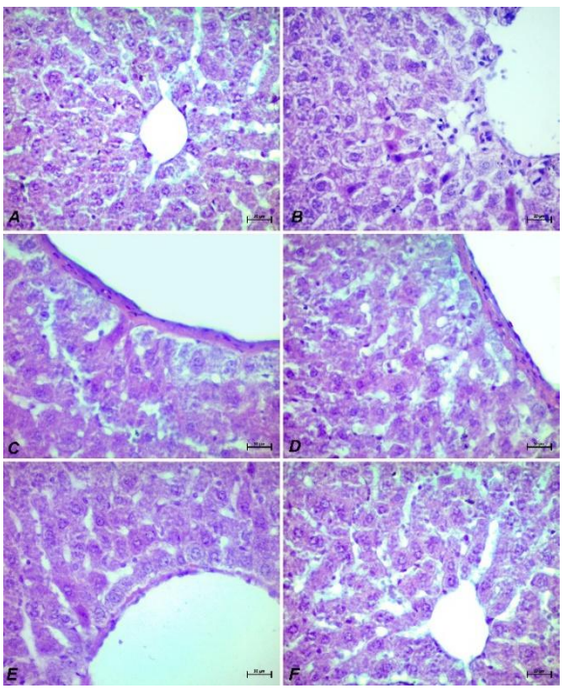

Fig. 1. Cross-sections of liver tissue in different experimental groups. Histopathological changes can be seen in control (**A**), diabetes (**B**), diabetes + metformin (**C**), diabetes + zingerone 25 (**D**), diabetes + zingerone 50 (**E**), and zingerone 50 (**F**) groups (Hematoxylin and Eosin staining, Bar: 10 µm).

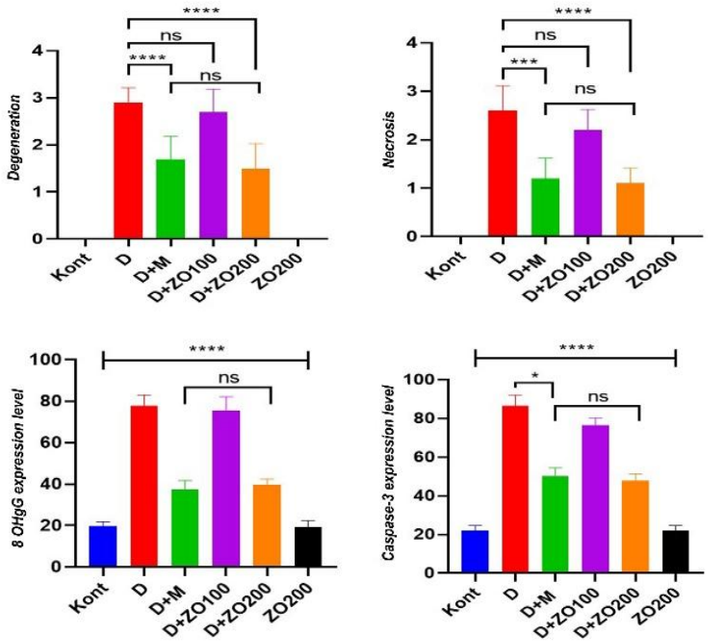

Fig. 2. Scoring of histopathological, immunohistochemical and immunofluorescence examinations in liver tissues of different experimental groups and statistical analysis data. **** $p < 0.0001$, *** $p < 0.001$, and * $p < 0.01$. ns: Non-standard deviation; Kont: Control; D: Diabetes; M: Metformin; ZO: Zingerone.

Immunohistochemical and immunofluorescence findings. Control: When liver tissues were analyzed immunohistochemically and immunofluorescently, caspase-3 and 8-OHdG expressions were evaluated as negative (Figs. 3 and 4). Diabetes: When liver tissues were examined, severe intra-cytoplasmic 8-OHdG expression was detected in hepatocytes by immunohistochemical staining, and severe caspase-3 intra-cytoplasmic expression was detected by immunofluorescent staining (Figs. 2 and 3). Diabetes + metformin: When liver tissues were analyzed, immunohistochemical staining revealed mild intra-cytoplasmic 8-OHdG expression in hepatocytes. In immunofluorescent staining, mild intra-cytoplasmic caspase-3 expression was detected (Figs. 3 and 4). Diabetes + zingerone 25: When liver tissues were analyzed, immunohistochemical staining showed moderate intra-cytoplasmic 8-OHdG expression in hepatocytes. Immunofluorescent staining showed moderate intra-cytoplasmic caspase-3 expression (Figs. 3 and 4). Diabetes + zingerone 50: When liver tissues were examined, mild intra-cytoplasmic 8-OHdG expression was detected in hepatocytes in immunohistochemical staining, and severe intra-cytoplasmic caspase-3 expression was detected in immunofluorescent staining (Figs. 3 and 4). Zingerone 50: When liver tissues were analyzed immunohistochemically and immunofluorescently, caspase-3 and 8-OHdG expressions were evaluated as negative (Figs. 3 and 4). Scoring of immunohistochemical and immunofluorescence findings and statistical analysis data are presented in Figure 2.

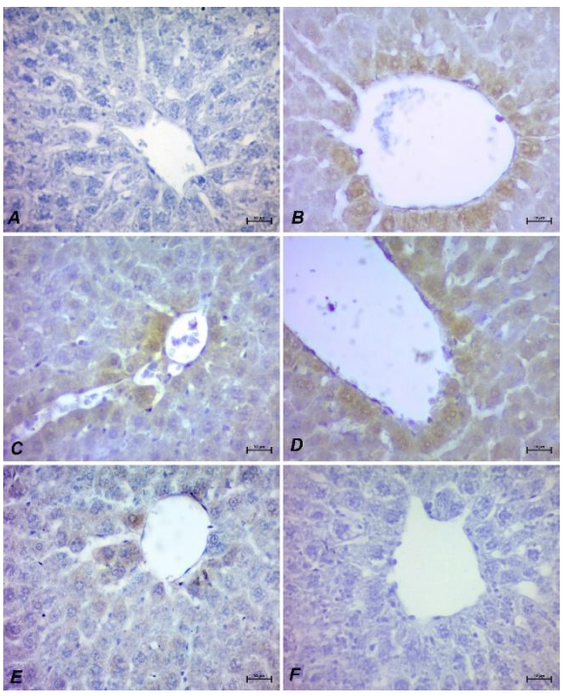

Fig. 3. Cross-sections of liver tissue in different experimental groups. The 8-OHdG expressions in hepatocytes of control (**A**), diabetes (**B**), diabetes + metformin (**C**), diabetes + zingerone 25 (**D**), diabetes + zingerone 50 (**E**), and zingerone 50 (**F**) groups (immunohistochemical staining, Bar: 10 µm).

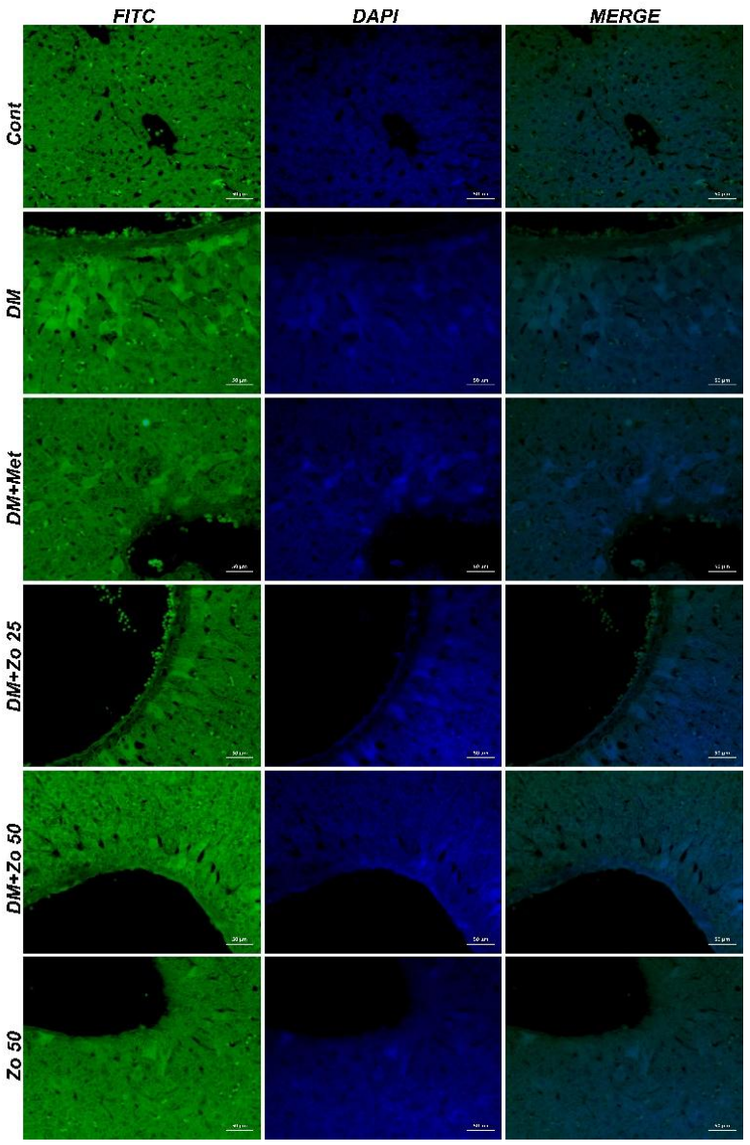

Fig. 4. Cross-sections of liver tissue in different experimental groups. Caspase-3 expressions in hepatocytes of control (Cont), diabetes (DM), DM + metformin (Met), DM + zingerone (Zo) 25, DM + Zo 50, and Zo 50 groups (immunofluorescent staining, Bar: 50 µm).

Discussion

Diabetes mellitus is an endocrine and metabolic disease characterized by disruptions in the metabolism of carbohydrates, lipids, and proteins. Chronic hyperglycemia can lead to imbalances in carbohydrate, fat, and protein metabolism, which in turn can cause serious health issues, such as neuropathy, retinopathy, hepatopathy, nephropathy, peripheral vascular disease, coronary artery disease, and atherosclerosis. Ginger contains approximately 9.25% vanillylacetone, a powerful anti-oxidant, anti-inflammatory, anti-cancer, and anti-microbial compound known as zingerone, belonging to the phenolic alkanone group. Due to these properties, numerous studies have been conducted on it in recent years. This study investigated the protective effect of zingerone against experimentally induced diabetes-related hepatopathy.

Zingerone, playing a significant role in the anti-oxidant properties of ginger, has been shown to have a healing effect on various tissue damages (12). Zingerone eliminates harmful oxidation agents, such as free radicals, reactive oxygen species (ROS), and peroxides, thereby preventing the destruction of damaged tissue caused by cell apoptosis (13). In this study, since zingerone was thought to be effective against diabetes-induced tissue damage, liver tissues were examined in terms of caspase-3, an apoptotic damage marker. It was demonstrated that zingerone treatment significantly reduced caspase-3 levels depending on the dose administered.

Some studies have reported that DM leads to oxidative stress in the liver by increasing ROS level, which in turn raises 8-OHdG levels (14). Zingerone, a compound playing a key role in the anti-oxidant properties of ginger, has shown a healing effect on various tissue damages. Zingerone reduces oxidative stress by eliminating harmful oxidation agents, such as free radicals and ROS (15). In this study, the efficacy of zingerone, a powerful anti-oxidant with additional anti-hyperglycemic, anti-lipophilic, anti-diabetic, and anti-apoptotic effects, in treating oxidative stress-induced tissue damage in diabetic hepatopathy was investigated. The 8-OHdG levels in liver tissues were evaluated, and when the findings of the diabetic and treatment groups were compared, it was determined that zingerone exhibited anti-oxidant activity in a dose-dependent manner.

In conclusion, it was determined that zingerone inhibits oxidative stress, apoptosis, and DNA and RNA damages through its anti-oxidant and anti-apoptotic activities in cases of diabetes-induced hepatopathy, thereby exhibiting a hepato-protective effect. Therefore, zingerone is considered a potential hepato-protective agent against diabetes-related complications.

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Efficacy of syringic acid against cadmium-induced testicular damage in rats

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Abstract

Cadmium (Cd) is a toxic heavy metal with strong toxicity and one of the most serious environmental pollutants. The Cd is defined as a toxic heavy metal because it has no biological activity and has serious side effects. The structures, and physiological and biochemical functions of various organs, such as liver, kidney, lung, pancreas, testis, placenta, and bone may be affected by Cd. Syringic acid (SA) is a non-flavonoid phenolic compound in the hydroxybenzoic acid derivative, being frequently found in fruits and vegetables. In the present study, Cd-induced testicular damage and the effectiveness of SA against this damage were investigated. For this purpose, 50 *Wistar* rats were randomly divided into five groups, including control, Cd, SA50+Cd, SA100+Cd, and SA100. At the end of the seven-day study, the testicular tissues of the rats were placed in 10.00% buffered formalin solution and examined histopathologically. At the end of the examinations, it was determined that severe degeneration and necrosis were observed in spermatocytes due to Cd, while this damage was reduced with SA application. Further, it was observed that 8-OHdG and caspase 3 levels in testicular tissues increased significantly due to Cd, while SA was found to reduce these parameters depending on the dose. As a result of the study, it was concluded that SA may be a reliable protective agent against Cd-induced testicular damage.

Keywords: Cadmium, Histopathology, Immunohistochemistry, Syringic acid

Introduction

The heavy metal cadmium (Cd) is an important toxicant associated with many industrial processes. Occupational exposure to this heavy metal, accumulating slowly in the body, usually occurs during the manufacture of batteries and pigments or in mines, while the general population is exposed by inhalation from the atmosphere or through contaminated drinking water and food. However, due to the high concentrations of Cd in cigarettes, exposure is doubled by active or passive smoking. Exposure to Cd has been associated with numerous harmful effects. The structures, and physiological and biochemical functions of various organs, such as liver, kidney, lung, pancreas, testis, placenta, and bone may also be affected by Cd (1). The Cd can cause direct cytotoxic effects, and apoptotic or necrotic events. One of the organs directly affected by Cd is the testis and it causes infertility by inducing various changes in testicular histo-architecture. It weakens spermatogenesis and negatively affects spermatid development. It induces apoptosis in Sertoli cells and may cause morphological damage in Leydig cells. In addition, some studies have shown that Cd damages the blood-testis barrier, disrupts tight junction complexes, and causes failure in spermiogenesis. Oxidative stress due to

the formation of free radicals is the main cause of Cd toxicity (2). Free oxygen radicals disrupt cell membrane structures by stimulating lipid peroxidation, and lipid peroxidation has a key role in male infertility and sperm dysfunction. Anti-oxidants are compounds preventing the formation of free radicals and reacting with existing radicals to prevent their transformation into more harmful forms (3).

Syringic acid is a non-flavonoid phenolic compound in the hydroxybenzoic acid derivative, being frequently found in fruits and vegetables (4). Syringic acid exhibits several pharmacological properties through its anti-oxidant, anti-proliferative, anti-endotoxic, anti-carcinogenic, and neuroprotective activities (5). In this study, it was aimed to reveal the toxic effects caused by Cd in rat testicular tissue and the protective mechanism of SA against these harmful effects.

Materials and Methods

In the study, 50 male Wistar rats (weight: 220-250 g and age: 10-12 weeks) were used. They were obtained from ATADEM Experimental Medicine Application and Research Centre. Rats were housed under standard laboratory conditions (24-25 °C constant temperature, 45-50% humidity, and 12 hr light-12 hr dark). Water and feed were provided *ad libitum*. The rats were randomly divided into five groups with 10 animals in each group, including control, Cd, SA50+Cd, SA100+Cd, and SA100. The Cd and SA doses were determined from previous studies.

Experimental groups. Control: Water was administered orally for 7 days. Cd: It was administered intra-peritoneally for 7 days at a dose of 25 mg/kg. Cd + SA50: The SA (50 mg/kg) was administered orally half an hr after intra-peritoneal administration of Cd (25 mg/kg) for 7 days. Cd + SA100: The SA (100 mg/kg) was administered orally half an hr after intra-peritoneal administration of Cd (25 mg/kg) for 7 days. SA100: It was administered orally for 7 days at a dose of 100 mg/kg. On the 8^{th} day, all rats were sacrificed by cervical dislocation under general anesthesia, and testicular tissues were removed for histopathological and immunohistochemical examinations.

Histopathological examination. Tissue samples taken at the end of the experiment were preserved in 10.00% formaldehyde solution for 48 hr and embedded in paraffin blocks after routine tissue follow-up procedures. From each block four mm thick sections were taken and the preparations prepared for histopathological examination were stained with hematoxylin and eosin and examined by light microscopy (Olympus BX 51, Japan). Sections were classified according to the histopathological features as absent (-), mild (+), moderate (+++), and severe (++++).

Immunohistochemical investigation. Tissue taken on adhesive (poly-L-lysin) slides for immunoperoxidase examination sections were deparaffinized and dehydrated. Then, they were kept in 3.00% H_2O_2 for 10 min and endogenous peroxidase was inactivated. The tissues were then boiled in 1.00% antigen retrieval solution (citrate buffer; pH: 6.10) and allowed to cool at room temperature. The sections were incubated with protein block for 5 min to prevent ground staining. Then, primary antibody (caspase 3 and 8-OHdG;Reconstitution ratio: 1/100; US) was used and incubated according to the instructions for use. The 3-3'-diaminobenzidine was used as a chromogen in the tissues. Stained sections were examined by light microscopy (Zeiss Axio, Germany).

Statistical analysis. The data were evaluated by GraphPad Prism 8.0.2 software for statistical analysis and $p < 0.05$ was considered significant. Duncan test was used for comparison between groups. Non-parametric test was used to determine group interaction. Kruskal-Wallis and Mann Whitney U tests were used to determine the differences between groups. Immunohistochemical staining images were obtained to determine the intensity of positive staining; five random fields from each picture were selected and evaluated in Zeiss Zen Imaging Software. The analyzed data were statistically expressed as mean and standard deviation.

Results

Histopathological findings. Control: When testicular tissues were examined histopathologically, normal histological appearance was observed (Fig. 1). Cd: When testicular tissues were examined histopathologically, severe thinning of the seminiferous tubules walls due to the spermatocytes degeneration and necrosis, inter-tubular spaces edema, hemorrhage, and hyperemia were observed (Fig. 1). SA50 + Cd: When testicular tissues were examined histopathologically, mild thinning of the seminiferous tubules walls due to the spermatocytes degeneration and necrosis, interstitial mild edema, and moderate hyperemia were seen (Fig. 1). SA100 + Cd: When testicular tissues were examined histopathologically, spermatocytes mild degeneration and mild hyperemia were observed (Fig. 1). SA100: When testicular tissues were examined histopathologically, normal histological appearance was found (Fig. 1).

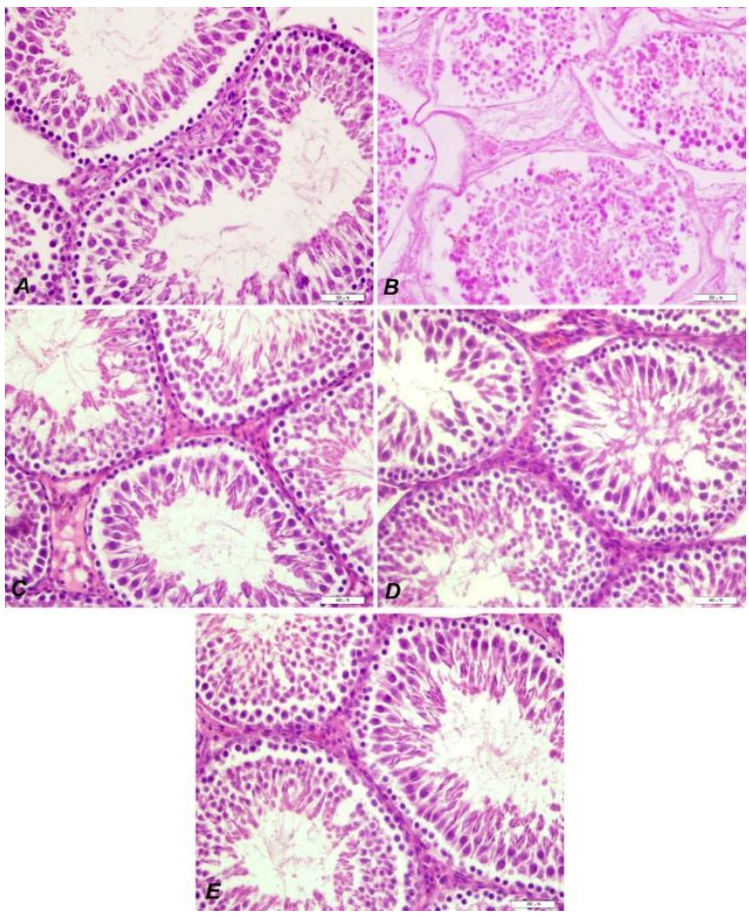

Fig. 1. Testicular tissue cross-sections in different experimental groups. Histopathological findings can be seen in control (**A**), cadmium (**B**), Syringic acid 50 + cadmium (**C**), Syringic acid 100 + cadmium (**D**), and Syringic acid 100 (**E**) groups (Hematoxylin and Eosin staining, bar: 50 μm).

Immunohistochemical results. Control: When testicular tissues were examined immunohistochemically, caspase 3 and 8-OHdG expressions were negative (Fig. 2). Cd: When testicular tissues were examined immunohistochemically, severe caspase 3 and 8-OHdG expressions were determined in the seminiferous tubules wall and spermatocytes cytoplasm (Fig. 2). SA50 + Cd: When testicular tissues were examined immunohistochemically, moderate caspase 3 and 8-OHdG expressions were detected in the spermatocytes cytoplasm (Fig. 2). SA100 + Cd: When testicular tissues were examined immunohistochemically, mild caspase 3 and 8-OHdG expressions were observed in the cytoplasm of spermatocytes (Fig. 2). Moreover, a statistically significant difference was detected compared to the Cd group. SA100: When testicular tissues were examined immunohistochemically, caspase 3 and 8-OHdG expressions were negative (Fig. 2).

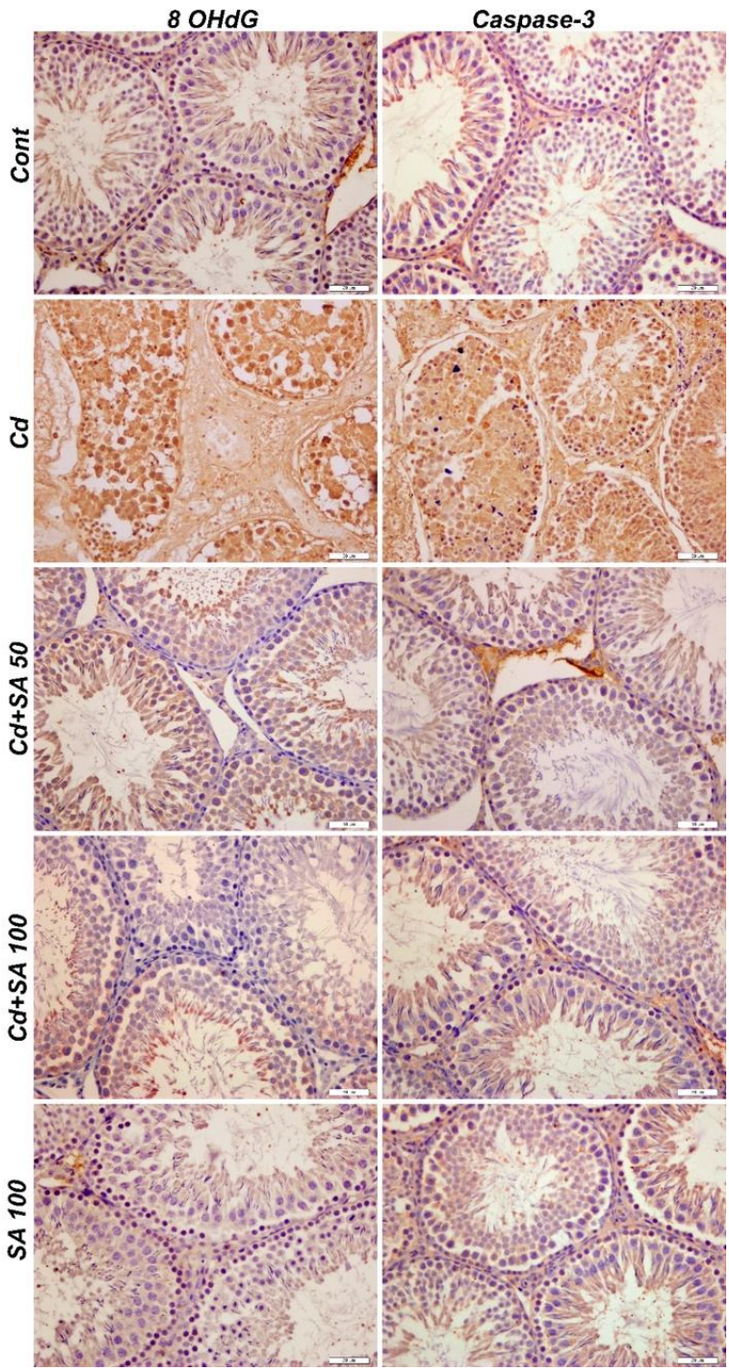

Fig. 2. Testicular tissue cross-sections in different experimental groups. The 8-OHdG and caspase 3 expressions levels can be observed in spermatocytes and seminiferous tubules wall (immunohistochemical staining, bar: 50μm). Cd: Cadmium; SA: Syringic acid; Cont: Control.

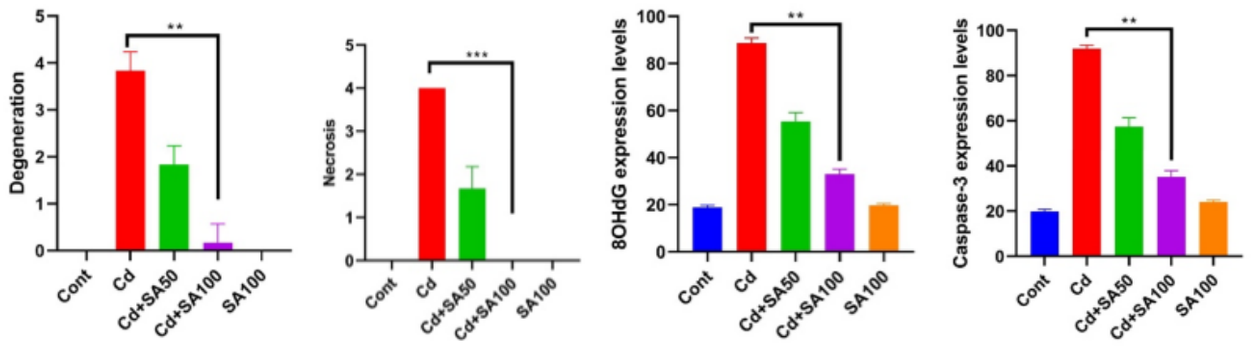

Fig. 3. Scoring of histopathological and immunohistochemical findings in testicular tissue of different experimental groups. *** $p < 0.001$; ** $p < 0.01$. Cd: Cadmium; SA: Syringic acid; Cont: Control.

Discussion

Cadmium is known to cause various complications in testicular tissue. In studies, it has been reported histopathologically that Cd causes severe degenerative and necrotic changes in testicular tissue, especially in spermatocytes (2). It has also been demonstrated that Cd has negative effects on spermatogenesis by causing these damages especially in spermatocytes. On the other hand, SA has been reported to suppress degenerative and necrotic changes in testicular tissue caused by toxic agents in some studies. In the present study, it was observed that Cd caused severe degeneration and necrosis in spermatocytes in testicular tissue and SA application prevented this damage picture.

Oxidative stress is a pathological process occurring due to excessive increase of free oxygen radicals (ROS) in the body and causes negative effects on many tissues, especially testicular tissue. The 8-OHdG is the most widely used biomarker to show DNA damage caused by oxidative stress in cells (6). In many studies, it has been reported that Cd also triggers oxidative stress by causing ROS increases in testicular tissue and causes increases in 8-OHdG expression levels (6). Syringic acid, which has proven to have anti-oxidant activity, has also been shown to suppress oxidative stress in testicular tissue caused by various complications (7). In the present study, it was determined that SA decreased 8-OHdG levels by showing anti-oxidant activity against Cd induced oxidative stress and 8-OHdG expression levels elevation in testicular tissues (8). Apoptosis is known as programmed cell death, but some toxic agents are known to interfere with this function and cause it to be pathological (8). The Cd is one of these toxic agents. Studies have shown that Cd is effective in the apoptotic process in many tissues and organs, especially in testicular tissue in the body. Although anti-apoptotic activity of SA has been reported in many studies, there is no study investigating its activity against Cd-induced apoptosis in spermatocytes. In the present study, it was observed that apoptosis occurred by increasing caspase 3 expression levels in spermatocytes and Leydig cells in Cd-induced testicular toxicity (9). It was determined that these expression levels were down-regulated by SA application. With this results, it was revealed that SA suppressed Cd-induced oxidative stress and apoptosis in rats testicular tissue in this study.

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Effects of quercetin against deoxynivalenol induced adverse effects on intestinal epithelial cells

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Abstract

The HCT116, a human colon cancer cell line often used in colon cancer research, was employed to investigate the protective effects of quercetin, an anti-oxidant present in various foods, against deoxynivalenol's harmful effects on these cancer cells. The study exposed the cells to increasing quercetin concentrations before simultaneous exposure to quercetin and deoxynivalenol. Cell viability was assessed using the MTT test, and oxidative and nitrosative stress levels were measured. Results showed a significant decrease in cell survival when exposed to deoxynivalenol compared to the control, while cells treated with quercetin exhibited notably higher survival rates. Groups treated with 50 and 100 μM quercetin demonstrated the ability to decrease malondialdehyde levels and enhance total anti-oxidant capacity compared to the deoxynivalenol control group. Moreover, various concentrations of quercetin were found to lower total lipid peroxidation levels in comparison with deoxynivalenol control group. Overall, quercetin exhibits the potential to mitigate the effects of deoxynivalenol in HCT-116 cell lines and may be considered for further investigation as a supplementary agent in the context of the aforementioned cancers.

Keywords: Deoxynivalenol, Epithelial cell, Intestine, Mycotoxin, Quercetin

Introduction

Mycotoxins, produced by fungi, pose a threat to humans and animals as prevalent contaminants in food supplies. Ingesting mycotoxin-contaminated food can have serious health effects and economic consequences. Over 300 types of mycotoxins have been identified, including deoxynivalenol (DON), which can cause symptoms like appetite loss, nausea, and cell death in blood progenitor cells, affecting the production of essential biomolecules, such as proteins, DNA, and RNA (1).

Quercetin, a common anti-oxidant found in fruits, vegetables, and grains, plays a vital role in protecting the body from free radical damage. Its anti-oxidant properties can help reduce inflammation, allergies, and symptoms of high blood pressure. Additionally, quercetin shows potential in lowering the risks of heart disease, cancer, and brain disorders. Flavonoids like quercetin have strong anti-oxidant effects, making them effective in combating the effects of mycotoxins. Quercetin's anti-oxidant properties suggest it may offer protection against the harmful effects of mycotoxins (2).

Considering the information provided, the objective of this study was to explore the potential impact of quercetin to counteract the adverse effects of the mycotoxin (DON) in intestinal epithelial cells (HCT-116). The findings from this investigation may serve as a referenced resource for further research and subsequent studies, particularly in the context of cancer patients.

Materials and Methods

Cell culture. The study involved culturing colon epithelial cells (HCT-116) in Dulbecco's Modified Eagle Medium supplemented with fetal bovine serum and penicillin-streptomycin. The cells were grown to 80.00% confluence, sub-cultured, and seeded in various culture vessels. Different concentrations of quercetin and mycotoxin (DON) were introduced into separate wells for testing. The substances were initially prepared in high concentrations with dimethyl sulfoxide (DMSO) and then, diluted in the cell culture medium. The cells were treated first with various quercetin concentrations for 24 hr, followed by exposure to both quercetin and mycotoxin for another 24 hr, with the highest quercetin concentration used as a control group.

MTT. In this study, 10^4 cells were initially seeded in a 96-well plate and incubated for 24 hr at 37 ˚C in a CO_2 incubator to allow them to adhere and proliferate. The cells were then exposed to varying concentrations of quercetin and mycotoxin for 24 hr. The MTT was added to the wells, and after a 4-hr incubation, the formazan crystals were dissolved with DMSO, creating a purple solution. The intensity of the purple color was measured using an ELISA reader at a wavelength of 570 nm to assess cell viability.

Malondialdehyde (MDA). In the MDA test procedure, the sample (cell supernatant) was initially diluted with distilled water in a test tube. This mixture was further diluted with a reagent solution containing trichloroacetic acid and thiobarbituric acid (TBA). The combined solution was heated in a bain-marie at 100 ˚C, cooled and then, centrifuged. The absorbance was measured at 532 nm using a spectrophotometer. A calibration step was performed using distilled water in place of the sample for device calibration (3).

Total nitrate-nitrite content (total NO value). In the process of determining the NO value, 50 µL of the sample was mixed with 50 µL of a reagent mixture in a 96-well plate. After a 10 min incubation at room temperature, measurements were taken at 540 nm using an ELISA reader. Standard values were established by dissolving sodium nitrite in water to create various concentrations, which were then analyzed in triplicate at 540 nm (4).

Total lipid peroxidation (TLP). In the TLP test, a reagent was prepared using substances, like ammonium ferrous sulfate, hydrogen peroxide, butylated hydroxytoluene, TBA, catalase, xylenol orange, and triphenylphosphine. The test involved mixing 10 µL of the treated cell supernatant with 90 µL of the reagent. After a 20 min incubation, readings were taken at 560 nm using a spectrophotometer. Standard solutions prepared with hydrogen peroxide at various concentrations were also measured in triplicate for accuracy.

Total anti-oxidant capacity (TAC). The TAC test involved blending 10 µL of the sample with phosphate buffer, sodium benzoate, Fe-EDTA complex, and hydrogen peroxide. After incubation, acetic acid and TBA were added, followed by a heat treatment and spectrophotometric measurement at 532 nm. Standards containing uric acid were used for calibration, and control groups were included in the analysis. Data analysis was conducted following Koracevic's method.

Statistical analysis. The data analysis involved performing a one-way ANOVA (analysis of variance) followed by post-hoc analysis to identify groups with notable mean variances. Statistical tools, such as Sigmastat version 3.5 and GraphPad Prism version 9 were employed for this analysis. The significance of differences between various groups and control group was indicated by $p \leq 0.05$.

Results

MTT. The 24-hr test showed that exposure to DON at certain concentrations led to a significant decrease in cell viability percentages compared to the control group (Fig. 1A). In contrast, cell survival percentages were notably higher in cells treated with quercetin at specific concentrations. The MTT test results over 24 hr further confirmed lower cell survival percentages in groups exposed to DON and quercetin compared to the control group. Notably, cells solely exposed to DON at certain concentrations showed distinct decreases in cell survival percentages (Figs. 1B and 1C).

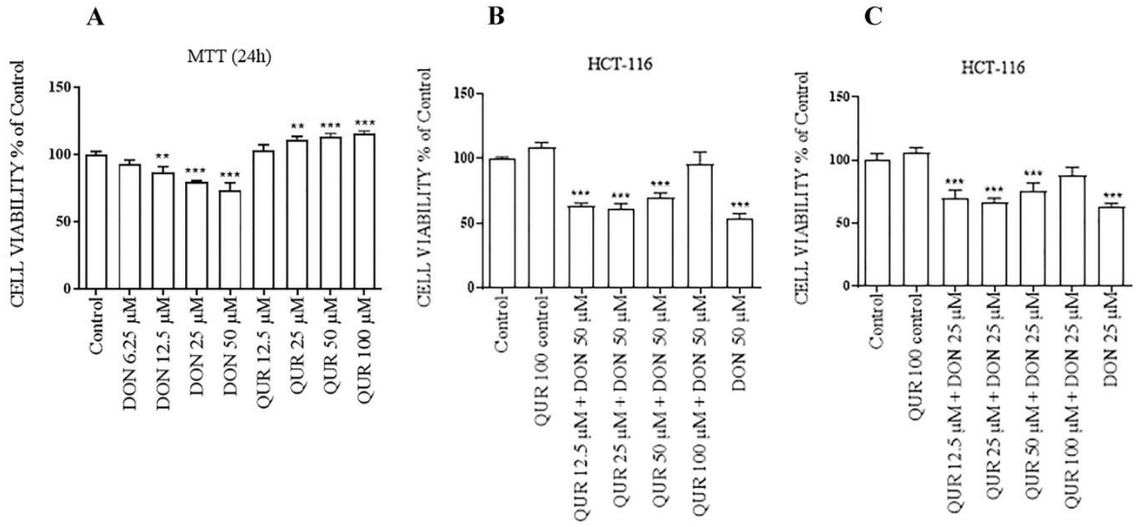

Fig. 1. A) The MTT test results after exposure to various concentrations of deoxynivalenol (DON) and quercetin over a 24-hr period in the control group; **B** and **C**) The MTT test results in different treatment groups after a 24-hr pre-incubation with different concentrations of quercetin, followed by a 24-hr incubation with 25 and 50 µM concentrations of DON. ** indicates significant differences with control group ($p \leq 0.01$) and *** indicates significant differences with control group ($p \leq 0.001$).

Malondialdehyde. The analysis revealed that the MDA concentration in the control groups, Q50 + DON25, Q100 + DON25, and Q100-control was significantly lower than that in the DON25-control group (Fig. 2A). The results from the 24-hr MDA test indicated that the MDA concentration in the groups treated with 12.50 and 25 µM of quercetin in combination with 50 µM of DON, as well as the control groups with 25 and 50 µM of DON was significantly elevated compared to the control group (Fig. 2B).

Total NO value. The assessment of the total NO value revealed that the DON 25-control group exhibited a significantly higher amount of this index compared to the Q50-DON25 and Q100-control groups (Fig. 2C).

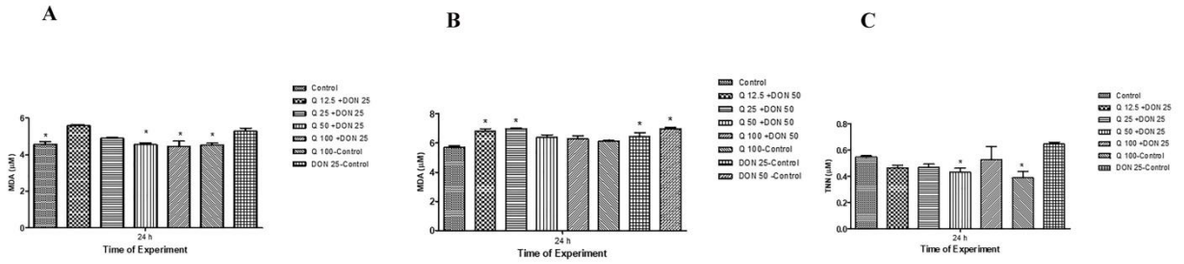

Fig. 2. A) The malondialdehyde (MDA) test results among various groups exposed to quercetin and deoxynivalenol (DON) over a 24-hr period (* differs from DON25-control [$p \leq 0.05$]); **B**) The MDA test results of various groups exposed to quercetin and DON over a 24-hr period (* differs from control [$p \leq 0.05$]); **C**) The results of NO tests in various groups after receiving quercetin and DON over a 24-hr period (* differs from DON-25 control [$p \leq 0.05$]).

Total lipid peroxidation. The results indicated that the TLP concentration in the Q25+DON25 and DON-25 groups was higher than that in the negative control groups and the Q-100 control group. Additionally, the TLP level in the Q100+DON 25 group was significantly lower than DON 25-control group (Fig. 3A). The statistical analysis revealed that the TLP concentration in all groups, with the exception of the group received 12.50 µM quercetin alongside 50 µM DON, was significantly lower than that in the control group containing 50 µM DON (Fig. 3B).

Total anti-oxidant capacity. The TAC measurement indicated that the groups treated with 25, 50, and 100 µM quercetin alongside 25 µM DON exhibited higher TAC compared to the negative control groups, DON-25 and DON-50 (Fig. 3C). The TAC measurement revealed that the groups administered with 50 and 100 µM quercetin alongside 50 µM DON demonstrated a higher TACcompared to the negative control groups, DON-25 and DON-50 (Fig. 3D).

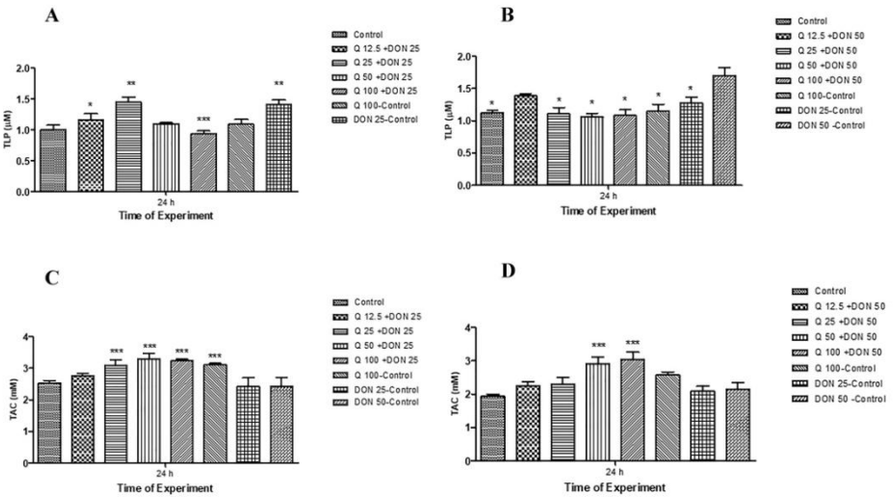

Fig. 3. A) The TLP test outcomes in distinct groups exposed to quercetin and deoxynivalenol (DON) over a 24-hr timeframe, * differs from control ($p \leq 0.05$), ** differs from control and Q100-control ($p \leq 0.05$) and *** differs from DON-25 control ($p \leq 0.05$); **B**) The TLP test results in various groups exposed to quercetin and DON over a 24-hr period, * differs from DON 50-control ($p \leq 0.05$); **C** and **D**) The total anti-oxidant capacity (TAC) test outcomes in diverse groups following exposure to quercetin and DON over a 24-hr period, *** indicates significant differences with all controls (3 controls) at $p \leq 0.05$.

Discussion

The DON disrupts mitochondrial function by generating reactive oxygen species (ROS), leading to apoptosis. Excess ROS can cause oxidative stress when anti-oxidant capacity is overwhelmed, resulting in damage to cell membranes and DNA through lipid peroxidation. This oxidative stress can further induce cell apoptosis, as evidenced by various studies (5, 6). Quercetin's anti-oxidant properties have been well-documented in both laboratory and animal studies (7). Quercetin is thought to mitigate ROS through two main mechanisms including direct neutralizing of intra-cellular radicals, such as superoxide anions and stimulating the production of anti-oxidant enzymes within cells (8).

Zhang *et al*., in 2019, discovered that quercetin concentrations at and above 100 μM exhibit cytotoxic effects on HCT-116 cells over 24, 48, and 72-hr periods (9). While, some researchers suggest cytotoxic effects of quercetin at concentrations exceeding 100 μM on HCT-116 cells (10). The present study found increased cell survival with quercetin concentrations ranging from 25 to 100 μM. Combining lower doses of quercetin with DON led to decreased viability of HCT-116 cells. In contrast, a study on non-tumorigenic gastric cells recommended further exploration of treatment involving a lower quercetin concentration (11). Variations in study outcomes could be influenced by the specific types of cells being tested.

The study evaluated the effects of various concentrations of quercetin and DON on MDA, NO, TLP, and TAC levels in cells. While MDA levels did not significantly differ in cells treated with specific quercetin and DON combinations compared to the control group, other treatments showed a significant increase in MDA levels. The NO levels decreased significantly in certain quercetin and DON treatment groups. Different quercetin concentrations effectively reduced TLP levels, with specific treatments showing significant decreases compared to controls. The TAC levels increased notably in certain quercetin and DON treatment combinations. Another study by Kumar Kalagatur *et al*., highlighted the impact of DON on MDA levels and anti-oxidant enzymes in SH-SY5Y cells, while specific quercetin and DON treatment regimens did not differ significantly from the control (12).

The accumulation of MDA, a pro-oxidant of lipids in cells, effectively demonstrates the oxidative stress caused by DON. Frankič *et al*., through an *in vivo* research highlighted DON-induced impairment in the liver, spleen, and lymphocytes of poisoned laboratory animals, attributing it to the formation of free radicals (13).

Quercetin plays a crucial role in maintaining cell membrane integrity by balancing ROS production, reducing lipid peroxidation, and sustaining high TAC levels (14). Its protective effects help combat stress induced by DON. Research shows quercetin's ability to scavenge free radicals, inhibit DON-induced oxidative damage in mouse lymphoma cells, and safeguard human neuroblastoma cells from oxidative stress by targeting KLF4 (15).

Collectively, these findings highlighted quercetin's potential in effectively modulating ROS levels to defend against DON-induced oxidative stress. In conclusion, this study found that using a concentration of 50-100 μM quercetin offers protection for intestinal epithelial cells against doses of 25-50 μM DON. Quercetin not only improved cell viability but also reduced MDA levels and increased TAC levels in the cells through its anti-oxidant properties. Therefore, it is suggested to administer doses of 50-100 μM/L to counteract the effects of DON (at concentrations of 50 μM and below) in intestinal epithelial cells (HCT-116).

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In vivo evaluation of superoxide dismutase and histopathological analysis of the large intestine in quinolone-treated Wistar rats

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Abstract

The use of quinolone natural compounds has been well-established for almost a century and is associated with various therapeutic purposes, showing protective effects. Among the quinolone-class drugs, ciprofloxacin, a widely used medication, has demonstrated anti-oxidant effects. In this study, the focus was on investigating the anti-oxidant levels, specifically superoxide dismutase (SOD), in the large intestine, along with histopathological assessment. The study involved twelve male *Wistar* rats divided into two groups including a control group received 1 mL of distilled and deionized water twice a week *via* gavage and a ciprofloxacin group received 1 mL of ciprofloxacin at a dose of 70 mg/kg twice a week over a span of 8 weeks. Following the 8-week period, all rats were sacrificed for histopathological evaluation and SOD measurement. The results showed a significant increase in the SOD levels in the large intestine of the ciprofloxacin-treated group compared to the control group. Moreover, normal histopathological conditions were observed in the colon of all groups. The findings of this study suggest that ciprofloxacin treatment could potentially elevate the anti-oxidant levels in organ tissues, indicating its potential use as a novel therapeutic agent in clinical trials.

Keywords: Anti-oxidant, Colon, Quinolone, Superoxide dismutase, Wistar rat

Introduction

Since the initial isolation of quinine, a diverse range of quinolone compounds produced by animals, plants, and microorganisms has been identified, some of which exhibit pharmacologically significant properties, such as anti-allergenic, anti-cancer, and anti-microbial activities. These discoveries have significantly contributed to the development of numerous drugs, including the successful class of fluoroquinolone antibiotics (1). Ciprofloxacin is one of the multi-faceted drugs of the quinolone class used in *in vivo* and *in vitro* studies, exhibiting anti-oxidant properties (2,3). In biological systems, the production of free radicals and reactive oxygen species is inevitable. The body has natural anti-oxidant defense mechanisms to neutralize their harmful effects to some extent. However, if the production of free radicals increases, or if the anti-oxidant factors decrease, the resulting damage increases in a condition known as oxidative stress. In essence, the balance between the production of free radicals and peroxide substances, on the one hand, and the anti-oxidant defense system, on the other, determines the occurrence of oxidative stress (4,5). Superoxide dismutase (SOD) is an important anti-oxidant enzyme shielding the body from damage caused by toxic superoxide generated in the mitochondria. Reduced blood and oxygen supply to the tissues leads to elevated intra-cellular calcium levels and triggers cell death. This damage in the heart and blood vessels can result in cardiovascular injury. The re-

introduction of oxygen leads to an increase in metal ions, such as iron and copper at the site, creating conditions favorable for the generation of oxygen free radicals. The SOD enzyme prevents lipid peroxidation by scavenging free radicals and sequestering iron and copper ions. Cells utilize these mechanisms to protect themselves from oxidative damage (6). The current research aimed to examine the impact of ciprofloxacin on the histopathological changes in the large intestine and assess its effect on the levels of SOD.

Materials and Methods

Chemicals. Ciprofloxacin was purchased from Sigma Aldrich in St. Louis, USA, and distilled and deionized water was purchased from Perssa Co., Iran.

Animals and diet. In this study, 12 male *Wistar* rats aged 10-12 weeks and weighing approximately 200 ± 20 g were obtained from the Laboratory Animals Reproduction and Breeding Center of the North Research Center, Pasteur Institute of Iran, Amol, Iran. They were housed for 1 week to be acclimatized before starting the experimental study under standardized conditions (relative humidity of 60%±10%, 12 hr light/dark period, and temperature of 23 ± 2 ˚C) and supplied with a standard diet purchased from Pars Animal Food Co., Iran, and water *ad libitum*. The desired ethical protocols were followed in all stages of the study, and all animals were treated under the guidelines of the Iranian National Committee for Ethics in Biomedical Research (7).

Experimental design. By the end of the acclimatization period, animals were randomly allocated into two groups (6 rats each). Animals in all groups were treated for 8 consecutive weeks as follows: The 1st group was served as a control and received 1 mL of distilled and deionized water twice a week by gavage for 8 weeks. The 2nd group (ciprofloxacin group) was given 1 mL of ciprofloxacin at a dose of 70 mg/kg twice a week for 8 weeks (8). At the end of experimental period (8 weeks), animals from all groups were euthanized. Then, the intestine tissues were instantly harvested and washed in cold saline. Organs were divided into two parts; the 1st parts were stored at – 80 ˚C to be used for oxidative stress biomarkers analysis and the other parts were fixed in a 10.00% neutral buffered formalin solution for histopathological examination.

Tissue homogenization. The collected tissue samples were stored in a freezer at – 80 ˚C and then, weighed in preparation for the oxidative stress tests. For every gram of tissue, 10 mL of phosphate-buffered saline was added to homogenize the tissue. A ULTRA-TURRAX® digital homogenizer (Model T-18, IKA Works Inc., USA) was used to homogenize the tissue samples. The resulting homogeneous tissue sample was transferred to a 15 mL Falcon tube and then, centrifuged using a refrigerated centrifuge at 2000-6000 rpm for 20 min. After centrifugation, the supernatant of the samples was transferred into 2 mL Eppendorf tubes and frozen to perform oxidative stress tests.

Superoxide dismutase level determination. The SOD Assay Kit (Navand Salamat Co., Iran) was used to measure SOD levels in large intestine tissues. The working solution was prepared by diluting Lysing Buffer 10x, being typically used to prepare tissue samples by mixing 1 mL with 9 mL of double distilled water and then vortexing. Reagent 1 and Reagent 2 were prepared based on the kit manual. The diluted Lysing Buffer was used to lyse tissue samples, with 100 mg of sample tissue and 500 µL of buffer being added and homogenized. The target sample was then centrifuged at 12,000 rpm for 5 min at 4 ˚C, and the supernatant was used as a sample. The materials were added to the wells of the 96-well plate following the kit guidelines.

After 5 min of incubation at room temperature and away from light, the microplate reader device measured the optical absorption of the samples at a wavelength of 405 nm.

Histopathology. The large intestine tissues were initially rinsed with sterile normal saline and then fixed in 10.00% neutral buffered formalin. Following this, they underwent dehydration, clearance, and embedding in paraffin. Tissue sections, 5 µm thick, were prepared using a rotatory microtome (DS4055, Did Sabz Co., Iran) and stained with hematoxylin and eosin. Subsequently, the tissue sections were examined using an optical microscope (Olympus CX31, Japan) and images were captured with a camera (Truechrome II; Tucsen, Fuzhou, China).

Statistical analysis. The data were analyzed using SPSS 27 based on one-sample *t*-test.

Results

Superoxide dismutase level in large intestine tissue. The oral administration of ciprofloxacin resulted in a significant increase ($p < 0.05$) in the SOD level of ciprofloxacin-treated rats compared to the control group (Fig. 1).

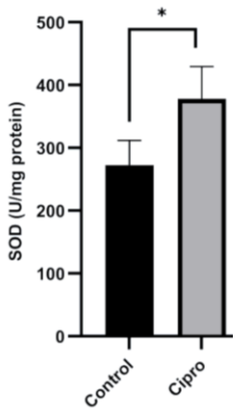

Fig. 1. Comparison between superoxide dismutase (SOD) levels in the large intestine tissue of different groups. Cipro: Ciprofloxacin. * Significant compared to the control group ($p < 0.05$).

Histological observations. All groups were scored and compared regarding the pathological lesions in large intestine tissue. Figure 2 illustrates the histostructure of the large intestine in the control and ciprofloxacin groups. In the large intestine of ciprofloxacin-exposed rats, no sign of necrosis, cell division, and aberrant crypt foci were observed. The same findings were also seen in the control group (Fig. 2).

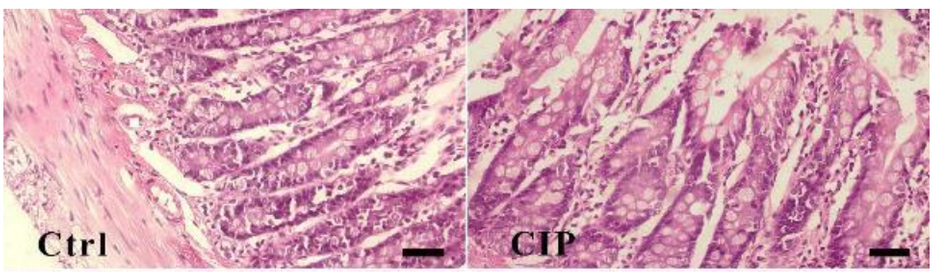

Fig. 2. Comparison of large intestine tissue lesions in different study groups. Normal tissue conditions were observed in the control (Ctrl) and ciprofloxacin (CIP) groups, and the absence of necrosis, cellular proliferation, and aberrant crypt foci is evident. Hematoxylin and Eosin staining; Scale bar = 50 um.

Discussion

Quinolone natural compounds have been recognized for nearly a century and associated with a range of functions (1). Among quinolone family members, ciprofloxacin possesses the maximum efficacy in apoptosis induction (9). Aranha *et al*., were the 1st to demonstrate that ciprofloxacin possesses a strong anti-proliferative effect and can induce apoptosis in certain cell lines (10, 11). Following the administration of ciprofloxacin, it was observed that the levels of Bax increased, resulting in alterations in the Bax: Bcl2 ratio, mitochondrial depolarization, and subsequent cleavage of PARP (10, 11). Moreover, another report has illustrated that ciprofloxacin induces topo-II mediated DNA or chromatin modification, consequently activating the ATM / p53 pathway, leading to apoptosis in lymphoblast-like cell lines (12). Oxidative stress is defined as an imbalance between the occurrence of reactive oxygen/nitrogen species and the organism's capacity to counter their action by anti-oxidant defense systems (13). The SOD is an anti-oxidant enzyme being present in most living cells and can detoxify superoxide. The research findings showed a significant increase in SOD levels in the ciprofloxacin-treated group. Consequently, it could imply the anti-oxidant properties of the ciprofloxacin-treated group compared to the control group. Additionally, based on the results of this study, receiving oral ciprofloxacin did not lead to changes in histopathology of rats large intestine. It could be due to the different routes of ciprofloxacin administration or induction dose (14). In conclusion, the results of this study indicated that oral administration of ciprofloxacin, as a member of the quinolone family, could have anti-oxidant features resulting in improving of the SOD level. In addition, no histopathological changes were seen in the colon. Therefore, more studies should be done to investigate the apoptosis-related mechanisms regarding the ciprofloxacin treatment.

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Investigation of the increase in the number of fibroblasts resulting from pulmonary fibrosis in offspring from pregnant rats poisoned by paraquat and protective effects of coenzyme Q10

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The lungs are one of the primary target organs in paraquat-induced toxicity in animal models and humans (1). Pulmonary fibrosis is characterized by a replacement of alveolar region cellular composition with excessive collagen deposition (2). Coenzyme Q10 is recognized as an anti-oxidant inhibiting oxidative injuries to DNA, lipids, and proteins (3). The present study determined the average number of interstitial lung fibroblasts in the infants of paraquat-treated pregnant rats and scrutinized possible protective effect of coenzyme Q10 in paraquat-induced pulmonary fibrosis. The average number of interstitial lung fibroblasts significantly increased in paraquat-treated group, indicating that paraquat causes pulmonary fibrosis in infant rats whose mothers have been received paraquat during pregnancy. Administration of coenzyme Q10 along with paraquat could not modulate the pulmonary fibrotic effects of paraquat (during 16 days). The increase in the average number of fibroblasts on the 1st and 2nd days in the paraquat-administered group was higher than the subsequent days, suggesting that the fibrotic effects of paraquat in the lung are palliated over time. The results suggest that coenzyme Q10 may need more than 16 days to exert the protective effect against lung fibrosis induced by paraquat.

Keywords: Coenzyme Q10, Fibroblast, Paraquat, Pregnant rats, Pulmonary fibrosis

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Evaluation of the number of mast cells in lung tissue in offspring from pregnant rats poisoned by paraquat and protective effects of coenzyme Q10

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Paraquat may cause acute respiratory distress syndrome, being determined by collagen deposition and pulmonary fibrosis leading to a decrease in expansion and vital capacities (1). It has been determined that mast cells number increases in the pulmonary interstitial tissue of humans and animals with pulmonary fibrosis. It has been indicated that mast cells can play a role in pulmonary fibrosis through fibroblast activation (2). The current study aimed to examine pulmonary mastocytosis in the infants of pregnant rats poisoned with paraquat and the possible protective effects of coenzyme Q10. Paraquat caused a significant increase in mast cells population of lung tissue, indicating that paraquat causes pulmonary mastocytosis in infants rats whose mothers have been administered paraquat during pregnancy. While, the average number of mast cells in the group received coenzyme Q10 plus paraquat decreased significantly compared to the paraquat-treated group. It can be suggested that paraquat may cause pulmonary fibrosis through mastocytosis induction and coenzyme Q10 can have pulmonoprotective effects *via* mastocytosis inhibition.

Keywords: Coenzyme Q10, Mast cell, Paraquat, Pregnant rats, Pulmonary fibrosis

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Protective effect of crocin on dimethylhydrazine-induced colon carcinogenesis in rats

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One of the most common tumors to cause death worldwide is colon cancer. Dimethylhydrazine (DMH) is widely used as a carcinogen to induce colon cancer in animal models (1). The herbal plants are the richest sources of natural remedies and bioactive compounds used to manage cancer with minimal side effects. Crocin is a bioactive compound naturally occurring in some medicinal plants, especially saffron, and has several pharmacological actions, including anti-inflammatory and cancer cell growth inhibitor properties (2). This study aimed to investigate the protective effects of crocin against DMH-induced structural lesions of colon. Forty male rats were randomly divided into 4 groups (n=10) being treated intra-peritoneally for 6 weeks including control, crocin (40 mg/kg/day), DMH (30 mg/kg/day), and DMH (30 mg/kg/day) + crocin (40 mg/kg/day) groups. Light microscopic examinations of DMH group showed enlarged and hyperchromatic nuclei and high grade dysplastic changes as well as severe lymphocytic infiltration in colon. The DMH + crocin group rats showed regenerated colon architecture and decrease of inflammatory cell infiltration. In conclusion, the results in the present study suggest a potential therapeutic approach of crocin in the prevention of colon carcinogenesis.

Keywords: Colon cancer, Crocin, Dimethylhydrazine, Histopathology, Rat

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The effect of the pharmacological effects of sulfasalazine and hydroalcoholic extract of the ivy plant on ulcerative colitis in Wistar rats experimental model

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Following acquiring the ivy plant, we cleaned it and dried it in a sanitary environment at room temperature. After one week, we filtered the resulting solution multiple times to eliminate plant residues. The rats were kept in a state of fasting for 24 hr, after which we induced ulcerative colitis using an acetic acid solution administered via an injector (1). There was a significant reduction in the disease severity index in the treatment group receiving ivy extract at doses of 50 and 100 mg, however, no statistically significant difference was observed between these two doses. Conversely, the group treated with 150 mg of ivy extract demonstrated a notable decrease in the disease severity index, exhibiting a significant difference from the other groups. The activity of the MPO enzyme and nitric oxide was the highest in the affected group and the lowest in the healthy group. A reduction in enzyme levels was observed in the treatment group receiving sulfasalazine, although there was no significant difference between the affected group and the 50 mg extract treatment group. However, no significant differences were found between the treatment groups receiving 100 mg and 150 mg. There was no significant relationship between the various concentrations of ivy extract except for IL-1 where the optimal concentration was determined to be 100 mg. Based on these findings, it coul be concluded that ivy extract could control inflammation and manage ulcerative colitis (2,3).

Keywords: Inflammation, Ivy plant, Ulcerative colitis

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Protective effects of crocin on aging damage in liver and kidney tissues of mice

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Aging is a gradual and irreversible pathophysiological process. This process appears with a decrease in tissue and cell function and a significant increase in the risk of related diseases. Although with the advancement of medical science, human health has improved and life expectancy has increased, however, increasing age has become the most important cause of disability and death in the elderly (1). In this study, 24 mice were divided into three groups of eight including young control group (8 weeks old), old control group (12 months old) and group treated with Crocin (12 months old). Crocin was administered (100 mg/kg/IP) in the treatment group and normal saline in the young and old control groups for 21 days. Serum levels of liver and kidney enzymes and markers of oxidative stress in liver and kidney tissue were evaluated using ELISA method. Also, the expression level of beta-galactosidase gene was evaluated by qRT-PCR method (2). The results showed that administration of Crocin could significantly reduce the level of liver enzymes and serum creatinine in old rats compared to the old control group. The amount of catalase and total antioxidant capacity were increased significantly and malondialdehyde decreased significantly in the treatment group compared to the aged control group ($p < 0.05$). Also, the lowest level of beta-galactosidase gene activity was obtained in the old control group. The findings showed that the administration of Crocin might have a protective effect on the liver and kidneys in aging-related damage in mice due to its antioxidant properties.

Keywords: Aging, Antioxidant capacity, Beta-galactosidase, Crocin, Mice

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Section 6

Food Hygiene and Quality Control

Evaluation of antimicrobial, antioxidant and chemical composition of coriander essential oil *(Bifora Testiculata L. Spreng)*

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Abstract

Recently, there has been an increased tendency to use natural preservatives which has led researchers to identify their compounds. This study aimed to determine the antimicrobial, antioxidant and constituents of coriander (*Bifora Testiculata L. Spreng*) plant essential oil *in vitro*. The essential oil of the coriander plant was extracted using the water distillation method and the chemical composition of the essential oil was analyzed using a GC-MS device. The amount of total phenol was determined using the Folin Ciocalteu method and the antioxidant properties of the essential oil were investigated using three methods: DPPH, FRAP, and ABTS. The antimicrobial effect of the essential oil was determined using the agar well diffusion, minimum growth inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) methods. Results showed that the main components of the essential oil were trans-2-dodecen-1-ol (12.29%), 2-dodecenoic acid (11.52%), hexadecanoic acid (10.03%), lauric acid (7.89%), phytol (7.28%), pheophytadine (1.85-6.83%), E-2-tetradecen-1-ol (4.68%), decanal (4.52%), Nonaldehyde (3.8%), dodecanal (3.45%) and 2-pentadecanone trimethylhexa (3.36%). The amount of total phenol in the essential oil was 74.72 ± 6.02 mg of gallic acid/g, and for DPPH, the IC50 value of the essential oil was calculated as 17.9 mg/mL. In the antimicrobial test, the largest diameter of the zone of inhibition was related to *Listeria monocytogenes* (22 mm) and *E. coli* (13.4 mm). On the other hand, *L. monocytogenes* was the most sensitive among bacteria in MIC and MBC. The results of this study showed that Bifora essential oil had significant antioxidant and antibacterial properties.

Keywords: Antimicrobial activity, Antioxidant activity, Coriander, Chemical compounds, Essential oil

Introduction

In recent years, due to the increasing awareness of consumers about food health and safety, food factories have paid much attention to the use of these natural preservatives of herb and microbial origin instead of chemical and synthetic preservatives in their products. Recently, the tendency of people to use natural preservatives, especially the essential oils of plants and spices and the identification of their constituent compounds has increased and has brought positive results in overcoming food pathogens and preventing spoilage and oxidation. Plant essential oils are volatile aromatic oily liquids that are obtained from different parts of plants such as flowers, leaves, roots, stems and seeds and are used as food flavorings and therapeutic properties. Various studies and researches have confirmed the antimicrobial, antifungal, antiviral, anti-parasitic and antioxidant properties of essential oils and plant compounds (1).

Coriander plant with the scientific name *Bifora testiculata L. Spreng* and the local name of mountain coriander is an herbaceous and annual plant of the genus Bifora. Coriander plant is native to the Mediterranean, parts of Europe, especially Iran and Arabia. This plant grows wildly in the northwestern regions of this plant are used in food by local people in the region.

Despite numerous studies and researches on the coriander plant and its various properties, however, no study has been done on the antimicrobial, antioxidant, cytotoxic properties and the constituents of the essential oil of the *B. testiculata L. Spreng* plant. Therefore, this research was done with this goal.

Materials and Methods

Preparing the plant and verifying its scientific name. Bifora plant was collected at the end of May 1400 from the fields of Naqara village in Garami city (Maghan), Ardabil province, Iran, and after its scientific name was confirmed by a botanist, and registered in Urmia Agriculture and Natural Resources Research Center with Herbarim number 10604.

Extraction of essential oil from Bifora plant using Clevenger device. Essential oil extraction lasted for 4 hr. At the end of the work, after dehydrating the essential oil with sodium sulfate (Merck, Germany), it was poured into a frosted glass container and kept in a refrigerator at a temperature of 4 ˚C until analysis and further use (2). The percentage of essential oil extraction efficiency was calculated based on the volume of essential oil obtained.

Identification of essential oil components. A gas chromatography device was used to separate and determine the percentage of each component of the essential oil and a gas chromatography device coupled with a mass spectrometer was used to identify the components of the essential oil.

Evaluation of antioxidant activity of essential oil. The antioxidant activity of Bifora essential oil was measured by three methods: DPPH, ABTS and FRAP, and total phenolic compounds were also measured.

Preparation of bacterial suspension. In order to achieve a fresh and active culture of the studied microorganisms, the target microorganism was transferred from the reserve culture medium to the brain heart infusion broth culture medium and incubated at a temperature of 37 ˚C or 18 hr. The turbidity of bacteria was visually compared and adjusted with the 0.5 McFarland standard.

Agar well diffusion method. After the cultivation of pathogenic bacteria on the surface of the Mollier Hinton agar culture medium, using a sterile punch, wells with a diameter of 5 mm were created in the culture medium with an appropriate distance from each other and from the pellet wall, then 30 µL of essential oils were added. It was poured into each well with different concentrations. DMSO solvent was used as a negative control and gentamycin as a positive control. The pellets were incubated at 37 ˚C for 24 hr. The well was measured in millimeters with a special caliper (3).

Microdilution method - determining the minimum growth inhibitory concentration (MIC) and determining the minimum lethal concentration (MBC). In order to determine the minimum inhibitory concentration (MIC), the microdilution method was used in a sterile 96-well microplate. 80 µL of nutrient broth and 20 µL of standardized bacteria suspension with 0.5 McFarland were added to each microplate well. Then, 100 µL of the prepared essential oil stock solutions with the study concentrations were added to each

well, respectively, from the highest concentration. Finally, the microplate was shaken for 20 sec at 300 rpm and incubated at 37 °C for 24 hr (4).

Statistical analysis. To analyze the data and results of this research, SPSS software version 20.0, IBM Corp., Armonk, NY, USA), was used, and for statistical analysis and comparison of treatment means, one-way analysis of variance (ANOVA) and Duncan's test were performed at the probability level ($p \geq 0.05$). To draw graphs, Microsoft Office software, Excel program were used.

Results

In total, 40 compounds of Bifora plant essential oil were identified, which constituted 97.9% of the total essential oil. The main compounds of Bifora essential oil were, respectively, trans-2-dodecen-1-ol (12.29%), 2-dodecenoic acid (52 11.0%), hexadecanoic acid (10.03%), lauric acid (7.89%), phytol (7.28%), pheophytadine (1.85-6.83%), E-2-tetradecen- 1-L (4.68%), decanal (4.52%), nonaldehyde (3.8%), dodecanal (3.45%) and 2-pentadecanone trimethylhexa (3.36%).

The results of free radical scavenging power (DPPH). The results of investigating the antioxidant effects of different concentrations of Bifora essential oil and butyl hydroxyanisole (BHT) with DPPH radical inhibition percentage are shown in Figure 1.

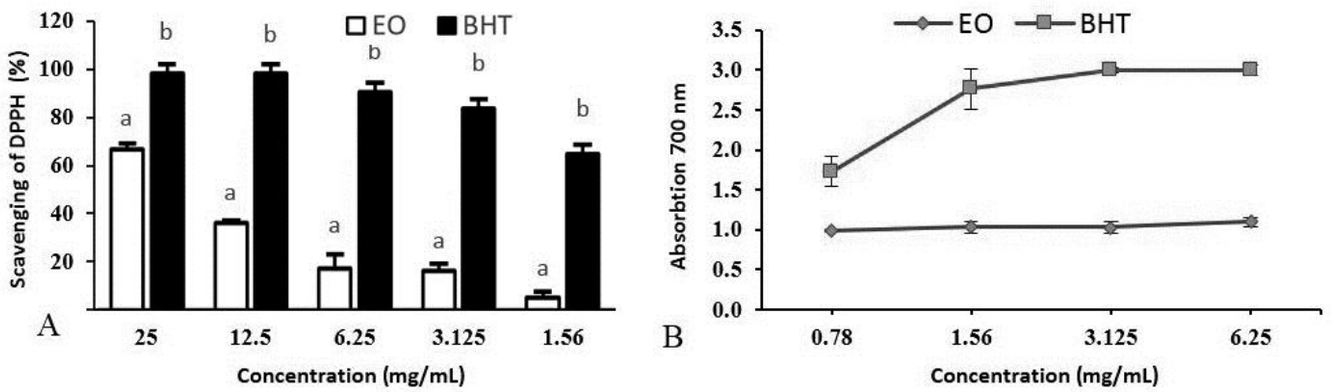

Fig. 1. A) DPPH radical scavenging rate of different concentrations of Bifora essential oil was compared to BHT. Different letters in each concentration indicate a significant difference ($p \leq 0.05$). **B)** The result of the reducing power of Bifora essential oil was compared to BHT.

The results of evaluating ABTS radical inhibitory power. The results of the evaluation of ABTS radical inhibitory power in terms of inhibition percentage and antioxidant capacity of Bifora essential oil and ascorbic acid are shown in Table 1.

Results of total phenol (TP). Based on standard curve of gallic acid, the amount of total phenol of Bifora essential oil was equal to 747.24 ± 60.21 mg of gallic acid per gram of essential oil.

Results of antimicrobial properties in agar well diffusion method. The results of the effect of Bifora essential oil on the diameter of zone inhibition of Gram-positive and Gram-negative bacteria investigated in comparison with the antibiotic ciprofloxacin in agar diffusion method are shown in Table 2.

The results of MIC and MBC. Table 3 shows the results of evaluating the antimicrobial property of Bifora essential oil against four bacteria *Salmonella Typhimurium, Listeria monocytogenes, Staphylococcus aureus* and *Escherichia coli* using microdilution method.

Table 1. Inhibition percentage of different concentrations of Bifora essential oil and ascorbic acid.

Concentrations (mg/mL)		Inhibition (%)
0.78	EO	8.09±1.59 ^{aA}
	Acid ascorbic	95.19±6.27 ^{bA}
1.56	EO	11.49±2.21 ^{aB}
	Acid ascorbic	97.33±2.07 ^{bA}
3.125	EO	16.26±3.38 ^{aC}
	Acid ascorbic	94.66±6.39 ^{bA}
6.25	EO	72.41±0.26 ^{aD}
	Acid ascorbic	100^{bA}
12.5	EO	41.05±6.06 ^{aE}
	Acid ascorbic	100^{bA}

Table 2. Results of antimicrobial properties. The diameter of the zone inhibition is the mean ± standard deviation.

Groups	Concentration	*S. aureus*	*L. monocytogenes*	*S. typhimurium*	*E. coli*
EO	100µg	18.15±1.3^{b}	22.0±1.2^{aA}	16.6±3.0^{bA}	13.4±0.9^{cA}
Ciprofloxacin	30 µg	19.6±1.2^{cA}	20.0±0.9^{cA}	24.0±0.6^{bB}	29.8±0.5^{aB}

In each row, non-similar lowercase letters indicate a significant difference at the $p \leq 0.05$ level between different bacteria at the same concentration. Non-similar uppercase letters also indicate a significant difference between the essential oil and the positive control at the $p \leq 0.05$ level.

Table 3. Results of MIC and MBC.

Bacteria	MIC	MBC
	EO (mg/mL)	
L. monocytogenes	2	4
S. typhimurium	64	-
S. aureus	32	-
E. coli	64	-
DMSO	-	-

MIC: minimum growth inhibition concentration and MBC: minimum bactericidal concentration.

Discussion

The characteristics of Bifora chemical compounds and essential oils of different aerial parts of the plant have been reported by Mandal et al. These differences may be due to difficulties in proper classification as well as in differentiation in isolation/extraction methods, plant origin and location, climatic and environmental conditions, humidity, radiation, harvest period time which significantly affects the chemical composition (5). In the comparison of free radical inhibition, there was no significant difference between essential oil and BHT only at a concentration of 25 mg/mL ($p \geq 0.05$) and there was a significant difference between essential oil and BHT in other concentrations ($p \leq 0.05$). In this test, the IC50 value (the concentration of essential oil in which 50% of free radicals were inhibited in the reaction medium) of Bifora essential oil and BHT were 17.9 and 13.78 mg/mL, respectively.

Koroghli et al. investigated the antioxidant capacity and total phenol of the diethyl extract and methanolic extract of Bifora Radian plant and DPPH free radical inhibition activity was reported to be 69.91 and 43.21%, respectively (6).

In another study, Virapago et al. showed a significant antioxidant activity in Bifora seed essential oil and the percentage of free radical inhibition for Bifora essential oil and standard ascorbic acid was 66.2% and 87.8%, respectively, and their IC50 values were 0.147 and 0.108. mg/mL (7). In several other reports, the antioxidant properties of Bifora essential oil have been attributed to compounds in addition to linalool, such as decanol, 1-decanol, trans-2-dodecanal, dodecanal and other compounds with antioxidant activities (8). The results of reducing power showed that the reducing power of essential oil and synthetic antioxidant BHT were increased with increase in concentration. In comparing the reductive power, there was a significant difference between essential oil and BHT ($p \leq 0.05$). In all these concentrations, the power of BHT synthetic antioxidant was more than essential oils. At a concentration of 6.25 mg/mL, the power of essential oil and BHT was equal to 0.937 and 3 mg/mL.

In the study by Shahwar et al., the reducing power of Bifora seed and leaf essential oil at a concentration of 500 µg/mL was reported as 1.274% and 1.146%, respectively (9). In the study of Hajlaoui et al., the IC50 value of the reducing power of Bifora essential oil was reported as 24 µg/mL (10). In one study, the antioxidant activity of three varieties of Bifora fruit, the reducing power of seed essential oil in Tunisian, Syrian, and Egyptian varieties was reported to be122.01, 54.20, and 56.11 µg/mL, respectively (11). In the study of Tang et al., investigating the antioxidant power of reducing iron in coriander root and leaf extracts, 0.129 and 0.136 mmol were found on heat (12).

In Hameed et al. study, the regenerative power of coriander methanolic extract was reported as 5.45 mg Torolex per gram (13). In the study of Marquez et al., the results of the evaluation of ABTS radical inhibitory power in coriander were stated as 22.9% (14). In another study, the content of total phenol equivalent to milligrams of gallic acid per gram was obtained in three cultivars of coriander fruit in Tunisian, Syrian and Egyptian cultivars, respectively, 1.09, 1 and 0.94 micrograms/mL (11).

In the study of Tang et al., oncoriander plant extract (root and leaf), the highest total phenol values of 31.38 and 24.57 mg of gallic bergamot were reported in the root and leaf of coriander plant, respectively (12). In the study of Zanganeh et al., in the well diffusion method for coriander essential oil, the zone of inhibition in *S. aureus, E. coli, S. typhimurium, and L. monocytogenes* bacteria was 34.2, 30, 28.8, and 35 mL, respectively, at a concentration of 128 mg/mL was reported (15).

In another study, the zone of inhibition for these microorganisms was 21, 11, 9 and 25 mm, respectively, at a concentration of 10 µL of essential oil (16).

In Pascal et al. study on coriander essential oil with four bacteria, *S. aureus, E. coli, S. typhimurium,* and *L. monocytogenes*, the MIC results for these four bacteria were 0.4, -0.23, and 0.47%, respectively. The highest antimicrobial effect of essential oil was against Listeria bacteria (1).

In another study conducted by Silva et al., coriander essential oil had antimicrobial activity against all tested bacteria. With the exception of *B. cereus* and *E. faecalis*, the MIC of *S. aureus*, *E. coli* and *S. typhimurium* bacteria were 0.4%, 0.2% and 0.4%, respectively, and the MBC of the above bacteria was 3.2%, 0.2% and 0.8%, respectively. The main mechanism of the effect of coriander essential oil was found to be disruption of the

membrane which contributes to cell death. (2). Yildiz et al. reported that the strong antibacterial activity of essential oil against Gram-positive and gram-negative bacteria was due to membrane permeability. In addition, essential oil was particularly effective against *L. monocytogenes*, possibly due to the presence of alcohols and aldehydes (16). The most abundant compound in this essential oil was (E)-2-decenal. This compound inhibits Gram-positive bacteria including *L. monocytogenes*. Essential oil compounds are a complex mixture of volatile substances including octanol, decanol, desanol, alcohols, and aldehydes which have a strong inhibition on Gram-positive bacteria and lack of effectiveness against Gram-negative bacteria. (1).

According to the results obtained in the present study, Bifora essential oil had good antioxidant properties and also showed a broad antimicrobial effect, especially against Gram-positive bacteria. Therefore, the cilantro plant, which has a natural source and has no side effects, can be used as an alternative to chemical preservatives and its use in the development of natural food preservation techniques that can improve the shelf life of food by delaying oxidation processes.

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Combined application of postbiotics and thyme essential oil nanoemulsion for extending rainbow trout fillet shelf life

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This research aimed to investigate the effects of postbiotics derived from *Lactiplantibacillus plantarum* (PL) and *Thymus diagenesis* Celak essential oil (TDEO) nanoemulsion (EON) on microbial, chemical and sensorial characteristics of rainbow trout fillets. The PL_{50} with a concentration of 50% w/v of lyophilised PL, $EON_{1.5}$ containing 1.50% v/v of TDEO, EON_3 containing 3% v/v of TDEO and PL_{50}-EON_S as combined forms of PL_{50} and EONs were sprayed on rainbow trout fillet. Total psychrotrophic count (TPC), total mesophilic count (TMC), pH, total volatile base nitrogen (TVB-N), thiobarbituric acid (TBA) and sensorial characteristics of control and treatment groups were evaluated during 9 days of storage at 4 °C. Shelf life of the control sample was up to 6 days, while at the same day, PL_{50}-EON_3 exhibited significantly lower TPC (2.59 $\log_{10}$ CFU/g), TMC (4.87 $\log_{10}$ CFU/g) and TVB-N (23.40 mg/100 g sample). Sensory evaluation showed that the treated sample with PL_{50}-EON_3 had a higher acceptance than the control sample. The obtained results from PL_{50}-EON_3 treated group can be related to the synergistic effect of the PL_{50} components, including plantarcin and organic acids (1, 2) and EON_3 components, such as thymol and carvacrol (3). The principal component analysis showed that EON_3 and PL_{50}-EON_3 had positive effects on the TBA, TMC, TPC, TVB-N, and pH parameters of fillets. In conclusion, PL_{50}-EONs prolonged the shelf life of fillets by >3 days based on quality parameters threshold limits. The findings demonstrate the potential of postbiotics in combination with EON to develop a preservative solution in fish products.

Keywords: Coating, Essential oil, Postbiotics, Rainbow trout, Shelf life

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Effect of green tea extract and cinnamon in different concentrations on the microbial, physicochemical and sensory characteristics of cookies

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Chemical preservatives like anti-oxidants are often used in food to extend shelf life, but their negative effects have led to research on natural alternatives. Green tea and cinnamon are examples of plants that can be used as natural preservatives due to their anti-fungal and anti-bacterial properties (1). Cookies, high in fat, are susceptible to oxidation which can shorten their shelf life. This study was conducted to assess the impact of green tea extract and cinnamon on the physicochemical and sensory properties of cookies. Results showed that adding 5.00% green tea extract with 4.00% cinnamon to the cookie formulation produced acceptable results, maintaining the quality of the cookie. Higher levels of green tea extract negatively affected the cookie quality, highlighting the importance of finding the right balance in natural preservative concentrations for optimal results. This study demonstrated that a combination of green tea and cinnamon at specific levels can effectively enhance the shelf life and quality of cookies without compromising their properties.

Keywords: Cinnamon, Cookies, Green tea extract, Microbial, Physicochemical properties

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Evaluation of antibacterial effects of dill (*Anethum graveolens* L.) essential oil in the presence of butylated hydroxyanisole and ethylenediaminetetraacetic acid

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In recent years, investigating the antimicrobial effects of plant essential oils (EOs) in the presence of other food preservatives has been considered by researchers (1-3). In this study, the antibacterial effects of dill EO alone and in combination with ethylenediaminetetraacetic acid (EDTA) and butylated hydroxyanisole (BHA) on *Staphylococcus aureus*, *Listeria monocytogenes*, *Escherichia coli* and *Salmonella typhimurium* were studied. Antimicrobial susceptibility tests were performed using disk diffusion and broth microdilution minimal inhibitory concentration testing methods. Dill EO showed antibacterial effects on the tested bacteria except for *L. monocytogenes*. The combination of dill EO with EDTA and BHA significantly increased the antibacterial properties of dill EO on *S. aureus* ($p < 0.05$). However, the combination of dill EO with EDTA and BHA did not enhance the antibacterial effects of dill EO on *S. typhimurium*. The combination of dill EO with EDTA significantly increased the antibacterial properties of dill EO on *L. monocytogenes* ($p < 0.05$), however, such an effect was not observed in its combination with BHA. The combination of dill EO with EDTA significantly increased the antibacterial properties of dill EO on *E. coli* ($p < 0.05$), however, such an effect was not observed in its combination with BHA. It could be concluded that dill EO had antibacterial effects and its antibacterial effects were increased significantly in combination with EDTA. Therefore, the combination of dill EO with EDTA in different food systems is suggested.

Keywords: Antibacterial effects, BHA, Dill, EDTA, Essential oil

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The effects of cinnamon (*Cinnamomum zeylanicum*) essential oil in the presence of nisin on the growth of *Salmonella typhimurium* in minced chicken meat during storage at refrigerator temperature

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In this research, the effects of cinnamon essential oil (EO), nisin and a mixture of cinnamon EO and nisin on the growth of *Salmonella typhimurium* in minced chicken meat during 10 days of storage at refrigerator temperature (+4 ˚C) were studied. Extraction and analysis of cinnamon EO, determination of the minimal inhibitory concentration, preparation of minced chicken meat containing *S. typhimurium* and various concentrations (100, 200, 400 ppm or i.u/g) of cinnamon EO, nisin and cinnamon EO and nisin mixture, *S. typhimurium* count, standard plate count (SPC) and pH measurement on different days (0, 4, 7, 10) of storage at the refrigerator were the methods used in this study (1-3). The major components of cinnamon EO were cinnamic aldehyde (35.23%), alpha bergamotene (15.06%) and trans cinnamyl acetate (12.08%). The MIC of cinnamon EO, nisin and a mixture of cinnamon EO and nisin were 1.6 mg/mL, 100 i.u/mL, and 0.8 mg/mL + 50 i.u/mL, respectively. The Fractional Inhibitory Concentration index showed that the mixture of cinnamon EO and nisin inhibited *S. typhimurium* in an additive manner. The mix of cinnamon EO and nisin was the most effective treatment in reducing the count of *S. typhimurium* and the SPC in minced meat during storage days. The pH value did not show any significant difference among the studied treatments. Also, the mixture of 400 ppm of cinnamon EO and 400 i.u/g of nisin was the most effective concentration in reducing *S. typhimurium* count and SPC.

Keywords: Cinnamon, Chicken meat, Nisin, *Salmonella typhimurim*, Shelf life

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A systematic review of the use of plant essential oil for probiotic yoghurt preservation

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Biopreservatives are currently used to improve the safety and quality of dairy products. It has been demonstrated that some essential oils (EOs) have antimicrobial properties that allow their use as food preservatives and flavoring agents. Therefore, to ensure the beneficial effects of EOs, they should be evaluated individually and in combination with other preservative agents such as probiotic bacteria to determine whether there are synergistic effects between these agents. This study was a systematic review of the feasibility of using EOs in probiotic yoghurt. Various studies have reported conflicting results regarding the effects of EOs on the survival of probiotics in yoghurt during storage (1-3). Nevertheless, EOs with probiotics have a synergistic function against pathogenic and spoilage microbes in yoghurt. Probiotic yogurt treated with the lowest concentration of EOs have the highest sensorial attribute score and viable count of probiotic bacteria. However, a high concentration of EOs is the most appropriate treatment for improving the microbial quality of probiotic yoghurt. According to the findings of this systematic review, using EO at optimum concentrations can be a suitable approach to enhance the shelf life and safety of probiotic yoghurt. Furthermore, EOs that eliminate competing microorganisms can increase the survival rate of probiotics in these products.

Keywords: Essential oil, Probiotic, Safety, Shelf-life, Yogurt

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Evaluation of the antifungal effects of savory (*Satureja hortensis*), dill (*Anethum graveolens*) and cinnamon (*Cinnamomum zeylanicum*) essential oils alone and in combination on *Aspergillus flavus* and *Penicillium chrysogenum*

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In this research, the effects of savory, dill, and cinnamon EOs alone and in combination on the growth of *Aspergillus flavus* PTCC 5004 and *Penicillium chrysogenum* PTCC 5033 mycelium were studied. The concentrations of 0.25, 0.5, and 1% (w/v) of the EOs were prepared in sterile molten potato dextrose agar (PDA). Then, the mycelium of tested molds was transferred to PDA containing different concentrations of EOs using a sterile puncher (Ø=5mm). The growth diameter of molds was measured on days 3, 5, 7, and 10 of incubation at 25 ˚C and the inhibition percentage of various concentrations of EOs was calculated. The Savory and dill EOs alone inhibited the growth of *A. flavus* and *P. chrysogenum* 100% on different days of incubation in the tested concentrations. Also, cinnamon EO alone in all concentrations inhibited the growth *of P. chrysogenum* by 100% and the EO alone could not inhibit the growth of *A. flavus* in concentrations of 0.25 and 0.5% on days 7 and 10 of incubation completely. The combination of cinnamon EO with savory and dill EOs in the same concentrations enhanced the antifungal effects of cinnamon EO. It could be concluded that savory, dill and cinnamon EOs alone had antifungal effects and their combination with each other in the tested concentrations, except for cinnamon EO, did not affect their antifungal activity. It is suggested to use savory, dill, and cinnamon EOs as flavoring and antifungal compounds in foods and evaluate their combined effects in concentrations less than 0.25%.

Keywords: Antifungal effect, *Aspergillus flavus*, *Penicillium chrysogenum*, Plant essential oils

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Evaluation and comparison of antioxidant effects of different extracts of *Vitis vinifera* L. var. Ghizil Uzum skin and seeds extracted by ultrasonic and deep eutectic solvents (DESs) methods

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Extraction of antioxidants from plant tissues can be done using traditional extraction processes such as solid-liquid extraction, solvents and steam distillation (1). One of the simplest techniques for extraction is ultrasonic extraction which is easy to perform using common laboratory equipment like an ultrasonic bath. In recent years, a new generation of green solvents called deep eutectic solvents (DES) has been introduced (2). For this study, samples of *Vitis vinifera* L. var. Ghizil grapes were collected from Urmia city. Grape seeds and skin extracts were extracted using ultrasonic and DESs methods. Antioxidant activity of different extracts was evaluated using total phenol, DPPH and reducing power tests. The results of this study showed that the extraction method played an important role in determining the antioxidant properties of the extract. There was a significant difference in the antioxidant capacity of skin and seed extracts that were extracted using each of the ultrasonic and DESs methods compared to the control group ($p < 0.05$). The highest antioxidant capacity per mg of grape skin and seed extract was observed in the extract prepared by the combined method of ultrasonic and deep eutectic solvents ($p < 0.05$). The highest amount of total phenol and antioxidant capacity was observed in the seed extract compared to the skin extract. Therefore, it could be concluded that Ghizil grape extracts extracted using the combined ultrasonic method and deep eutectic solvents had higher antioxidant effects compared to other conventional methods of extract preparation.

Keywords: Antioxidant, Deep eutectic solvents, Extract, Ghizil Uzum, Ultrasonic

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Section 7

Large Animals

The health benefit and application of ginger in animal production

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Abstract

Zingiber officinale, commonly known as ginger, is a medicinal plant with a wide range of beneficial properties in livestock production. The rhizome of ginger contains a variety of bioactive compounds, including essential oils (such as zingiberene, curcumin, geraniol, citronyl acetate, terpinolene, linalool, borneol, neral, and geranial), phenolic compounds, and flavonoids. Additionally, ginger is rich in organic acids, such as malic, citric, and tartaric acids. These constituents confer numerous therapeutic properties, including antiviral, anti-inflammatory, anti-ulcer, antioxidant, antibacterial, antidiarrheal, antispasmodic, astringent, hepatoprotective, fungicidal, cyclooxygenase inhibition, and lipoxygenase inhibition. In ethnoveterinary practices, ginger rhizomes have been used to treat a variety of conditions in livestock, such as coughs, colds, retained placenta, bloat, diarrhea, and sprains in poultry, ruminants, and swine. Both the leaves and rhizomes are used in the prevention of mastitis and the treatment of numerous ailments, including wounds, hemorrhagic septicemia, pneumonia, asthma, cough, nasal mucosa swelling, gastrointestinal disturbances (e.g., stomach pain, tympanitis, constipation, dysentery, and loss of appetite), lumbar fractures, and urinary retention. Beyond its medicinal uses in humans, the bioactive molecules in ginger play a pivotal role in improving livestock and fisheries production. Supplementation of animal feed with ginger and its derivatives aligns with modern agricultural practices, particularly organic animal husbandry. This review compiles evidence on the effects of ginger and its extracts on various performance parameters in animals, including poultry, horses, ruminants, and fish.

Keywords: Ginger, *Zingiber officinale*, large animals, poultry, fish.

Introduction

The application of antibiotic growth promoters (AGPs) in animal feeds represents one of the most significant biotechnological advancements in animal husbandry during the 20th century. AGPs serve multiple functions, including enhancing animal performance, promoting disease prevention, and facilitating treatment, and have been widely used in both livestock and aquaculture for extended periods. However, the overuse of AGPs has led to the accumulation of drug residues and the development of bacterial resistance, which have substantial implications for the quality of animal products and aquatic organisms, as well as posing serious risks to food safety and human health. As a result, concerns over the long-term use of antibiotics—particularly regarding bacterial resistance and drug residues—have led to the enactment of regulations banning the use of antibiotics in certain livestock sectors, such as pig production (1).

Ginger (*Zingiber officinale*) has a long history of medicinal use, dating back more than 2,500 years in cultures such as those of China and India, where it has been employed to treat a variety of conditions, including headaches, nausea, rheumatism, and colds (2).

Ginger contains several bioactive compounds, including terpenes, oleoresin, zingiberol, zingibcrone, zingiberene, gingerol, shogaol, zingerone, and paradol, which function as antioxidants, natural antibiotics, and immune stimulants. These compounds support animal health and growth, making ginger a potential alternative to AGPs in animal feed, aligning with the growing demand for sustainable and antibiotic-free animal husbandry practices (3).

Discussion

Ginger and large animals. In one study, ginger extract seemed to decrease the recovery time in exhaustive bout of exercise in horses. Ginger extract did not significantly affect other physiological responses to exercise, such as heart rate, blood pressure, time to fatigue, plasma lactate concentration, core temperature, or rectal temperature. However, further investigation into the specific compounds present in ginger extract is necessary to assess whether these substances could potentially be detected in drug tests commonly administered during competitive events (4).

Another study evaluated the effects of *Zingiber officinale* rhizome methanolic extract (ZOR) on the in vitro growth of bovine Babesia species (*B. bovis*, *B. bigemina*, *B. divergens*) and equine piroplasms (*B. caballi* and *Theileria equi*), as well as on the growth of *Babesia microti* in mice. The potential synergistic interaction between ZOR and either diminazene aceturate (DA) or potent Medicines for Malaria Venture (MMV) hits from the malaria box was also explored. In vitro, ZOR inhibited the growth of *B. bovis*, *B. bigemina*, *T. equi*, and *B. caballi* in a dose-dependent manner, with *B. divergens* being the most susceptible to the inhibitory effects of ZOR. Furthermore, DA and MMV compounds enhanced the in vitro antibabesial activity of ZOR. When 12.5 mg/kg DA was administered in combination with ZOR in mice, it resulted in significantly greater inhibition ($P < 0.05$) of *B. microti* growth compared to 25 mg/kg DA monotherapy. These findings suggest that ZOR may serve as a promising medicinal plant for the treatment of babesiosis, particularly when combined with a modest dose of DA or potent anti-*B. bigemina* MMV compounds (5).

A current study demonstrated significant enhancements in ram growth, nutrient digestion efficiency, immune responses, antioxidant status, and ruminal fermentation following the supplementation of ginger powder. The findings suggest that supplementing rams with 5 or 7g/kg body weight of ginger powder is advisable. Furthermore, further research is warranted to explore ginger's impact on various farm animals and to develop novel feed additives (6).

The objective of another study was to assess the impact of hydroalcoholic ginger extract on the contraction and motility of the reticulum and rumen in ruminants. Hypomotility of the reticulorumen is associated with compromised physiological function of the digestive system. The prokinetic properties of ginger have been well-documented in both laboratory animals and humans. Reticulum and rumen samples were collected from eight sheep and analyzed in vitro. At concentrations of 0.1 and 1.0 mg/L, the extract showed no significant effect on motility. However, in vitro results indicated that the hydroalcoholic ginger extract contained both

spasmogenic and spasmolytic compounds. In contrast, in vivo findings suggested that the extract exhibited a stimulant effect on reticulorumen motility at a concentration of 40 mg/kg (7).

A recent study conducted by our team investigated the effects of *Zingiber officinale* aqueous extract (ZOAE) on cecal smooth muscle contractions in healthy cows. In vitro experiments were performed by suspending cecal muscle strips in an organ bath, where the ginger aqueous extract induced a concentration-dependent contraction in the cecal smooth muscle. To investigate the underlying mechanisms of the contractile effect, the administration of atropine, 1,1-dimethyl-4-diphenylacetoxypiperidinium iodide (4-DAMP), and verapamil completely inhibited the smooth muscle contractions induced by the extract. However, hexamethonium had no impact on the contraction process. The absence of reduced contraction in the presence of hexamethonium suggests that the extract contains acetylcholine-like components that act independently of nicotinic receptors. The inhibitory effects of atropine and 4-DAMP imply that part of the prokinetic action of the extract is mediated through muscarinic receptors, particularly M3 receptors. Additionally, the inhibitory action of verapamil suggests that the extract exerts its effects through L-type calcium channels. These findings indicate that ZOAE possesses a potential prokinetic effect, which could serve as a pharmacological foundation for its use in treating or preventing cecal motility disorders (8).

According to results of a study, supplementation of *Zingiber officinale* improved ruminal fermentation by reducing NH_3-N levels, methane production, and the protozoal population. Results demonstrated that 60 mg of *Z. officinale* significantly enhanced the potential extent of gas production, while methane production decreased by 21.0% and 6.3% in the 30 mg and 60 mg ginger treatments, respectively. The Entodiniinae and Diplodiniinae subfamilies had the greatest influence on methane production, with a strong correlation observed between methane production and protozoal populations. Nevertheless, further research is needed to fully validate the positive nutritional effects of ginger, particularly in relation to animal responses (9).

Another study investigated the effect of ginger root supplementation on milk production, milk composition, and physiological blood parameters in local Friesian dairy cows. Three non-pregnant cows, all of the same live weight and in either their fourth production season or first month of calving, were divided into three treatments. The second and third treatments involved supplementing the standard ration with 75 or 150 g of ginger root powder per cow per day, respectively, while the first treatment received only the standard ration. The cows were fed individually over three periods (28 days per period) in a 3x3 Latin square design. The results showed that feed intake, uncorrected and corrected daily milk yields, milk fat, protein, lactose percentages, red and white blood cell counts, hemoglobin concentration, packed cell volume, lymphocyte percentage, total protein, and globulin levels were significantly increased in treatments T2 and T3 compared to T1. However, the percentages of milk fat and blood glucose were significantly reduced in T2 and T3 compared to T1. These findings suggest that ginger root supplementation positively influences milk production and composition, as well as certain physiological blood parameters in dairy cows (10).

Ginger and poultry. Although reports on the efficacy of ginger in poultry diets are mixed, there is evidence suggesting that feeding it to animals can enhance growth performance in broilers, improve egg production and laying characteristics in hens, and contribute to better gut function and antioxidative capacity in poultry. However, to draw definitive conclusions regarding its effectiveness, the optimal doses, methods of application (feed or water), and extraction processes of ginger need to be standardized. Future research should

focus on establishing these standards, enabling the optimal use of ginger in poultry diets for the benefit of producers (11).

Ginger root extract (GRE) represents a potential alternative to conventional growth promoters like BMD in poultry production, aiming to enhance growth performance and intestinal development while mitigating oxidative stress in broiler chickens. In one study, inclusion of up to 1.5% GRE in broiler diets has been shown to improve feed efficiency, support intestinal function, and reduce the susceptibility to intestinal integrity issues and oxidative cell damage (12).

Ginger and probiotics are generally considered safe, as evidenced by the absence of acute toxic side effects during the experimental period. The results of another study indicate that experimental diets led to a reduction in gizzard weight and abdominal fat in broilers. However, these diets did not affect broiler growth performance or carcass characteristics significantly. Serum biochemistry remained largely unaffected, although there was a tendency towards healthier profiles in broilers fed the experimental diets. Moreover, both ginger and probiotics influenced the immune response of broilers; ginger likely exerted this effect through its potent antioxidant activity, while probiotics stimulated the production of natural antibodies (13).

Ginger and fish. One study corroborated the enhanced growth performance, feed efficiency, and health status of striped catfish administered a ginger-supplemented diet. The observed improvement in growth performance was attributed to a significant upregulation in digestive enzyme activity and the potentiation of antibacterial defense mechanisms associated with dietary ginger. Furthermore, the study demonstrated a notable enhancement in the antioxidative capacity of striped catfish, evidenced by increased activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), alongside a reduction in malondialdehyde (MDA) levels (14).

Another study was conducted in outdoor tanks (20 m^2) using a completely randomized design with three replicates per experimental group. Supplementation of the diet with ginger powder (GP) at 15 g/kg (GP15) resulted in a significant improvement in the growth performance of *Labeo rohita* fingerlings. Additionally, feeding the GP15 diet significantly enhanced the health status of the fish, as evidenced by notable changes in various hematological indices, including higher levels of red blood cells (RBC), white blood cells (WBC), hemoglobin (Hb), and hematocrit (Ht). The diet also improved oxidative status, indicated by increased superoxide dismutase (SOD) and decreased lipid peroxidation (LPO) levels. Furthermore, biochemical analyses revealed increased high-density lipoprotein (HDL) levels and reduced cholesterol and triglyceride concentrations, while liver enzyme activities, such as aspartate aminotransferase (AST) and alanine aminotransferase (ALT), were significantly lower. Overall, the results suggest that dietary GP supplementation can positively impact both growth and health in *L. rohita* fingerlings, highlighting its potential as a natural nutraceutical for promoting sustainable carp farming (15).

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A review on therapeutic perspectives of *Nigella sativa* in animals

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Abstract

Nigella sativa seeds (black cumin seeds, BCUS) and their bioactive constituents have been utilized for centuries as phytomedicinal agents in the treatment of various diseases in both humans and animals. *Nigella sativa* L., an ancient herb from the Ranunculaceae family, is native to Eastern Europe and Asia. Historically, BCUS have been referenced in multiple traditional medical systems, including Iranian, Indian and Chinese medicine. Scientific studies have identified BCUS as rich in antioxidants, which play a crucial role in protecting tissues from various pathologies, including hypertension, dermatological conditions, gastrointestinal disorders, and immunological dysfunctions. Additionally, BCUS have demonstrated anticancer properties and protective effects against renal and hepatic diseases. Recent research suggests that phytogenic feed additives, such as BCUS, can enhance the growth and health of animals by promoting beneficial bioactivities, including probiotic, prebiotic, and immune-enhancing effects. This review aims to examine the potential benefits of BCUS when used as feed additives in different animal models, including fish, poultry, and ruminants.

Key words: black cumin, *Nigella sativa,* ruminants, poultry, fish.

Introduction

Medicinal plants are a significant source of secondary metabolites, which can be applied in veterinary medicine as growth enhancers and potential next-generation antibacterials. These plants are widely utilized across various industries, including food, feed, pharmaceuticals, and cosmetics, due to their antiviral, antitumor, anti-inflammatory, and antioxidant properties. Within the feed industry, medicinal plants are increasingly valued for their bioactive compounds, which exhibit unique biological activities, such as antimicrobial, immunomodulatory, anti-stress, and growth-stimulating effects. Among these, black cumin (*Nigella sativa* L.) seeds (BCS) have garnered growing interest as a feed supplement due to their high oil content and diverse phytochemical composition (1). In addition to its historical significance, black cumin also holds religious importance, as it is mentioned in both Islamic and Christian texts. Beyond its cultural relevance, it has been used in traditional medicine for a variety of ailments, including asthma, fever, bronchitis, cough, chest congestion, dizziness, paralysis, chronic headaches, back pain, and inflammation. The significance of this plant has prompted the scientific community to conduct extensive phytochemical and biological investigations on *N. sativa*. Pharmacological studies have confirmed its antidiabetic, antitussive,

anticancer, antioxidant, hepatoprotective, neuroprotective, gastroprotective, immunomodulatory, anti-inflammatory, analgesic, antimicrobial, spasmolytic, and bronchodilator activities (2).

Discussion

BCUS as ruminants feed. Ruminants such as sheep, goats, and cattle are raised in cultural farms for meat, milk, and its production worldwide including the Middle East. Therefore, diets from plant sources are good choice for improving the production of these animals (3).

A study investigated the effects of dietary treatments on the productivity and health of Spanish goats, specifically in relation to the management of gastrointestinal parasites, including *Haemonchus contortus* and *Eimeria* spp. (coccidia). The results indicate that sericea lespedeza leaf meal pellets (SL), both alone and in combination with black seed meal (BS-SL), may serve as a viable alternative to conventional deworming treatments. While these diets did not significantly affect adult *H. contortus* numbers, they reduced fecal egg counts (FEC), and the SL diet also decreased fecal oocyst counts (FOC). Goats on the SL treatment exhibited higher body weights and average daily gains. Furthermore, the SL and BS-SL groups had similar packed cell volume (PCV) values to the dewormed control group (CONT), suggesting these dietary treatments helped maintain red blood cell counts and improved resilience to parasitic infections. Future research should focus on long-term studies to assess the sustained efficacy and broader impacts of these treatments on goat production and health. While the BS-SL treatment was equally effective against gastrointestinal nematodes as the SL treatment, the addition of black seed meal did not enhance the anti-parasitic effects of sericea lespedeza leaf meal. Given that black seed meal is more costly than sericea lespedeza, its inclusion in long-term treatments may be limited by economic factors (4).

Soybean meal (SBM) contains more protein than *Nigella sativa* meal (NSM), which has lower ruminal degradability and distinct amino acid profiles. NSM can partially replace SBM as a protein source in the diet of growing lambs. Replacing 75% of SBM with NSM improved feed efficiency, growth performance, and economic viability compared to other replacement levels. Thus, using NSM as an alternative protein feed for Ossimi lambs is economically advantageous. However, further long-term studies are needed to confirm its effects and explore the specific mechanisms underlying its impact on animal performance across different growth stages (5).

BCUS as poultry feed. In the recent days poultry such as chicken, pigeon, quail, and duck are preferable to humans in comparison to livestock meat due to its low cost and healthy quality of protein. The methanolic extract of *Nigella sativa* induced the expression of iNOS transcripts and increased nitric oxide production in chicken peripheral blood mononuclear cells. Additionally, the ethanolic extract of *Nigella sativa* demonstrated the potential to enhance macrophage functions, which may play a role in regulating adaptive immunity and controlling infectious diseases. These findings suggest that the methanolic extract of *Nigella sativa* could be explored as an adjuvant in conjunction with vaccines or antigens for poultry (6).

Dietary supplementation with *Nigella sativa* seeds in our recent study led to significant changes in hemogram parameters. The group fed 16% *Nigella sativa* seeds exhibited the highest values of these parameters on day 21, continuing until the conclusion of the experiment. Specifically, *N. sativa* resulted in a reduction of white blood cells (WBC) and lymphocyte counts, while increasing percentages of heterophils,

heterophil-to-lymphocyte (H/L) ratio, monocytes, eosinophils, and basophils. When broiler diets were supplemented with up to 2% *N. sativa* seeds, serum levels of these parameters were elevated. However, supplementation at 2–4% resulted in decreased serum levels of these same parameters. In conclusion, the inclusion of 1-2% *N. sativa* seeds in broiler diets serves as an effective natural growth promoter, enhancing overall performance, boosting humoral immune responses, modifying serum biochemical profiles, and inducing changes in hemogram and leukogram without adverse, residual, or harmful effects (7).

It has been reported that 5% black cumin seed (BCS), 20% kefir, and their combination effectively reduced necrotic enteritis (NE) lesion scores and mortality without negatively impacting broiler performance compared to the infected positive control. The treatments with 5% BCS, 20% kefir, and their combination were as effective as antibiotic treatment in reducing mortality during NE infection. This suggests that *Nigella sativa* (black cumin) and authentic kefir could serve as potential alternatives to commonly used antibiotics, such as BMD, for improving broiler performance, especially in cases of necrotic enteritis. The combination of kefir and BCS showed promising synergistic effects, making it the most effective approach for disease control. Therefore, the successful use of *N. sativa* and authentic kefir could contribute to improved poultry health and help address the growing issue of antibiotic resistance in the poultry industry (8).

The results of another study indicate that black cumin seed oil (BCSO) effectively reduced necrotic enteritis (NE) lesion scores and mortality compared to the infected control, although it was less effective than the antibiotic BMD treatment when NE infection was more severe. Broiler performance, particularly weight gain, was improved with BCSO supplementation in the presence of *Clostridium perfringens* (Cp) infection. However, BCSO may require a longer period to improve broiler health compared to BMD treatment. Broilers infected with Cp strains Cp#4 and Cp#6 exhibited subclinical NE, with Cp#4 causing severe disease outcomes and impairing performance more than Cp#6. The differences in disease severity and performance may be due to the impact of Cp strains on intestinal bacterial communities and shifts in specific bacterial groups, which in turn affect gut health. This warrants further investigation. The virulence of the Cp strain is an important factor to consider in animal trials. Overall, *Nigella sativa* (black cumin) as a phytogenic additive shows promise as a potential alternative to in-feed antibiotics like BMD, particularly for enhancing broiler performance in conditions prone to NE (9).

One study demonstrated the properties of chitosan/WPG nanoparticles, formed by the ionic gelation method, for encapsulating *Nigella sativa* extract (NSE). Higher chitosan concentrations (at a 2:1 molecular ratio) resulted in larger particle sizes, and the zeta potential values confirmed the stability of the nanoparticles. FTIR and XRD analyses verified the electrostatic interaction between chitosan and WPG, as well as the successful encapsulation of NSE by the wall materials. Encapsulation significantly enhanced the antiviral properties of NSE. These nanocarriers effectively protected the bioactive compounds of NSE from adverse environmental conditions. However, conducting a comparative study with the IBV vaccine could provide a deeper understanding of this approach. Given the evidence that components with antiviral properties against animal coronaviruses are also effective against human coronaviruses, NSE-loaded nanoparticles may offer a promising solution not only for the poultry industry but also for potential human medical applications (10).

The developed chitosan-loaded *Nigella sativa* (CNP-NS) nanoparticles enhanced intestinal mucosal immunity and provided protection to broiler chicks against virulent and multidrug-resistant *Salmonella*

Enteritidis (SE) infection. Oral administration of CNP-NS nanoparticles modulated both innate and systemic immune responses, leading to complete clearance of SE from the liver and fecal droppings. The CNP-NS preparation effectively recruited immune cells to the intestinal cell wall and improved villi morphology. Upregulation of the MUC-2 gene, which is involved in mucin production, microbial adherence, and colonization prevention in intestinal epithelial cells, was observed, along with increased expression of TLR-4, which is responsible for microbial recognition and cytokine signaling. Additionally, cecal cytokines (IFN-γ, IL-4, and IL-1β) and IgA gene expression were modulated. These improvements collectively enhanced broiler chick protection against SE infection. Based on these findings, we recommend the use of CNP-NS as a prophylactic and therapeutic alternative to antibiotics for managing SE infections in broiler chicks (11).

BCUS as fish feed. Fish represents one of the most important nutritive sources for humans since its flesh contains healthy fatty acids such as omega-3 and vitamins such as vitamin D. Therefore, researchers put into consideration studies on health of fish. It has been reported that supplementing Nile tilapia with a diet containing 3%/ black seed oil/body weight for 45 days significantly improved the immunity parameters measured and protected their livers and kidneys from the oxidative damage caused by *Burkholderia cepacia* bacterial infection (12).

The results of another study provide new insights into the use of black seed as a natural growth promoter, antioxidant, and hepatic-nephric protector in the aqua feed of rohu. The findings demonstrated that both 1% and 2.5% black seed levels are safe and positively impact the growth performance, antioxidant activity, and histo-biochemical parameters of rohu. Black seed is suggested as a promising feed additive for intensive fish farming practices, helping to mitigate stress-related losses and ultimately enhance overall fish production (13).

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Therapeutic effects of ethanol extract of propolis on experimental cutaneous candidiasis in horse

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Candida albicans is an opportunistic flora in the skin and mucous membranes which under specific condition causes clinical symptoms of candidiasis in human and animals (1). Prophylactic and therapeutic administrations of some anti-fungal drugs have caused drug resistance (2); therefore, there is a need to introduce novel therapeutic agents for treatment of candidiasis. The appearance of horse skin is very important from conformational point of view; hence, the present study was conducted to examine the therapeutic effects of ethanol extract of propolis on experimental cutaneous candidiasis in horse. Two six-year-old female horses approximately 400 kg were included into the present study. The immune system of animals was suppressed with dexamethasone. Thorax and flanks on the right and left sides were shaved. The *C. albicans* (CPTC: 5027) suspension was inoculated intra-dermally (3) 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 were treated with nystatin ointment, and the lesions on the right flank were treated with glycerin. The lesions on the left flank were left untreated. Cutaneous candidiasis was established within 5 days. Ethanol extract of propolis during 5 days and nystatin within 8 days improved the clinical signs of cutaneous candidiasis. It was concluded that ethanol extract of propolis could improve injuries of horse cutaneous candidiasis in a shorter time compared to nystatin. Therefore, it could be considered as a suitable alternative to other synthetic anti-fungal products.

Keywords: *Candida albicans*, Cutaneous candidiasis, Ethanol extract, Horse, Propolis

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Therapeutic effects of ethanol extract of propolis on experimental cutaneous dermatophytosis in cattle

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Trichophyton verrucosum has been introduced as a main cause of ringworm in the cattle (1). Prophylactic and over-dose administrations of some anti-fungal drugs have caused drug resistance (2). So, there is a need to introduce newer drugs for the treatment of dermatophytosis. Since the ringworm is an important zoonotic disease (3) and also causes economic disadvantages in leather industry, this study was conducted to examine the therapeutic effects of ethanol extract of propolis on experimental dermatophytosis. For this purpose, two six-month-old female calves with a weight of approximately 150 kg were used. At first, thorax and flanks on the right and left sides were shaved. After weakening the immune system of animals, 0.50 McFarland of *T. verrucosum* spore solution was inoculated dermally in each area. After the development of cutaneous dermatophytosis, the lesions on the left thorax were treated with ethanol extract of propolis, the lesions on the right thorax were treated with ketoconazole ointment, and the lesions on the left flank were treated with glycerin, daily for 14 days. The lesions on the right flank were not treated. The results of this study showed that the ethanol extract of propolis, similar to ketoconazole, was able to remove *T.verrucosum* and improve cutaneous lesions of cattle. The anti-fungal activities of propolis might be due to the pinobanksin and aromatic acids presented in it.

Keywords: Cattle, Dermatophytosis, Propolis, Ringworm, *Trichophyton verrucosum*

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Treatment of experimental *Candida* keratitis with ethanol extract of propolis in goat

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Fungal keratitis is a serious clinical infection on the cornea mainly caused by *Candida albicans* and is one of the leading causes of blindness (1). The treatment options are currently limited to a few anti-fungal agents. With the increasing incidence of drug-resistant infections, many patients fail to respond to antibiotics (2). Recent studies have shown that propolis has the potential of anti-fungal activity (3). However, only a few clinical trials have been initiated in fungal keratitis treatment using propolis. In this study, the therapeutic effects of ethanol extract of propolis on *Candida* keratitis in goats were investigated. For this purpose, 8 male goats with an approximate weight of 50 kg were used. The immune system was suppressed with dexamethasone. The cornea was scraped and contaminated with *C. albicans* (PTCC 5027). Corneal lesions were confirmed by fluorescein strip. Affected animals were organized into four distinct groups (n=2). Group I was treated topically with propolis ethanol extract dissolved in glycerin (1000 µg/mL), Group II was treated topically with nystatin ointment, Group III was treated with glycerin, and Group IV was designed as an untreated control group. The results of this study showed that ethanol extract of propolis in comparison with nystatin, could eliminate *C. albicans* and improve clinical sings of *Candida* keratitis in goats in a shorter duration period.

Key words: *Candida albicans*, *Candida* keratitis, Goat, Propolis

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Beyond antacids: The promise of herbal therapies for equine gastric ulcers

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Equine gastric ulcer syndrome (EGUS) is characterized by two distinct diseases, equine squamous gastric disease (ESGD) and equine gastric glandular disease (EGGD). The EGUS is highly prevalent especially in training horses where up to 100% can be affected (1). Stressful factors such as intense exercise, NSAIDs use and improper nutrition can increase the risk of EGUS development (2). The EGUS symptoms include loss of appetite, poor coating, diarrhea and reduced performance. However, gastroscopy should be used for definitive diagnosis. Antacids are the most common treatment for EGUS, but, their high cost and unclear effectiveness have led scientists to search for alternative treatments (3). Herbal medicines are alternative treatments that have received increasing attention. In this study, we attempted to collect a list of medicinal plants with the potential to be used in the treatment of EGUS by searching for scientific sources. Herbal materials including chamomile (*Matricaria chamomilla*), fenugreek (*Trigonella foenum graecum*) liquorice (*Glycyrrhiza glabra*), marshmallow (*Althaea officinalis*), Pot Marigold (*Calendula officinalis*), St. john's wort (*Hypericum perforatum*), flaxseed (*Linum usitatissimum*), peppermint (*Mentha piperita*), *Griffonia Simplicifolia* ,olive (*Olea Europaea*), Seabuckthorn (*Hippophae rhamnoides*), aloe vera , fermented rice extract, mixture of chinese herb alone or in combination could be significantly effective in the prevention or treatment of EGUS. However, turmeric (*Curcuma longa*) and Devil's claw (*Harpagophytum species*) had no significant effect on EGUS treatment. There seems to be good potential for effective plant substances in the treatment of EGUS and further research in this field can promise more effective and affordable treatments with fewer side effects.

Keywords: Equine, Equine gastric ulcer syndrome, Herbal medicine, Treatment

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Section 8

Small Animals

Effects of *Alpinia officinarum Hance* on sex hormones and sperm quality indices in adult dogs

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Nowadays, one of the primary concerns of reproductive researchers is organismal fertility (1). This study aimed to investigate the effects of consumption of *Alpinia officinarum Hance* extract on spermatogenesis in dogs. For this purpose, 20 adult dogs were divided into 4 groups of five including control group, which did not consume the extract and three other groups took doses of 150, 250, and 500 mg/kg (2, 3). At the beginning, a sample of venous blood (5 mL) and a sperm sample were collected. Serum samples were used to measure testosterone, malondialdehyde, glutathione peroxidase (GPX), total anti-oxidant capacity (TAC), superoxide dismutase, and catalase. To assess the effects of extract, samples were collected weekly, and after eight weeks of feeding the extract. The sperm count, plasma membrane damage, DNA damage, motility, and morphological indices were also studied (2). The findings showed that the 3rd group experienced the greatest improvement in spermatogenesis when the extract dose was increased among the groups. Significant increases in curvilinear velocity, straight-line velocity, path velocity, and TAC were observed in the 2nd and 3rd groups, respectively. Additionally, it was observed that dogs given a high dose of extract had significant increases in their levels of testosterone, GPX, and parameters like straightness/beat cross frequency throughout the course of treatment. Generally, the oral consumption of *Alpinia officinarum Hance* extract was shown to benefit adult male dogs, according to our analysis of quantitative and qualitative sperm parameters as well as blood serum biochemical parameters in different treatment groups.

Keywords: *Alpinia officinarum Hance*, Dog, Sex hormones, Sperm quality indices

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Herbal therapy for treatment of neoplasia of the urogenital system in small animals

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Cancers have attracted a great deal of attention over time due to scientific progress and increasing prevalence. Thus, investigating potent treatments has always been a major issue in veterinary medicine research. Using medicinal plants revealed many opportunities in the discovery of anti-cancer drugs and significant benefits associated with lesser side effects. Urogenital tumors notably have been important targets in those evaluations. Particular herbal medicines used for kidney, bladder, or prostate cancers mostly inhibit tumor growth by immunomodulatory effects. Mistletoe (*Viscum album*) extracts have been useful medicaments for tumors in decades. They are associated with immune system stimulation due to the plant's lectin. In a study, mistletoe extract was administrated intraperitoneally in various dose levels, and significantly inhibited tumor growth in colon, testicular, and renal cell carcinomas (1). Astragalus, Cnidium, and black cumin have also been found to have remarkable efficacy in renal carcinogenesis treatment. Some investigations claim that special herbs like garlic and kava have noteworthy therapeutic value for treating bladder cancer. In particular, kava (*Piper methysticum*) flavokawains led to more than 50% inhibition of bladder tumor cells in nude mice models (2). Furthermore, studies to develop prostate cancer treatment have proven therapeutic applications of some medicinal plants like Saint John's wort, milk thistle, red clover, etc. Saint John's wort (*Hypericum perforatum*) demonstrated significant tumor growth and metastases number reduction due to serotonin reuptake inhibition (3). As a result, medicinal plants have shown to be natural phenomena and valuable resources for developing anti-cancer therapeutics.

Keywords: Herbal medicine, Small animals, Urogenital cancer

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Therapeutic potential of *Paronychia kurdica* subsp. *kurdica* var. *kurdica* boiss in treating oral papillomatosis in dogs

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The *Paronychia* genus is represented by approximately 110 species worldwide. *Paronychia* species can adapt well to arid regions and are typically distributed in high mountainous areas. In Türkiye, *Paronychia* species are known colloquially as Boz kepekotu, kepekotu, etyaran, and siğil otu (1). We are working with *Paronychia kurdica* subsp. *kurdica* var. *kurdica* Boiss. being distributed in southeastern Türkiye, Iran, Iraq, and northern Syria (2). *Paronychia* species are used traditionally as anti-viral agents in papilloma treatment, and for their anti-bacterial and anti-fungal properties in the treatment of paronychia (1-3). An ethnobotanical study indicated that the aerial parts of the plant are used by locals for treating warts. A research conducted on cows demonstrated that a water extract prepared from the aerial parts of *P. kurdica* is effective against nipple and mammary papillomatosis (3). In our study, a water extract prepared from the aerial parts of *P. kurdica* subsp. *kurdica* var. *kurdica* Boiss. applied at least three times, with a three-day interval, was observed to almost completely heal oral papillomatosis in dogs.

Keywords: Anti-viral, Dog, Papillomatosis, *Paronychia kurdica*

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Clinical report of a successful treatment of mebendazole toxicity, with silymarin drug in kittens, referred to Veterinary Hospital of Shahid Chamran University of Ahvaz, Ahvaz, Iran

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Mebendazole is an anti-parasitic drug used to treat infections caused by worms and other parasites. It is given at a dose of 22 mg/kg in pets (1). A dose of several times has the risk of hepatotoxicity and in some cases death. In the present study, during the years 2022-2023, six kittens with symptoms of toxicity were referred to the Department of Small Animal Medicine. The most important clinical symptoms were illness, depression, lethargy, anorexia, saliva secretion and dehydration. Toxicity with mebendazole was confirmed by clinical examination and taking history. Out of six kittens, three cases were referred to the hospital less than 6 hr (group A) and other three cases, after more than 6 hr (group B). Blood samples were taken and measurement of alkaline phosphatase, alanine transaminase and aspartate aminotransferase was done. Supportive treatments were done in them including fluid therapy and administration of group B vitamins. Concurrently, they were treated with silymarin (Livergol) at a dose of 30 mg/kg (only once). In group A, liver enzymes returned to normal range within 48 hr, whereas in group B, liver enzymes were still high up to 96 hr and the process of treatment was longer. This study showed that early silymarin treatment correlated with quicker recovery. It is recommended to study on more animals and longer enzyme monitoring periods to fully understand silymarin's effects in treating mebendazole toxicity.

Keywords: Cat, Hepatotoxicity, Mebendazole, Silymarin, Toxicity

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Section 9

Theriogenology

Amelioration of sperm parameters alterations by *Malva sylvestri* hydroalcoholic extract in mature mice following testicular torsion

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Abstract

The torsion of the testicle is one of the urological emergencies that often occurs in children. Early diagnosis and detorsion surgery are very important in preventing testicular tissue damage and resulting infertility. This study aimed to investigate the effects of the hydroalcoholic extract of *Malva sylvestris* on the sperm parameters in mature mice following testicular torsion. In this study, 24 male mice were randomly divided into 4 groups (n=6) including the control group, where no intervention was performed, the torsion/detorsion group, in which the left testicle along with the spermatic cord was rotated 720 degrees counterclockwise for 1 hr, the group received *M. sylvestri* extract at a dose of 200 mg/kg and the treatment group received the extract through gavage for 35 days after torsion/detorsion. The comparison of sperm parameters in all groups indicated that sperm count, viability, motility, maturity, and DNA integrity were significantly decreased in torsion/detorsion group compared to the other groups. In torsion/detorsion + *M. sylvestri* group, all above-mentioned sperm parameters were significantly increased and improved compared to the torsion/detorsion group, but had significant differences with control and *M. sylvestri* groups. The percentage of abnormal sperm was significantly decreased in torsion/detorsion group compared to the other groups. In torsion/detorsion + *M. sylvestri* group, abnormal sperm percentages were significantly decreased compared to the torsion/detorsion group, but had significant differences with control and *M. sylvestri* groups. In conclusion, the use of *M. sylvestirs* extract due to its anti-oxidant properties can be an approach to reduce complications caused by ischemia/reperfusion injuries.

Keywords: *Malva sylvestri*, Mice, Sperm, Torsion

Introduction

The torsion of the testicle is one of the urological emergencies that often occurs in children and adolescent boys. The symptoms of this disorder include acute pain in the scrotum, nausea and vomiting (1-3). Early diagnosis and detorsion surgery are very important in preventing testicular tissue damage and resulting infertility (4). The primary pathological mechanism of testicular torsion/detorsion is ischemia/reperfusion injury. In other words, the rotation of the testicle around the longitudinal axis of the spermatic cord leads to a decrease in blood flow and the creation of ischemic conditions (5). After the detorsion surgery, the blood flow is restored to the testicular tissue, and this change causes neutrophils to be called to the testicular tissue and

the release of pro-inflammatory cytokines (6-8). After settling in the tissue, neutrophils produce a large amount of reactive oxygen species (9). Reactive oxygen species affect the structure and function of germ cells, and with their apoptosis, they lead to disruption in the spermatogenesis process of the affected testicle (10). Ischemia caused by torsion of the testis also disrupts the function of the testicular blood barrier (5). As a result, the sperm is released into the bloodstream, leading to the activation of the body's immune system and production of anti-sperm antibodies. Following these events, the spermatogonial cells of the opposite side testicle are also at risk (11-13). According to the mentioned cases, torsion of the spermatic cord not only has a negative effect on the spermatogenesis of the testicle of the same side, but also on the function of the opposite side testicle. It also leads to defects in spermatogenesis, biochemical and tissue changes (14-16). A significant increase in the apoptosis of sperm cells, decreases in the number and motility of sperms, and capillary congestion and bleeding of the testicular tissue due to the torsion injury have been reported formerly (12, 14). Since these factors lead to infertility, maintaining testicular function is considered very important (13, 15).

Malva sylvestris, also known as marshmallow, is classified as a painkiller due to its mucilage and compounds, such as flavonoids and anthocyanins. Among other properties of this plant, its diuretic, anti-pyretic and laxative properties have been highlighted. The effects of this plant have been confirmed in the treatment of mild infections and colds. Also, in traditional medicine, this plant is used to solve bladder problems (16). Various studies have shown that the *M. sylvestris* works as an immune system booster and also a cascade supplement in activating anti-inflammatory effects, stimulating white blood cells and macrophages; so, this plant can be used to relieve digestive system irritations, and urogenital ducts and respiratory tract inflammations and also treat various diseases, such as bronchitis, colitis, migraine, cold and mucous diseases (17). It has been stated that the aqueous extract of the *M. sylvestris* has been used to treat mucous diseases, cysts and diarrhea (18). Also, in India, the extract of this plant is used to treat cough, cold, and respiratory and digestive problems (19). In Brazil, *M. sylvestris* is also used to treat bronchitis, ulcers, colitis and hemorrhoids. It has been stated that this plant is effective in reducing pain and discomfort by having many properties and vitamins, and secreting special painkillers, causing faster recovery (20).

Since the *M. sylvestris* reduces apoptosis, cell damage and oxidative stress and has anti-inflammatory properties, and controlling the aforementioned factors can play an important role in preventing testicular damage, in the present study for the first time, the effect of the hydroalcoholic extract of the *M. sylvestris* on the sperm parameters in mature mice following testicular torsion was investigated.

Materials and Methods

The male mice (weighting 20-25 g, and aging 8-10 weeks) were used for the experiment. Mice were kept in the Animal Breeding Center of Bu-Ali Sina University, Hamadan, Iran, under standard conditions (temperature: 20-25 ˚C, relative humidity: 55-60% and light/dark cycle: 12 hr). Water and standard pellet diet were freely available to the animals. In this study, 24 male mice were randomly divided into 4 groups of 6 mice including the control group, where no intervention was performed, the torsion/detorsion group, in which the left testicle along with the spermatic cord was rotated 720 degrees counterclockwise and after 1 hr, it was returned to the initial state, the group received *M. sylvestri* extract at a dose of 200 mg/kg of body weight, and the treatment group received the extract through gavage for 35 days after torsion/detorsion surgery.

Surgery was performed in completely sterile conditions and under anesthesia with a single intra-peritoneal dose of ketamine hydrochloride at a dose of 100 mg/kg and xylazine at a dose of 10 mg/kg. The left scrotum and the areas close to it were shaved and disinfected. To apply torsion of the spermatic cord, a midline incision was made on the left scrotum. The left spermatic cord was rotated 720 degrees counterclockwise for 1 hr. The tunica albuginea and dartos were temporarily sutured together to fix the torsion. After 1 hr, to return the testicles to their original state, the left ischemic testicles were rotated 720 degrees clockwise and returned to their original anatomical location in the scrotum.

The *M. sylvestri* L. was collected from the plains of Hamadan province, Iran, and its hydroalcoholic extract was extracted at the Faculty of Veterinary Medicine of Bu-Ali Sina University, Hamadan, Iran. For this purpose, the collected plants were placed in 96.00% alcohol for 12 days after being converted into the powder in dry shade. Finally, after filtering the mixture, the solvent was removed using the operator's rotary device and the resulting extract was lyophilized to prepare the powder.

One day after the end of the treatment period, in order to study the count, viability, motility and morphology of the sperms, after animal euthanasia, the tail of the epididymis was cut and transferred to a micro-tube containing 1 mL of pre-heated human tubal fluid medium. Sperm suspension was used to analyze different sperm parameters. The investigations carried out included counting the average number of sperm *per* unit volume and constant dilution using hemocytometer slide, and determining the percentage of sperm motility, sperm viability (eosin nigrosin staining method), and sperms with intact DNA (DNA integrity; using acridine orange staining method), and nucleus maturation rate (Aniline Blue staining method).

The results of this study were statistically evaluated using SPSS version 19 software package and the results were expressed as mean ± standard deviation. To compare between groups, one-way analysis of variance followed by Tukey's supplementary test was used, and $p < 0.05$ was considered as a criterion for statistical inference.

Results

According to the Tables 1 and 2, the comparison of sperm parameters in all groups indicated that sperm count, viability, motility, maturity, and DNA integrity were significantly decreased in torsion/detorsion group compared to the other groups. In torsion/detorsion + *M. sylvestri* group, all mentioned sperm parameters were significantly increased and improved compared to the torsion/detorsion group, but had significant differences with control and *M. sylvestri* groups. The percentage of abnormal sperm was significantly decreased in torsion/detorsion group compared to the other groups. In torsion/detorsion + *M. sylvestri* group, abnormal sperm percentages were significantly increased and improved compared to the torsion/detorsion group, but had significant differences with control and *M. sylvestri* groups ($p < 0.05$; Tables 1 and 2).

Table 1. Comparison of the mean count, viability and motility of sperms in the experimental groups.

Groups	Count (10^6/mL)	Viability (%)	Motility (%)
Control	14.10 ± 1.65 [a]	81.33 ± 4.56 [a]	75.50 ± 6.49 [a]
Torsion/detorsion	6.49 ± 1.21 [b]	39.66 ± 6.85 [b]	34.83 ± 7.12 [b]
Malva sylvestri	15.30 ± 2.31 [a]	79.83 ± 4.76 [a]	76.58 ± 5.34 [a]
Torsion/detorsion + *Malva sylvestri*	10.46 ± 1.58 [c]	68.17 ± 6.32 [c]	64.50 ± 6.86 [c]

Different letters indicate significant differences ($p < 0.05$).

Table 2. Comparison of the mean percentage of mature and abnormal sperms, and sperms with DNA integrity in the experimental groups.

Groups	Maturity (%)	DNA integrity (%)	Abnormality (%)
Control	99.17 ± 0.85 [a]	92.16 ± 1.86 [a]	5.66 ± 0.66 [a]
Torsion/detorsion	89.66 ± 2.35 [b]	69.66 ± 5.41 [b]	26.16 ± 2.16 [b]
Malva sylvestri	98.83 ± 1.43 [a]	89.83 ± 3.78 [a]	6.83 ± 1.11 [a]
Torsion/detorsion + *Malva sylvestri*	94.33 ± 1.13 [c]	81.50 ± 2.65 [c]	12.33 ± 2.16 [c]

Different letters indicate significant differences ($p < 0.05$).

Fig. 1. Staining of sperm samples (×400). **A)** Eosin-nigrosin staining: Live sperm (yellow arrow) and dead sperm (red arrow) are obvious; **B)** Aniline blue staining. Mature sperm (dark blue arrow) and immature sperm (light blue arrow) are seen; **C)** Acridine orange staining. Sperm with intact DNA (green arrow) and sperm with damaged DNA (yellow arrow) are observed.

Discussion

The aim of this study was to evaluate the protective effect of *M. sylvestris* extract against damages inflicted by spermatic cord torsion/detorsion on sperm parameters in mature male mice. The various studies have proved the anti-oxidant and anti-inflammatory effects of *M. sylvestris*. Under our experimental conditions, testicular dysfunction caused by torsion/detorsion was confirmed by decreased sperm motility and count. Moreover, sperm count and motility were improved in torsion/detorsion + *M. sylvestri* animals.

On the other hand, animals exposed to *M. sylvestris* extract at a dose level of 200 mg/kg showed elevation in sperm count and motility reductions induced by torsion/detorsion and a subsequent recovery towards normalization compared to the torsion/detorsion mice. The anti-oxidants in *M. sylvestris* extract are likely able to counteract or minimize the undesirable effects induced by ischemia-reperfusion.

The modulatory effect of *M. sylvestirs* extract administration on sperm parameters alterations provoked by testicular ischemia-reperfusion was quite noticeable. Supplementation of *M. sylvestirs* extract in the diet of torsion/detorsion mice improved the sperm parameters and reduced sperm damage, which could be attributed to the anti-oxidant proprieties of *M. sylvestirs* extract (19).

Overall, it was concluded that *M. sylvestirs* extract exhibits anti-oxidant and anti-peroxidative properties, which could have a beneficial effect against oxidative damage induced by testicular ischemia-reperfusion. The findings of our study showed that one-sided torsion of the spermatic cord leads to dysfunction of the testicles and reduction of the spermatogenic process. This disorder is probably related to primary ischemia/reperfusion injury and the resulting autoimmune response. The use of *M. sylvestirs* extract can be a new approach in the

clinic by reducing complications caused by ischemia/reperfusion injury through blood-testis-barrier repair and spermatogenesis stimulation (20). The present study clearly demonstrated that administration of *M. sylvestris* extract attenuates testicular ischemia-reperfusion injuries through counteraction with free radicals *via* its anti-oxidant potentials. It seems that the torsion of the spermatic cord leads to the destruction of the tissue structure and reduction of spermatogenic potential. Although the exact mechanism involved in the process has not been correctly determined, it can be attributed to the damage of blood vessels and blood supply disruption, as well as early ischemia-reperfusion injury and the resulting autoimmune response. The use of *M. sylvestirs* extract due to its anti-oxidant properties can be an approach to reduce complications caused by ischemia/reperfusion injury in the clinic.

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Protective effects of *Malva sylvestri* hydroalcoholic extract on spermatogenesis indices in mature mice following testicular torsion

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Abstract

The important cause of infertility in males is torsion of the testicle in children. The medicinal plants are one of the approaches to improve the torsion adverse effects. This study aimed to investigate the effects of the hydroalcoholic extract of the *Malva sylvestris* on the spermatogenesis indices in mature mice following testicular torsion. In this study, 24 male mice were randomly divided into four groups (n=6) including the control group, where no intervention was performed, the torsion/detorsion group, in which the left testicle along with the spermatic cord was rotated 720 degrees counterclockwise for 1 hr, the group received *M. sylvestri* extract at a dose of 200 mg/kg and the treatment group received the extract through gavage for 35 days after torsion/detorsion. The comparison of spermatogenesis indices and histomorphometrical parameters in all groups indicated that seminiferous tubules diameter, germinal epithelium height, tubular differentiation index, repopulation index, and spermiation index were significantly decreased in torsion/detorsion group compared to the other groups. In torsion/detorsion + *M. sylvestri* g, all above-mentioned parameters were significantly increased and improved compared to the torsion/detorsion group, but had significant differences with control and *M. sylvestri* groups. The comparison of testicular capsule diameter showed that there were no significant differences between groups. Finally, it can be concluded that torsion and detorsion and complications caused by testicular ischemia/reperfusion cause damage and disorders in the male reproductive system, and it seems that *M. sylvestirs* hydroalcoholic extract due to the anti-oxidant and anti-inflammatory properties can improve the adverse effects of ischemia.

Keywords: Infertility, Testicular torsion, *Malva sylvestris*, Spermatogenesis, Histomorphometry

Introduction

Infertility is a common problem affecting approximately 15.00 % of couples trying to conceive a baby. In more than 50.00 % of couples having difficulty getting pregnant, the problem is at least partly related to male reproductive issues (1). Different factors contribute to male infertility or sub-fertility; including testicular torsion (2), which is one of the most common urological issues targeting young men. Annual incidence of testicular torsion among those less than 25 years old was reported to be 1 in 4000 (3). The highest peak was observed around puberty (accounted for 65.00 % of all torsions) with another much smaller peak in the first year of life (3). It is estimated that around 400 boys lose their testis because of testicular torsion in the United Kingdom annually (4). Therefore, this high rate of testicular torsion makes it an important factor leading to

infertility in men. It seems to be the second most common reason for emergency surgery in young men in Britain (5). During testicular torsion, unilateral or bilateral testicles get twisted in the scrotum leading to an interruption of testicular blood flow and therefore ischemia-related dysfunctions (6). The injuries caused by testicular torsion are severe enough to lead to ipsilateral damages, which are the consequences of significant increase in blood flow after detorsion (7). The success rate of treatment depends on the duration of torsion and early diagnosis, which could be a routine management for preservation of spermatogenesis and fertility (8). Although reperfusion is necessary for the survival of ischemic tissue, it can also trigger destructive pathophysiological cascades including generation of oxygen-derived free radicals (9). Reactive oxygen species (ROS) distort organ function owing to DNA damage, endothelial cell injury, and germinal cell necrosis (10). Researches focusing on stem cells have generated great optimism in the treatment of many human diseases in the near future. Particularly, bone marrow stem cells are well defined and have long been used therapeutically (11). The merits of stem cell therapy for ischemia have been proved in several tissues, such as muscle, heart, and even brain.

Malva sylvestris, also known as marshmallow, is classified as a painkiller due to its mucilage and compounds, such as flavonoids and anthocyanins. Among other properties of this plant, its diuretic, anti-pyretic and laxative properties have been highlighted. The effects of this plant have been confirmed in the treatment of mild infections and colds. Also, in traditional medicine, this plant is used to solve bladder problems (12). Various studies have shown that the *M. sylvestris* works as an immune system booster and also a cascade supplement in activating anti-inflammatory effects, stimulating white blood cells and macrophages; so, this plant can be used to relieve digestive system irritations, and urogenital ducts and respiratory tract inflammations and also treat various diseases, such as bronchitis, colitis, migraine, cold and mucous diseases (13). It has been stated that the aqueous extract of the *M. sylvestris* has been used to treat mucous diseases, cysts and diarrhea (14). Also, in India, the extract of this plant is used to treat cough, cold, and respiratory and digestive problems. In Brazil, *M. sylvestris* is also used to treat bronchitis, ulcers, colitis and hemorrhoids. It has been stated that this plant is effective in reducing pain and discomfort by having many properties and vitamins, and secreting special painkillers, causing faster recovery (15).

Since the *M. sylvestris* reduces apoptosis, cell damage and oxidative stress and has anti-inflammatory properties, and controlling the aforementioned factors can play an important role in preventing testicular damage, in the present study for the first time, the effect of the hydroalcoholic extract of the *M. sylvestris* on the spermatogenesis indices in mature mice following testicular torsion was investigated.

Materials and Methods

The male mice (weighting 20-25 g, and aging 8-10 weeks) were used for the experiment. Mice were kept in the Animal Breeding Center of Bu-Ali Sina University, Hamadan, Iran, under standard conditions (temperature: 20-25 ˚C, relative humidity: 55-60% and light/dark cycle: 12 hr). Water and standard pellet diet were freely available to the animals. In this study, 24 male mice were randomly divided into 4 groups of 6 mice including the control group, where no intervention was performed, the torsion/detorsion group, in which the left testicle along with the spermatic cord was rotated 720 degrees counterclockwise and after 1 hr, it was

returned to the initial state, the group received *M. sylvestri* extract at a dose of 200 mg/kg of body weight, and the treatment group received the extract through gavage for 35 days after torsion/detorsion surgery.

Surgery was performed in completely sterile conditions and under anesthesia with a single intra-peritoneal dose of ketamine hydrochloride at a dose of 100 mg/kg and xylazine at a dose of 10 mg/kg. The left scrotum and the areas close to it were shaved and disinfected. To apply torsion of the spermatic cord, a midline incision was made on the left scrotum. The left spermatic cord was rotated 720 degrees counterclockwise for 1 hr. The tunica albuginea and dartos were temporarily sutured together to fix the torsion. After 1 hr, to return the testicles to their original state, the left ischemic testicles were rotated 720 degrees clockwise and returned to their original anatomical location in the scrotum.

The *M. sylvestri* L. was collected from the plains of Hamadan province, Iran, and its hydroalcoholic extract was extracted at the Faculty of Veterinary Medicine of Bu-Ali Sina University, Hamadan, Iran. For this purpose, the collected plants were placed in 96.00% alcohol for 12 days after being converted into the powder in dry shade. Finally, after filtering the mixture, the solvent was removed using the operator's rotary device and the resulting extract was lyophilized to prepare the powder.

One day after the end of the treatment period, in order to perform histomorphometrical study, animals were euthanized. Then, left testis tissue samples were fixed in 10.00% buffered formalin. After tissue passage and preparation of tissue sections (thickness of 7 µm), they were stained with hematoxylin and eosin. All samples were evaluated with multiple magnifications (400x and 1000x). The average seminiferous tubules diameter (STsD), germinal epithelium height (GEH), and testicular capsule diameter (TCD) were determined in micrometers by Dino-Lite camera and Dino Capture software (version 2). In order to check the tubular differentiation index (TDI), the percentage of seminiferous tubules with more than three layers of differentiated germinal cells from type A spermatogonia was counted and the TDI was considered positive. Also, to calculate the repopulation index (RI), the ratio of active spermatogonia (type B spermatogonia with dark nuclei) to inactive spermatogonia (type A spermatogonia with light nuclei) was calculated in the seminiferous tubules. To determine the spermiation index (SI), the percentage of seminiferous tubules with normal spermatogenesis was considered as positive SI.

The results of this study were statistically evaluated using SPSS version 19 software package and the results were expressed as mean ± standard deviation. To compare between groups, one-way analysis of variance followed by Tukey's supplementary test was used, and $p < 0.05$ was considered as a criterion for statistical inference.

Results

According to the Tables 1 and 2, the comparison of spermatogenesis indices and histomorphometrical parameters in all groups indicated that STsD, GEH, TDI, RI, and SI were significantly decreased in torsion/detorsion group compared to the other groups. In torsion/detorsion + *M. sylvestri* group, all mentioned parameters were significantly increased and improved compared to the torsion/detorsion group, but had significant differences with control and *M. sylvestri* groups. The comparison of TCD showed that there were no significant differences between groups ($p < 0.05$; Tables 1 and 2, and Fig. 1).

Table 1. Comparison of the mean seminiferous tubules diameter, germinal epithelium height, and diameter of the testicular capsule in the experimental groups.

Groups	Seminiferous tubules diameter (µm)	Germinal epithelium height (µm)	Testicular capsule diameter (µm)
Control	235.42 ± 10.85 [a]	65.38 ± 4.68 [a]	10.06 ± 2.69 [a]
Torsion/detorsion	149.26 ± 12.32 [b]	36.54 ± 3.27 [b]	12.68 ± 3.89 [a]
Malva sylvestri	237.90 ± 13.57 [a]	61.76 ± 6.20 [a]	9.79 ± 1.95 [a]
Torsion/detorsion + *M. sylvestri*	205.68 ± 9.56 [c]	51.32 ± 5.13 [c]	11.23 ± 1.52 [a]

Different letters indicate significant differences ($p < 0.05$).

Table 2. Comparison of the mean tubular differentiation index, repopulation index, and spermiation index in the experimental groups.

Groups	Tubular differentiation index (%)	Repopulation index (%)	Spermiation index (%)
Control	86.80 ± 2.56 [a]	92.20 ± 3.85 [a]	93.60 ± 4.67 [a]
Torsion/detorsion	59.20 ± 3.09 [b]	53.80 ± 3.18 [b]	53.20 ± 3.84 [b]
Malva sylvestri	88.20 ± 4.75 [a]	89.60 ± 2.04 [a]	88.60 ± 4.55 [a]
Torsion/detorsion + *M. sylvestri*	77.60 ± 2.16 [c]	81.40 ± 1.59 [c]	79.80 ± 3.26 [c]

Different letters indicate significant differences ($p < 0.05$).

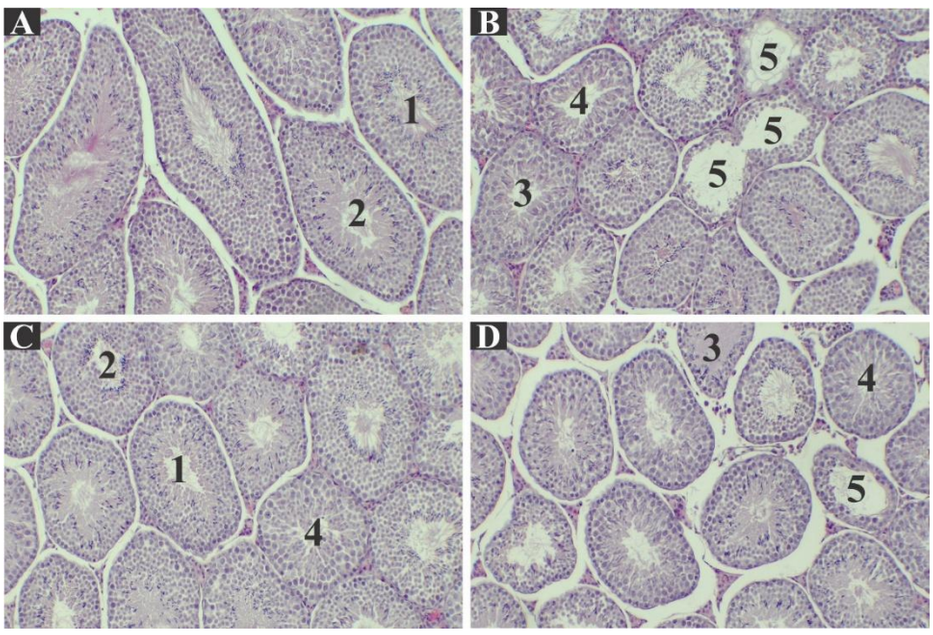

Fig. 1. Tissue sections of the testis in the experimental groups. Hematoxylin and eosin staining (×400). **A)** Control group; **B)** Torsion/detorsion group; **C)** *Malva sylvestri* extract received group (200 mg/kg); **D)** Treatment group received the extract after torsion/detorsion 1: Tubular differentiation index (TDI)-positive; 2: Spermiation index (SI)- positive; 3: TDI-negative; 4: SI-negative; 5: Degenerated seminiferous tubules.

Discussion

The results of this study showed that twisting the testicle around the axis of the spermatic cord causes the destruction of the testicular tissue and reduces the quality of spermatogenesis, while using *M. sylvestirs* extract after twisting improves the condition of the testicular tissue and the quality of spermatogenesis. The main

mechanisms of testicular injury after torsion include ischemia complications followed by hypoxia, as well as injuries caused by torsion due to increased oxygen pressure when blood flow is restored (16).

Ischemia of the testicular tissue causes the death of the related tissue's germ cells, which is mainly caused by the lack of oxygen supply needed for metabolic activities, depletion of stored cellular energy, and accumulation of toxic metabolites. As a result of ischemia and reperfusion, the increase in ROS production and cascading release of apoptosis-promoting enzymes such as caspases significantly cause cell damage caused by ischemia/reperfusion in the testicular tissue. Previous research showed that the severity of tissue damage under ischemia has a direct relationship with the duration and degree of torsion 17.

In a study in 2012, Filho *et al*., showed that ischemia and reperfusion for 1 hr produced results similar to previous studies (18). Studies have shown that ischemia/reperfusion caused by torsion and detorsion of the testicle by inducing destruction in the cells of the germinal epithelium of the seminiferous tubules of the testicular tissue causes extensive tissue changes such as a decrease in parameters like the thickness of the germinal epithelium of the seminiferous tubules and spermatogenic indices (19).

The results of the morphometrical examination of the present study are also consistent with these studies and showed that ischemia for 1 hr and then blood flow restoration caused changes such as decreases in the thickness of the germinal epithelium and diameter of the seminiferous tubules, as well as RI, SI, and TDI indices. Today, significant efforts are being made to identify factors and compounds that can inhibit or reduce the effects of ischemia/reperfusion in the testicular tissue as a new treatment method to reduce the unwanted effects of testicular torsion in the fertility process. The present study showed that the simultaneous use of *M. sylvestirs* extract along with surgical treatment may improve the effects of ischemia and reperfusion.

Finally, it can be concluded that torsion and detorsion and complications caused by testicular ischemia/reperfusion cause damage and disorders in the male reproductive system, and it seems that *M. sylvestirs* hydroalcoholic extract *via* anti-oxidant and anti-inflammatory activities can improve the adverse effects of ischemia.

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A review of medicinal plants used for the restoration of reproductive functionality following agricultural toxin-induced reproductive toxicity

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Abstract

Agricultural toxins, including pesticides and heavy metals significantly threaten reproductive health leading to infertility, hormonal imbalances and testicular damage. This review explores the potential of medicinal plants such as *Withania somnifera* (Ashwagandha), *Tribulus terrestris*, *Ficus carica*, and *Mucuna pruriens* (Velvet bean) to restore reproductive functionality compromised by these toxins. These plants contain bioactive compounds like flavonoids, saponins and alkaloids which offer antioxidant, anti-inflammatory and hormone-regulating properties. In addition, the review highlights the role of antioxidants such as curcumin, resveratrol, vitamin E and N-acetylcysteine (NAC) in enhancing the therapeutic effects of these plants. Antioxidants help mitigate oxidative stress, improve sperm quality and enhance testosterone levels, making them valuable in counteracting toxin-induced reproductive damage. The synergistic use of medicinal plants and antioxidants allows for lower doses and reducing potential side effects while maximizing efficacy. Detailed information on specific doses used in various studies is provided demonstrating the effectiveness of these natural therapies in reversing reproductive toxicity. However, further research is necessary to optimize treatment regimens, assess long-term safety and conduct clinical trials to confirm efficacy in humans. In conclusion, combining medicinal plants with antioxidants presents a promising approach to restoring reproductive health following exposure to agricultural toxins. With ongoing research, these therapies could become a practical solution for improving reproductive outcomes in affected populations.

Keywords: Agricultural Toxin, Medicinal plants, Reproductive Toxicity

Introduction

Agricultural toxins, such as pesticides, herbicides and heavy metals are widely used to enhance crop yields and control pests. However, these substances severely affect human health, particularly the reproductive system (1). Reproductive toxicity due to exposure to these chemicals manifests in various forms including hormonal imbalances, reduced fertility, testicular damage and embryonic malformations (2). The growing concern over these reproductive issues has led to a surge in research focused on finding effective remedies.

Medicinal plants have long been used in traditional medicine to treat various ailments including reproductive disorders (3). These plants contain bioactive compounds such as flavonoids, saponins, alkaloids and tannins which possess antioxidant, anti-inflammatory and hormone-regulating properties (4). These properties are particularly useful in counteracting the effects of agricultural toxins on reproductive health.

Additionally, using antioxidants which help reduce oxidative stress has been recognized as a crucial aspect of restoring reproductive functionality (5).

This review examines the medicinal plants and antioxidants that have been studied for their potential to restore reproductive health following exposure to agricultural toxins. The discussion includes the specific doses of these treatments used in various studies and their effectiveness in mitigating reproductive toxicity.

Medicinal plants in restoring reproductive functionality. Medicinal plants have been extensively researched for their potential to reverse reproductive toxicity induced by agricultural toxins. One of the most studied plants is *Withania somnifera* (Ashwagandha). Kumar et al. (6) investigated the effects of Ashwagandha root extract on male rats exposed to pesticide-induced reproductive toxicity. The study used a dose of 200 mg/kg body weight of Ashwagandha extract administered orally for 60 days, resulting in significant improvements in sperm count, motility and serum testosterone levels. Additionally, markers of oxidative stress in the testes were markedly reduced indicating the plants strong antioxidant properties.

Tribulus terrestris is another plant that has shown promise in mitigating reproductive toxicity. Kumar et al. (7) reported that administering of *T. terrestris* extract for 45 days to male rats exposed to heavy metals restored normal sperm morphology, increased testosterone levels, and reduced oxidative stress in the testes. This improvement was attributed to the plant high content of saponins which have been shown to enhance androgen production and protect against oxidative damage. *Ficus carica* is another medicinal plant that has been studied for its effects on reproductive toxicity. A study by Mahanem et al. (8) administered *F. carica* fruit extract to male rats exposed to cadmium, a heavy metal known to cause testicular damage. The treatment for 30 days led to a significant reduction in testicular oxidative stress and an increase in sperm count and motility. The study suggested that the flavonoids and phenolic compounds in *F. carica* were responsible for its protective effects. Another important plant is *Mucuna pruriens*, which has been traditionally used to enhance fertility. Research by Eze et al. (9) evaluated the effects of *M. pruriens* seed extract on male rats exposed to lead-induced reproductive toxicity. After 90 days of treatment, there was a marked improvement in sperm quality including increased sperm count, motility and viability. The study also reported a significant reduction in testicular lipid peroxidation indicating the plants antioxidant potential.

Role of antioxidants in treatment regimens. Antioxidants play a pivotal role in neutralizing the oxidative stress caused by agricultural toxins, thereby, aiding in the restoration of reproductive health. The use of antioxidants in combination with medicinal plants has been shown to enhance the therapeutic effects. *Curcumin*, the active compound in *Curcuma longa* (turmeric), has been extensively studied for its potent antioxidant properties. Lonare et al. (10) administered curcumin for 60 days to male rats exposed to organophosphate pesticides. The study found that curcumin supplementation significantly reduced oxidative stress in the testes, improved sperm parameters and increased testosterone levels. When combined with Ashwagandha, the effects were more pronounced, demonstrating the synergistic potential of curcumin and medicinal plants. *Resveratrol*, a polyphenolic compound found in grapes and berries, has also shown protective effects against reproductive toxicity. Jabłońska–Trypuć et al. (11) used resveratrol for 45 days in a rat model of pesticide-induced reproductive toxicity. The treatment preserved spermatogenesis reduced testicular apoptosis and enhanced the antioxidant defense system. Combining resveratrol with *T. terrestris* further improved reproductive outcomes, highlighting the benefits of combining antioxidants with medicinal

plants. *Vitamin E* is another well-known antioxidant that has been used in studies to mitigate reproductive toxicity. In a study by Ain et al. (12) on rats exposed to organophosphates, vitamin E was administered for 30 days. The results showed a significant reduction in testicular oxidative stress and improved sperm quality. The study suggested that vitamin E when combined with *F. carica*, could provide a comprehensive approach to restoring reproductive functionality. Another antioxidant, *N-Acetylcysteine* (NAC), has been studied for its role in protecting against reproductive damage. A study by Ji et al. (13) administered of NAC for 30 days to male rats exposed to cadmium. The treatment significantly improved sperm motility, reduced testicular apoptosis and restored serum testosterone levels. Combining NAC with *M. pruriens* seed extract was particularly effective in enhancing reproductive outcomes.

Discussion

The effectiveness of medicinal plants in restoring reproductive functionality varies based on the type of toxin and the specific plant used. For example, *W. somnifera* has shown significant efficacy against pesticide-induced reproductive toxicity likely due to its adaptogenic and antioxidant properties (. In contrast, *T. terrestris* has demonstrated greater effectiveness in cases of heavy metal-induced reproductive damage possibly due to its ability to enhance testosterone production and combat oxidative stress (6).

Other plants, such as *F. carica* and *M. pruriens*, have also shown promising results in restoring reproductive health compromised by various toxins. The active compounds in these plants, including flavonoids, saponins and alkaloids work through multiple pathways to exert their protective effects. For instance, *F. carica* has been effective in reducing oxidative stress and improving sperm parameters in cadmium-exposed rats (8), while *M. pruriens* has been particularly effective in mitigating lead-induced reproductive toxicity by enhancing antioxidant defenses and improving sperm quality (9).

The bioactive compounds in medicinal plants often exert their effects through multiple mechanisms. For example, the withanolides in *W. somnifera* are known to modulate the hypothalamic-pituitary-gonadal axis, improving hormone balance and reducing stress-induced reproductive dysfunction (7). Similarly, the saponins in *T. terrestris* stimulate the release of nitric oxide which improves blood flow to the reproductive organs and enhances erectile function (6).

Combining medicinal plants with antioxidants such as curcumin, resveratrol, vitamin E and NAC can result in synergistic effects that enhance the overall efficacy of the treatment. For example, curcumin potent antioxidant properties complement the adaptogenic effects of Ashwagandha leading to a more comprehensive approach to mitigating reproductive toxicity (10). Similarly, the combination of resveratrol with *T. terrestris* has been shown to amplify the protective effects on reproductive health, providing a dual approach to combating oxidative stress and hormonal imbalances (11).

The synergistic effects of combining medicinal plants with antioxidants also allow for lower doses of each component which can minimize potential side effects while maximizing therapeutic benefits. For example, combining vitamin E with *F. carica* has been shown to enhance the antioxidant defense system in the testes leading to improved sperm quality and reduced oxidative damage (12). This approach improves reproductive outcomes and reduces the risk of adverse effects associated with higher doses of individual treatments.

In conclusion, medicinal plants offer a promising approach to restoring reproductive functionality following agricultural toxin-induced reproductive toxicity. Plants such as *W. somnifera, T. terrestris, F. carica* and *M. pruriens* have demonstrated significant potential in reversing reproductive damage particularly when combined with antioxidants like curcumin, resveratrol, vitamin E and NAC. The use of these natural therapies provides a dual benefit by restoring reproductive health and protecting against oxidative stress which is a key contributor to reproductive toxicity. Further research is needed to optimize the use of these plants and antioxidants including determining the most effective doses and combinations for specific types of reproductive toxicity. Additionally, clinical trials in humans are essential to confirm the efficacy and safety of these treatments. With continued research and development, medicinal plants and antioxidants could become a valuable part of the therapeutic arsenal against reproductive toxicity in agricultural settings.

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A review of using medicinal plant extracts to preserve ram semen

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Abstract

Preserving ram sperm for artificial insemination and breeding programs is critical in enhancing genetic diversity, increasing productivity, and ensuring the sustainability of sheep farming. This process is far from routine, requiring careful attention to detail to maintain the quality and viability of stored sperm. In recent years, research has increasingly focused on using medicinal plant extracts due to their natural antioxidant properties which can mitigate oxidative stress—a leading cause of sperm degradation during storage. Antioxidants are vital because they neutralize free radicals which can damage sperm cells and reduce fertility rates. This review emphasizes the significance of incorporating plant-based antioxidants into sperm preservation techniques. It explores various medicinal plant extracts such as green tea (*Camellia sinensis*), rosemary (*Rosmarinus officinalis*), garlic (*Allium sativum*), and turmeric (*Curcuma longa*), all of which have shown promise in extending sperm longevity. Highlighting critical studies and the optimal dosages necessary to achieve the best outcomes, the review provides an in-depth analysis of both the advantages and potential limitations of using these natural compounds in ram sperm storage. Through this comprehensive examination, the review underscores the growing importance of plant-based solutions in improving reproductive efficiency in livestock, offering valuable insights for future research and practical applications in sheep breeding programs.

Keywords: Medicinal plant, Ram, Semen, Storage

Introduction

Artificial insemination (AI) in sheep is a widely used reproductive technology that allows for livestock-controlled breeding, thereby, improving genetic diversity and productivity (1). However, the success of AI depends significantly on the quality of stored sperm, which is often compromised by oxidative stress during storage (2). Oxidative stress, caused by an imbalance between reactive oxygen species (ROS) and antioxidants leads to lipid peroxidation, DNA damage and reduced sperm motility and viability (3). To reassure the audience about the effectiveness of the proposed solution this review emphasizes the crucial role of antioxidants in mitigating these effects. Recent studies have explored the potential of medicinal plant extracts as a source of natural antioxidants (4). Medicinal plants, with their centuries-old history in traditional medicine, have been recognized for their therapeutic properties including their antioxidant effects. These plants contain a variety of bioactive compounds such as flavonoids, phenolic acids and terpenoids which can scavenge ROS and protect cells from oxidative damage. In the context of sperm preservation, these natural antioxidants offer a promising alternative to synthetic antioxidants, which can have cytotoxic effects at higher concentrations. This review

examines the use of medicinal plant extracts in the preservation of ram sperm, with a focus on their antioxidant properties and optimal dosages, highlighting the potential benefits of this approach.

Thyme (*Thymus vulgaris*). A study explored the effects of *T. vulgaris* (thyme) extract on ram sperm motility, viability and membrane integrity during cryopreservation. The study demonstrated that thyme extract significantly enhanced sperm motility and membrane integrity after cryopreservation. This effect was attributed to thymol, a primary component of thyme, which acts as a potent antioxidant, reducing lipid peroxidation and protecting spermatozoa from oxidative stress (5). The ability of thymol to mitigate the negative effects of freezing on sperm cells makes thyme a promising plant extract for sperm preservation protocols.

Rosemary (*Rosmarinus officinalis*). *Rosmarinus officinalis*, or rosemary, is another medicinal plant whose extract has been evaluated for its effects on ram sperm preservation. Motlagh et al. reported that the inclusion of rosemary extract in sperm extenders resulted in a significant improvement in sperm motility and a reduction in reactive ROS levels after cryopreservation. The phenolic compounds in rosemary, particularly carnosic acid and rosmarinic acid, are thought to provide these protective effects by scavenging free radicals and preventing oxidative damage (6). These results suggested that rosemary could be an effective supplement for reducing oxidative stress in cryopreserved sperm, improving the overall quality and viability of the stored semen.

Green tea (*Camellia sinensis*). The antioxidant effects of *C. sinensis* (green tea) extract on ram sperm during liquid storage were examined by Mehdipour et al. This study found that green tea extract at an optimal concentration effectively maintained sperm motility and reduced ROS levels during cryopreservation. The polyphenols in green tea, particularly epigallocatechin gallate, were highlighted as critical components contributing to the antioxidant effect. However, it was noted that higher concentrations of green tea extract could be detrimental to sperm quality indicating the importance of using appropriate dosages to avoid negative effects on sperm viability (7). This finding emphasizes the delicate balance needed in antioxidant supplementation to ensure maximum benefit without harmful side effects.

Garlic (*Allium sativum*). Known for its wide range of health benefits, garlic (*A. sativum*) has also been studied for its potential in sperm preservation. Jerez-Ebensperger et al. investigated the effects of garlic extract on ram sperm. They found that adding the extract to the sperm extender significantly improved sperm motility, viability and membrane integrity after storage in the refrigerator. The antioxidant activity was attributed to the organosulfur compounds in garlic, particularly allicin which scavenged ROS and reduced oxidative stress on the sperm cells (8). Garlic potent antioxidant properties make it a valuable natural supplement for improving the preservation of ram sperm, offering a simple and natural approach to counteracting the negative effects of oxidative stress during storage.

Aloe vera (*Aloe barbadensis*). Aloe Vera (*A. barbadensis*) has been recognized for its antioxidant and anti-inflammatory properties, and its potential benefits in sperm preservation have been explored in several studies. Câmara et al. examined the effects of Aloe Vera extract on ram sperm during cryopreservation and reported significant improvements in sperm motility and membrane integrity. The beneficial effects were attributed to the polysaccharides and phenolic compounds present in Aloe Vera, which protected sperm cells from oxidative stress (9). Aloe Vera wide availability and potent antioxidant properties make it a practical choice for enhancing sperm preservation techniques.

Discussion

Medicinal plant extracts in ram sperm preservation offer a promising approach to enhancing sperm quality and longevity during storage. The antioxidant properties of these extracts, derived from their bioactive compounds, are crucial in combating oxidative stress, a significant factor in sperm degradation (10). However, the effectiveness of these extracts depends on their concentration, the specific bioactive compounds they contain and the conditions under which the sperm is stored.

One of the challenges in using medicinal plant extracts is identifying the optimal dosage that provides maximum antioxidant protection without inducing cytotoxicity (11). As seen in the studies reviewed, the effectiveness of plant extracts like thyme, rosemary, *Ginkgo biloba*, green tea and saffron varies depending on the concentration used. Therefore, future research should focus on optimizing the dosage of these extracts to maximize their benefits while minimizing potential risks (12). Another consideration is the possible synergistic effects of combining different plant extracts or combining plant extracts with synthetic antioxidants (13). However, this approach requires careful consideration of the interactions between different compounds and their combined effects on sperm.

Additionally, while most studies have focused on the effects of medicinal plant extracts during liquid storage or cryopreservation of ram sperm, there is a need for more research on their long-term effects on sperm function and fertility after AI (10). The ultimate goal of using these extracts is to improve reproductive outcomes, and future studies should assess the fertility rates and offspring quality following the use of plant-extract-preserved sperm.

Medicinal plant extracts offer a natural and potentially effective means of enhancing the preservation of ram sperm through their antioxidant properties. Key findings from the reviewed studies indicated that these extracts could improve sperm motility, viability and integrity during both liquid storage and cryopreservation by mitigating oxidative stress. However, the success of these extracts depends on the appropriate dosage and the specific bioactive compounds they contain. Future research should focus on optimizing dosages, exploring synergistic effects and assessing the long-term impact of these extracts on reproductive outcomes. As the use of natural antioxidants in sperm preservation becomes more prevalent, it holds the potential to improve the efficiency of AI in sheep and contribute to the sustainability of sheep farming by enhancing genetic diversity and productivity.

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Medicinal plants in the treatment of bovine mastitis: Promising alternatives to conventional antibiotics

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Abstract

Mastitis, a widespread and costly disease in dairy cattle, negatively impacts animal welfare and milk production. It is primarily caused by bacterial infections, categorized into contagious and environmental pathogens which provoke inflammation in the mammary gland leading to reduced milk yield and quality. While antibiotics have been the traditional treatment for over 50 years, the rising threat of antibiotic resistance and the presence of antimicrobial residues in milk and the environment pose potential risks to human health that have prompted the exploration of alternative therapies. Phytotherapy, using medicinal plants, has gained attention for its antibacterial, anti-inflammatory and immunomodulatory properties. This review highlights several medicinal plants including *Ocimum sanctum*, *Tinospora cordifolia*, *Origanum vulgare*, *Aloe vera*, *Curcuma longa*, *Morinda citrifolia, Withania somnifera*, *Fumaria indica*, and *Murraya koenigii* for their potential in treating bovine mastitis. These plants have shown significant reductions in bacterial loads, somatic cell counts and udder inflammation offering a promising alternative to conventional antibiotics. The use of medicinal plants may help mitigate the risks of antimicrobial resistance and support more sustainable farming practices. Further research is required to optimize their veterinary applications.

Keywords: Cow, Ethno veterinary, Mastitis, Medicinal plants

Introduction

Mastitis is the most common and costly disease in dairy cattle worldwide with a detrimental effect on both animal welfare and food security. It is a complex disease influenced by multiple factors such as environment, management practices, udder physiology and overall cow health. This condition is marked by mammary gland inflammation leading to reduced milk production which can be temporary or persist throughout the lactation period. The production decline is due to the damage caused by microorganisms in the mammary tissue increasing somatic cell counts (SCCs) in the milk as leukocytes rise during infection and cells die. The causative agents of mastitis are divided into two categories: Contagious and environmental. Contagious pathogens typically reside on the udder or teat skin and are transmitted during milking where they multiply and spread through the mammary gland. In contrast, environmental pathogens thrive in the surroundings and enter the udder by propulsion through the teat canal (during milking by capillary action or through the insertion of antibiotic tubes or teat cannulas) or by passive penetration right after milking. While there are some differences in the classification of these microorganisms and the list may vary by author, they are generally

categorized as contagious pathogens including non-aureus *Staphylococcus, Corynebacterium bovis, Mycoplasma spp., Staphylococcus aureus, Streptococcus agalactiae,* and *Streptococcus dysgalactiae*. On the other hand, environmental agents typically include *Citrobacter spp., Enterobacter spp., Escherichia coli, Klebsiella spp., Pasteurella spp., Pseudomonas aeruginosa, Streptococcus faecalis, Streptococcus uberi*s, along with yeasts and molds. Among these, *S. aureus* remains one of the most prevalent pathogens due to its ability to produce a wide array of virulence factors that facilitate bacterial invasion. It is associated with subclinical, acute, chronic and toxic bovine intramammary infections causing significant economic losses (1).

Historically, more than 80% of drug substances originated from natural sources and between 1981 and 2006, over half of FDA-approved drugs were either natural or derived from natural products. These substances are especially important in antibacterial drug discovery with 66% of approved antibacterial agents stemming from them. Phytochemicals which operate through mechanisms different from conventional antibiotics are particularly effective in combating resistant bacteria and can be used alongside existing medications. Although there are currently no plant-derived antibacterial drugs on the market, many promising phytochemicals have been identified, positioning plants as a vital source for future antibiotics (2).

In the treatment of mastitis in dairy cattle, antibiotics have been utilized for over 50 years both to manage outbreaks during lactation and as dry cow therapy to prevent new infections. However, resistance to commonly used antibiotics such as penicillin, clindamycin, and cefotaxime has emerged in many mastitis pathogens. This has prompted the exploration of alternative treatments. Herbal remedies which contain strong antibacterial and anti-inflammatory properties have proven to be effective in treating or preventing mastitis and offer a promising alternative to conventional antibiotics in some instances (3).

Material and methods

Articles published between 2010 and 2024 on the effects of medicinal plants on bovine mastitis were reviewed and analyzed using search results from Google Scholar, ScienceDirect, and the National Library of Medicine (PubMed).

Ocimum sanctum. *Ocimum sanctum,* commonly known as tulsi or holy basil, belongs to the Lamiaceae family and has long been recognized as a valuable traditional herbal remedy used in the treatment of both human and animal diseases. Its therapeutic potential is largely due to its active constituents including triterpenes, volatile oils and flavonoids. The aqueous extract of *O. sanctum* is particularly effective in treating bovine subclinical mastitis by enhancing phagocytic activity and the phagocytic index leading to a reduction in total bacterial count. Furthermore, both the powdered and aqueous extracts of *O. sanctum* have been shown to decrease milk electrical conductivity. Oral administration of *O. sanctum* leaf powder to cows with mastitis, at a dose of 600 mg/kg body weight twice daily for seven days, eliminated 69% of infections, reduced SCCs improved milk quality and enhanced the phagocytic activity of milk neutrophils as well as lactoperoxidase and myeloperoxidase activity (4).

Tinospora cordifolia. *Tinospora cordifolia* is an herbaceous vine of the family Menispermaceae indigenous to tropical regions of the Indian subcontinent. This medicinal plant is widely used in traditional medicine. *Tinospora cordifolia* possesses strong antibacterial, antioxidant and immunomodulatory properties. The hydro-methanolic extract of *T. cordifolia* shows therapeutic potential in treating bovine subclinical mastitis. Phytochemical analysis

of the extract revealed the presence of alkaloids, phenols, polysaccharides and proteins. When administered intramammary to cows with mastitis, the extract reduced SCCs and total bacterial counts in the milk while enhancing the phagocytic activity of milk polymorphonuclear cells, increasing lysosomal content in milk polymorphonuclear cells and elevating levels of lysosomal enzymes and IL-8 in milk serum (5).

***Origanum vulgare*.** *Origanum vulgare*, commonly known as Oregano, is a species from the Lamiaceae family native to the Mediterranean and western Eurasia. This aromatic herb has been widely used as both a remedy and a spice in various traditional healing systems around the world. Since ancient times, *O. vulgare* has been utilized in herbal medicine to treat conditions such as indigestion, painful menstruation, rheumatoid arthritis, scrofula, and urinary tract disorders. Oregano essential oil (OEO) has shown a therapeutic effect on clinical bovine mastitis caused by *S. aureus* and/or *E. coli*. After intramammary treatment with 0.9 mL of OEO ointment, physical udder conditions greatly improved. In the control group, SCCs, white blood cell (WBC) counts and bacterial levels were increased. However, in the OEO-treated groups, SCCs and WBC counts were significantly decreased and *S. aureus* and *E. coli* were not detected in the milk, similar to the gentamicin treatment group. The results revealed that OEO might be a useful alternative to antibiotics for controlling subclinical bovine mastitis caused by *S. aureus* and/or *E. coli* (6).

***Curcuma longa* and *Aloe vera* (L.) Burm.f.** *Curcuma longa Linn. (C. longa)*, commonly known as turmeric, is a member of the Zingiberaceae family and has a long history of medicinal use for treating various diseases. *C. longa* is utilized for a range of conditions, including cough, cold, dental problems, indigestion, skin infections, blood purification, asthma, piles, bronchitis, tumors, wounds, hepatic disorders, and as an antiseptic. Curcumin, the primary active component of *C. longa*, is renowned for its therapeutic potential in numerous ailments. *Aloe vera* (L.) Burm.f. (*Aloe barbadensis* Miller), a member of the Xanthorrhoeaceae family, is a perennial green herb with bright yellow tubular flowers commonly found in hot and arid regions of North Africa, the Middle East, the Southern Mediterranean and the Canary Islands. Traditionally, *A. vera* has been used for treating skin injuries such as burns, cuts, insect bites, eczema, and digestive issues due to its anti-inflammatory, antimicrobial and wound-healing properties. A study investigated the effect of an externally applied ethno-veterinary formulation consisting of a paste made from *A. vera* leaves, *C. longa* rhizome and calcium hydroxide in the treatment of bovine mastitis. The selected parameters (pH, SCC, and Electric Conductivity) in animals with mastitis returned to normal within 6-7 days of treatment. Milk production was nearly recovered to pre-mastitis levels. Therefore, this formulation effectively managed mastitis. An analysis of the intervention impact revealed an 18 to 49% reduction in antibiotic residues in the milk (7).

***Morinda citrifolia*.** *Morinda citrifolia*, commonly known as Great Morinda, is a small evergreen tree in the Rubiaceae family. It thrives from India to Malaysia, extending through Fiji and Eastern regions. Almost all parts of the plant are used for their medicinal and nutraceutical benefits and its fruit juice is highly sought after in alternative medicine for treating various illnesses. A study assessed the effect of *M. citrifolia* fruit juice on the milk of healthy and mastitis-affected dairy cows. Twenty-five cows (13 healthy and 12 with sub-clinical mastitis) were given 100 mL/day of *M. citrifolia* juice. The juice significantly reduced mastitis-affected milk pH and electrical conductivity, lowered the total bacterial count and decreased the total protein concentration. The findings suggested that feeding *M. citrifolia* fruit juice improved milk quality and biophysical parameters in mastitis-infected cows (8).

Withania somnifera. Withania somnifera, known commonly as ashwagandha, is an evergreen shrub in the Solanaceae or nightshade family that grows in India, the Middle East and parts of Africa. Roots, leaves and whole plants of Withania somnifera are used for medicinal purposes because of their adaptogenic, astringent, antibacterial, anti-inflammatory, antioxidative, antistress, antitumor, hemopoietic and immunomodulatory properties. Oral administration of W. somnifera root powder (500 mg/kg body weight daily) eliminated 64.28% of intramammary infections and significantly reduced SCC and ceruloplasmin concentration, thereby, reducing udder inflammation and improving milk quality (9).

Fumaria indica. *Fumaria indica* (Hausskn.), Fumitory, is a member of the Papaveraceae family and is an annual herb commonly found in India and Pakistan. Widely used in traditional medicine, it is known for its anthelmintic, diuretic, laxative, cholagogue and sedative properties as well as for blood purification and treating liver obstruction. Cows treated with intramammary administration of aqueous extract of *F. indica* demonstrated clinical recovery as indicated by improved California Mastitis test scores and reduced SCCs on day 5 compared to day 0 of therapy (10).

Murraya koenigii. *Murraya koenigii*, commonly known as curry leaf, is a significant leafy vegetable from the Rutaceae family, native to India and Southeast Asia. Beyond its use as a natural flavoring agent, curry leaves offer numerous health benefits. They possess medicinal properties including anti-diabetic, antioxidant, antimicrobial, antifungal, anti-inflammatory, anti-carcinogenic, hepatoprotective, cardioprotective, anti-ulcer, anti-diarrheal and phagocytic activities. Treatment of bovine subclinical mastitis with orally administered curry leaf powder at 60 mg/kg body weight resulted in a significant reduction in total bacterial count, California Mastitis Test scores, SCC, electrical conductivity, pH and lymphocyte levels. Thus, therapy with *M. koenigii* effectively reduced udder inflammation and improved milk quality (11).

Fig.1. Medicinal plants used in the treatment of bovine mastitis

Mastitis remains a significant challenge in dairy farming due to its impact on animal health, milk quality and economic productivity. While conventional antibiotic treatments have been the primary method for managing this disease, increasing antibiotic resistance and the presence of antimicrobial residues in milk and the environment have spurred the need for alternative therapies. This review highlighted the promising role of medicinal plants such as *O. sanctum*, *T. cordifolia*, *O. vulgare*, *A. vera*, *C. longa*, *M. citrifolia*, *Withania somnifera*, *F. indica*, and *M. koenigii* in the treatment of bovine mastitis. These plants, with their strong antibacterial, anti-inflammatory and immunomodulatory properties have demonstrated significant improvements in udder health and milk quality, reducing SCCs, bacterial loads and inflammation in mastitis-infected cows. Phytotherapy offers a natural, effective alternative to traditional antibiotics potentially reducing the risk of antimicrobial resistance and promoting more sustainable dairy farming practices. Further research is essential to explore their full potential and to optimize their application in veterinary medicine.

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Effect of rutin/cyclodextrin ınclusion complex on short-term storage of ram semen

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Rutin is one of the bioactive flavonoid compounds found in many fruits and vegetables. Numerous pharmacological characteristics of rutin, including anti-bacterial, anti-oxidant, anti-inflammatory and immunomodulatory effects have been reported previously (1). However, rutin's low water solubility, limits its systemic bioavailability (2). Cyclodextrins (CDs) are natural cyclic oligosaccharides obtained from the enzymatic breakdown of starch (3). The CDs act as complexing compounds increasing the permeability and adsorption of a substance across the membrane barrier, improving chemical stability. In the present study, it was aimed to investigate the effect of the rutin/CD inclusion complex on spermatological parameters during the short-term storage of ram semen. Eight Tuj breed rams being free of systemic and genital organ diseases with the age of 1.50-2 years were used as animal material. Ejaculates were taken from each ram by artificial vagina during non-breeding season. The semen was divided into 4 equal parts as control, 0.50 mM rutin/CD, 1 mM rutin/CD and 1.50 mM rutin/CD groups. After the addition of rutin/CD to the groups, the temperature of all groups was gradually reduced from 37 to 5˚C. Analyses of semen at 0, 24, 48, 72 hr and oxidant-anti-oxidant capacity (TOS/TAS) at 72 hr were carried out. When the results were analyzed, an increase in TAS, a decrease in TOS and an increase in sperm quality were observed in 0.50 mM rutin/CD group. In conclusion, rutin/CD administered to rams sperm at a dose of 0.50 mM improved semen quality during short-term storage of semen.

Keywords: Cyclodextrin, Ram, Rutin, Sperm

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A review of the effects of Aloe vera on mastitis in cattle

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Aloe vera is a succulent plant species of the genus aloe. It is widely distributed and is considered an invasive species in many world regions. Various therapeutic effects have been found with the use of *A. vera*. It is known that the primary etiological agents associated with bovine mastitis show high levels of antimicrobial resistance. The results of the present study showed that treatment with *A. vera* gel extract disrupted the cell membrane causing lysis in 75% of *Staphylococcus aureus*, in 88% of *Escherichia coli*, in 97% of Streptococcus uberis, and in 88% of Methicillin-resistant *S. aureus* cells (1). Cell membrane disruption is attributed to the presence of anthraquinones. The combination of *A. vera* and ceftiofur or cloxacillin delayed the appearance of chromosomal resistance in *S. aureus* strains (2). In a study conducted by Chikwanda *et al*. (2013), it was observed that *A. vera* possessed some antibacterial properties that could assist in controlling mastitis pathogens *S. aureus*, *S. agalactiae* and *E. coli*. The highest levels of inhibition were shown in *E. coli* organelles (3). The use of *A. vera* along with antibiotics could have positive effect on the control and treatment of mastitis in cattle.

Keywords: *Aloe vera*, Mastitis, *Staphylococcus aureus*

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Treatment of metritis in the feline with medicinal plants

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Metritis is a common reproductive disorder in cats, characterized by inflammation of the uterus, often resulting from bacterial infections. In recent years, medicinal plants have gained attention as alternative treatments for managing this condition due to their anti-inflammatory, antimicrobial and antioxidant properties (1). Aloe vera is widely used for its antibacterial and anti-inflammatory effects effectively managing cat uterine infections. When applied topically or orally in 1-2 mL doses per day, it can significantly reduce uterine inflammation and bacterial load. Additionally, Curcuma longa contains curcumin, a bioactive compound known for its potent anti-inflammatory and antimicrobial properties. Studies indicate that oral administration of 100 mg/kg of curcumin can reduce uterine inflammation in cases of metritis. Antioxidants like vitamin E (50 IU/day) and resveratrol (10 mg/kg/day) have been shown to complement herbal treatments by minimizing oxidative stress, commonly elevated during infections like metritis. These antioxidants accelerate recovery and help prevent further complications in feline reproductive health (2). Their role in reducing oxidative damage enhances the overall effectiveness of medicinal plant treatments. Combining medicinal plants and antioxidants presents a promising approach for treating feline metritis. This highlights the potential of natural therapies to improve reproductive health outcomes while minimizing side effects associated with conventional treatments. However, further studies are needed to confirm these findings and optimize treatment protocols.

Keywords: Feline, Medicinal plants, Metritis

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Section 10

Surgery

Radiological evaluation of the Common sage (*Ziziphora tenuior*) extract effects in Myna hypothyroidism

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Abstract

The present study aimed to investigate the radiological effects of common sage (*Ziziphora tenuior*) extract on hypothyroidism in myna (*Acridotheres tristis*). This descriptive-cross sectional study involved 20 male myna with an average age of 11.00 ± 9.00 months and a mean weight of 241.00 ± 42.00 g. The birds were randomly and equally divided into five groups including a negative control group, a positive control group, and three treatment groups receiving alcoholic extract of common sage at 1.00, 2.00, and 3.00% concentrations. The alcoholic extract of common sage was administered to the three treatment groups for 40 days. Propylthiouracil was used to induce hypothyroidism. After induction and onset of hypothyroidism symptoms, radiographs in lateral and ventrodorsal positions were obtained from all myna on days 0 and 45. The results of this study showed that following the induction of hypothyroidism and the development of osteoporosis in mynas, thin and shell cortical signs were observed in the radius, ulna, tibia, and fibula bones. With the initiation of treatment with alcoholic extract of common sage, the signs of osteoporosis were reduced in the study groups, and this reduction was significant in the treatment group received 3% of the extract from this plant. The findings of this study suggest that alcoholic extract of common sage can stimulate the thyroid gland and increase serum thyroid hormone levels in mynas.

Keywords: *Acridotheres tristis*, Hypothyroidism, Osteoporosis, Propylthiouracil, *Ziziphora tenuior*

Introduction

Thyroid hormones play a crucial role in growth, cell division, biological activities, and bone metabolism. In some cases, hypothyroidism occurs in mynas (1) and causes multiple complications, such as xanthomatosis, lipomatosis, increased beak growth, osteoporosis, fatty liver, chronic weight gain, and lipemia accompanied by anemia. The flavonoids and other constituents of common sage (*Ziziphora tenuior*), including caffeine, theanine, vitamins, and saponins are recognized as anti-inflammatory, anti-oxidant, anti-mutation, and anti-cancer agents (2). Common sage leaves contain alkaloids and methylxanthines, particularly caffeine, being considered important alkaloids in the quality of this plant (3). Bone loss due to osteoporosis is the leading cause of fractures, reducing lifespan and increasing mortality in animals. Disruption of the balance between bone formation and resorption can have several consequences. Increased or decreased triiodothyronine (T3) and thyroxine levels can potentially be harmful to bone tissue. Considering that the T3-strengthening effects

have been reported in the properties of common sage (4), but the mechanisms of these effects have not been fully elucidated, we aimed to investigate the effects of common sage extract on experimental hypothyroidism in mynas (*Acridotheres tristis*) and study the thyroid hormone-induced bone changes in these birds.

Materials and Methods

Ethical consideration. The procedures were carried out based on the guidelines of the Ethics Committee of the International Association for the Study of Pain (5). The study was registered under registration code of Ir.iau.urmia.rec 1401.1234 in Ethical Committee of Islamic Azad University, Urmia Branch, Iran.

Study design and birds. This descriptive cross-sectional study involved 20 male Mynas with a mean age of 11.00 ± 9.00 months and a mean weight of 241.00 ± 42.00 g. The diet for all birds consisted of millet and linseed in a 4:1 ratio. After a one-week acclimatization period, the birds were randomly and equally divided into five groups including a negative control group (Fig. 1), a positive control group (Fig. 2), and three treatment groups receiving alcoholic extract of common sage (*Z. tenuior*) at 1.00, 2.00, and 3.00% concentrations (Figs. 3-5). The alcoholic extract of common sage was administered to the three treatment groups for 14 days. The alcoholic extract of common sage was prepared by maceration, and propylthiouracil was used to induce hypothyroidism. The propylthiouracil dose used for each bird was 0.07 mg/kg. To administer the drug, it was mixed with distilled water and each bird was given 50 µL of the solution orally. The drug was administered daily in a single dose to the mynas for 40 days (6). After induction and onset of hypothyroidism-related symptoms, radiographs in lateral and ventrodorsal positions were obtained from all mynas on days 0 and 45. The X-ray machine used for this purpose was a digital model GXR-SD 152 DDR (Varian N.V. Co, Made in South Korea). The focal film distance was 100 cm, and the peak kilovoltage and milliampere-seconds were 42 and 5, respectively.

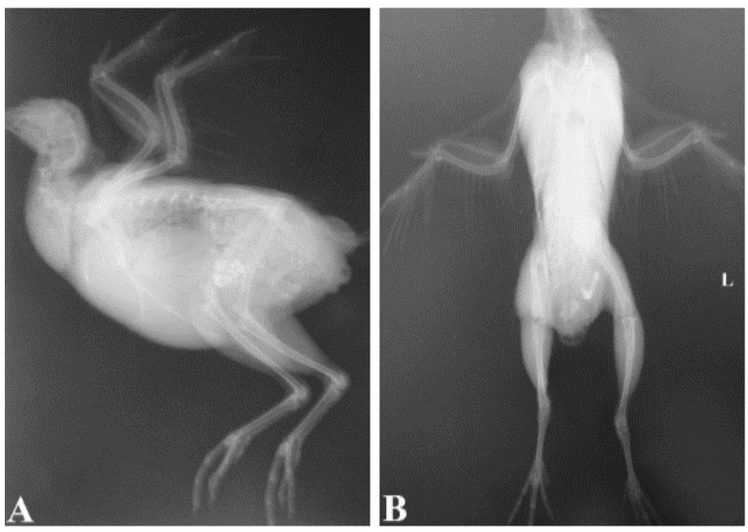

Fig. 1. Lateral (**A**) and ventrodorsal (**B**) radiographs of the negative control group.

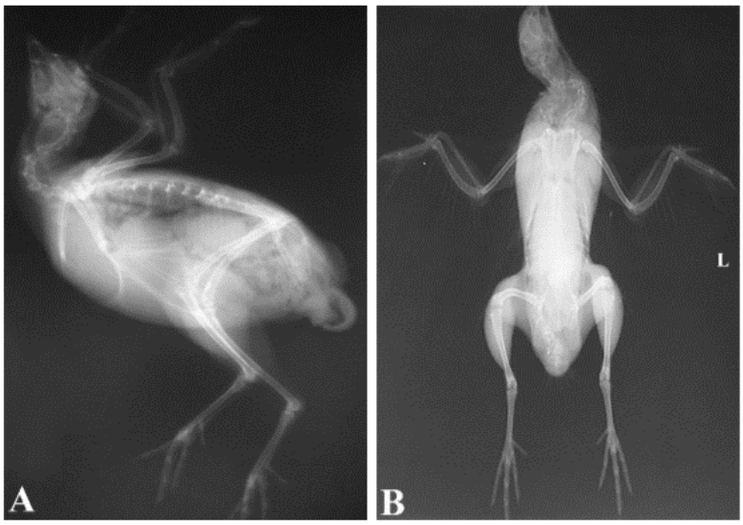

Fig. 2. Lateral (**A**) and ventrodorsal (**B**) radiographs of the positive control group.

Results

The results obtained from the radiographs indicated that osteoporosis was relatively observed in all bones of all study groups, being more evident in the radius, ulna, tibia, and fibula bones compared to the other parts of the body. After treatment, the bone cortex repair process was relatively observable; in the treatment groups received alcoholic extract of common sage (*Z. tenuior*) at 1.00, 2.00, and 3.00% concentrations, the degree of repair or increase in cortical plates showed an increasing trend (Figs. 3-5). In fact, based on the radiographs obtained from the control and treatment groups, it can be concluded that hypothyroidism leads to osteoporosis, which can be diagnosed in the radius and ulna bones in the wing and the tibia and fibula bones in the leg.

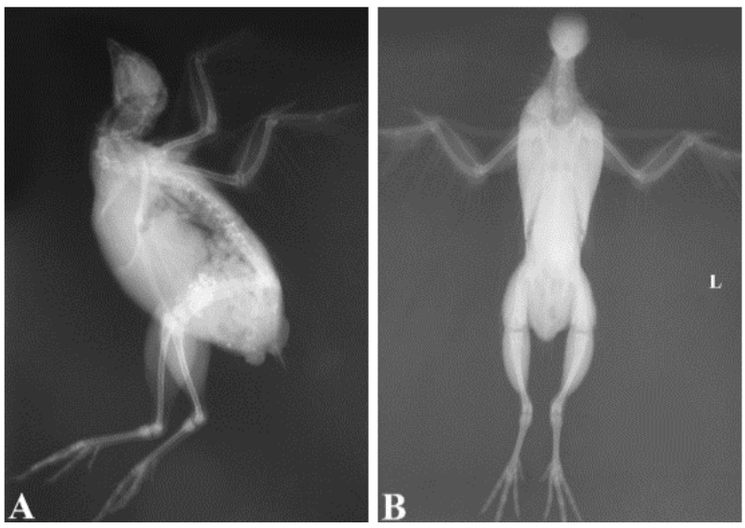

Fig. 3. Lateral (**A**) and ventrodorsal (**B**) radiographs of the 1.00% common sage received group.

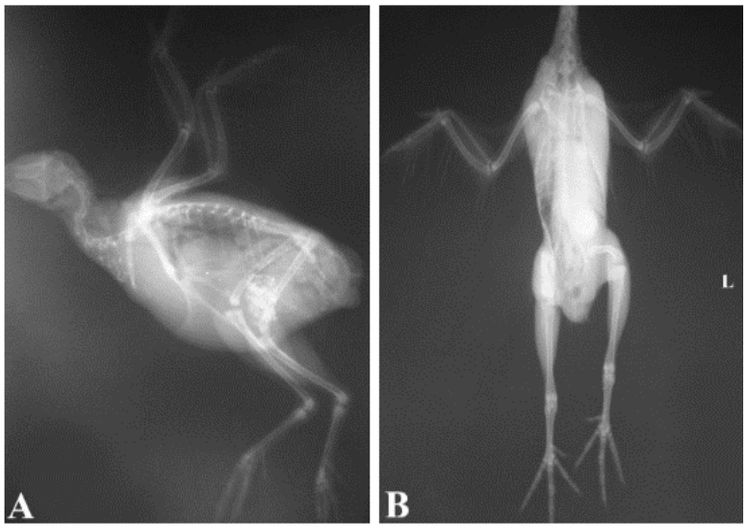

Fig. 4. Lateral (**A**) and ventrodorsal (**B**) radiographs of the 2.00% common sage received group.

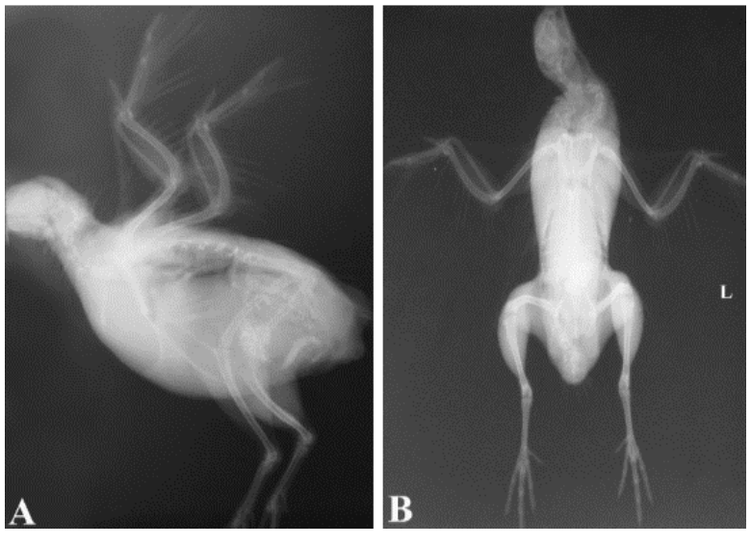

Fig. 5. Lateral (**A**) and ventrodorsal (**B**) radiographs of the 3.00% common sage received group.

Discussion

In the course of this study, the symptoms induced by propylthiouracil administration in mynas began with lethargy, mild decreased activity, and reduced feed intake. These symptoms gradually worsened over time. Later, changes in the appearance and quality of feathers and leg scales became evident. In the final days of propylthiouracil administration, the general symptoms observed in the experimental birds consisted of decreased physical activity, reduced feed and water intake, and an unkempt appearance. After the initiation of common sage (*Z. tenuior*) alcoholic extract administration, the extent of bird recovery varied depending on the dilution levels, which were 1.00, 2.00, and 3.00%. In the 3.00% treatment group, which received the highest extract concentration, recovery began with an increase in feed and water intake, accompanied by a concurrent rise in physical activity. In the 2.00% treatment group, the recovery process started slightly later. In this group as well, the onset of improvement was associated with increased water and feed intake. The restoration of feather quality and improvement varied among the birds in the different groups. In the 3.00% and 2.00% treatment groups, this recovery process commenced more rapidly. In the case of the 1.00% treatment group, there was a delay in recovery, and the overall improvement was weaker compared to the

other groups. Based on the radiographic findings, the induction of hypothyroidism using propylthiouracil was effective, and osteoporosis was evident in the radiographs of the birds. This condition was more pronounced in the wing bones (radius and ulna) and leg bones (tibia and fibula). After the treatment phase began and alcoholic plant extract was administered, the course of repair and treatment also varied depending on the dilution level. The extent of bone repair in the treatment group received alcoholic extract of common sage (*Z. tenuior*) at 3.00% was greater than the other treatment groups. This improvement was evident in the radiographs obtained based on the extent of bone cortex repair. Flavonoids, plant-derived compounds, have the ability to alter thyroid function (7). In one study, researchers stated that common sage (*Z. tenuior*) extracts affect the structure and function of the thyroid gland by inducing hypertrophy or hyperplasia of thyroid gland follicles and inhibiting thyroid peroxidase activity. In light of this information and the high flavonoid content of common sage (*Z. tenuior*), its effectiveness against hypothyroidism is confirmed. Thyroid hormones are lipophilic and easily cross cell membranes. The receptors for these hormones are located within cells and in the nucleus. Their binding to their receptors affects gene transcription and consequently protein synthesis. However, studies have shown that thyroid hormones also have a direct effect on mitochondria and membrane transport proteins (8). Integrin αVβ3 is a membrane receptor specific for thyroid hormones, and the binding of thyroid hormones to these receptors leads to the activation of the intra-cellular cascade (mitogen-activated protein kinase). The result is the regulation and modulation of membrane potential through the regulation of ion channels, activation of sodium/potassium exchangers, Ca^{2+} *ATPase*, and regulation of cytoskeletal components. On the other hand, T3-activated mitogen-activated protein kinase is translocated to the nucleus and causes serine subunit phosphorylation, leading to the induction of angiogenesis and cell proliferation (9). In another study, the corrective effect of common sage extracts on the structure and function of the thyroid gland by inducing hypertrophy or hyperplasia of thyroid gland follicles and inhibiting thyroid peroxidase activity was also confirmed (10). Investigations by researchers in children with hypothyroidism indicated that failure to treat the condition can lead to delayed or halted growth, short stature, and impaired endochondral bone formation (11).

The results showed that common sage plant extract can affect thyroid function and increase thyroid hormones in mynas with hypothyroidism. It is hoped that in the course of future studies, a better understanding of the mechanisms of these changes in the regulation of thyroid hormones in mynas hypothyroidism could be gained. Based on the results of this study, common sage plant extract can be used as a supplement in the diet of mynas to prevent thyroid and bone disorders.

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Analgesic herbal remedies in small animal surgery

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Pain is a fundamental and distressing sensation serving as a signal to protect the body from potential harm. Throughout history, plant-based remedies have been used to alleviate pain. This review aims to gather information from published articles and provide a comprehensive literature review on the use of folk remedies from plants as analgesics and in the treatment of pain-related conditions. Analgesics are substances relieving pain without causing loss of consciousness, although they may have effects on the central nervous system that impact awareness. In veterinary medicine, there is a wide range of analgesic medications available. The major classes of analgesics in this field include opioids, non-steroidal anti-inflammatory drugs, and local anesthetics. Several herbs with potential analgesic properties have been investigated (1). These include corydalis, Jamaican dogwood (*Piscidea erythrina*), Saint John's wort, California poppy, and Indian pipe (*Monotropa uniflora*). It is worth noting that Saint John's wort is more commonly known as an anti-depressant, but it has also demonstrated analgesic and anti-inflammatory activities in laboratory animals. Similarly, California poppy has not been extensively studied for its analgesic properties, but the United States dispensatory stated its effectiveness as a safe soporific and analgesic, containing small amounts of morphine and other alkaloids (2). However, the specific mechanisms of action for these analgesic herbal remedies have not been well-described, and further evaluation is necessary to fully understand their efficacy and safety.

Keywords: Analgesic herbs, Herbal medicine, Small animal surgery

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The antinociceptive effects of thymoquinone on acute postoperative pain: Roles of spinal cord oxidative stress, inflammatory and apoptotic mechanisms involved

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Pain is an important outcome of surgery, which in case of insufficient treatment can lead to chronic pain and its consequences (1). Natural products with fewer side effects are used to optimize post-operative pain management. In the present study, the effect of thymoquinone (TQ) on acute post-operative pain was investigated. The possible mechanisms were followed by measuring biomarkers of oxidative stress, inflammation and apoptosis in the spinal cord. Celecoxib (CLX), an inhibitor of the cyclooxygenase pathway, was used to compare the effects. Acute post-operative pain was induced by surgical incision of the plantar skin and muscles (2). The TQ and CLX were orally administered and mechanical allodynia was measured using von Frey filaments on days 1,2, 3, 5, 7, 9 and 11 after surgery. Biomarkers of oxidative stress, inflammation and apoptosis were determined on post-operative days 1, 3 and 7. Locomotor activity was also performed using an open-field test. In the vehicle-treated group, the fifty percentage of paw withdrawal threshold (PWT 50%) decreased at days 1, 2 and 3 and then, gradually returned to the pre-operative level. The TQ (5 and 10 mg/kg) and CLX (10 mg/kg) increased PWT 50%, and improved the spinal cord alterations of superoxide dismutase, malondialdehyde, tumor necrosis factor-alpha and caspase-3. Locomotor activity was not affected by the above-mentioned treatments. Oral administrations of TQ and CLX exhibited similar anti-allodynic effects. These mitigating effects might have occurred due to the inhibition of oxidative stress, inflammation and apoptosis in the spinal cord.

Keywords: Acute post-operative pain, Celecoxib, Mechanical allodynia, Rats, Thymoquinone

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Effects of linalool on oxidative stress parameters in a rat model of cutaneous wound healing

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Chronic and non-healing skin wounds have consistently posed a financial burden on patients, insurance providers, and governments (1). This study aimed to investigate the beneficial effects of linalool on oxidative stress parameters in wound healing in rats. Linalool is a monoterpene alcohol present in various aromatic plant species with the most common plant families being Lamiaceae, Lauraceae, and Apiaceae. It has some beneficial effects including anti-inflammatory, anticancer, lipid-lowering, sedative, antimicrobial, antidepressant and neuroprotective properties (2,3). Male mice (n = 24) were divided into three groups: 1-Sham: the wound was created using an 8 mm skin biopsy punch with no treatment. 2- Soyabean: The wound was created and soyabean oil was applied on the wound daily for 9 days. 3- Linalool group: after wound creation, linalool+soybean was rubbed on the wound daily for 9 days. On day 14, the animals were sacrificed and skin samples were taken and malondialdehyde (MDA), total oxidant status (TOS), glutathione peroxidase (GPx) and total antioxidant capacity (TAC) were evaluated. Linalool significantly reduced the levels of MDA and TOS compared to the sham and soybean groups ($p < 0.05$). Levels of GPx were increased in Linalool group compared to other groups nonsignificantly ($p > 0.05$). Moreover, TAC levels were significantly increased in the treatment group compared to other groups ($p < 0.05$). Overall, linalool treatment could beneficially reduce oxidative stress and improved wound healing compared to the sham and soyabean groups. Therefore, topical administration of linalool could be recommended for wound healing due to reducing oxidative stress biomarkers and wound healing acceleration.

Keywords: Linalool, Rat, Wound healing

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Histopathological evaluation of the effect of oral administration of sericin on the bone defect healing in rat

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This study investigated the effects of silk sericin on bone healing in rats considering its potential applications in biomedicine. Fifty male Wistar rats were randomized into five groups. After anesthesia, a cavity was created in the right femur using a dental drill (1). The sham group underwent surgery without a defect, while the control group had an untreated defect. Experimental groups received daily oral doses of sericin at 100, 150, and 200 mg/kg (2). Histopathological evaluations showed that the control group defect was filled with connective tissue, with newly formed trabecular bone observed deeper within (3). In the 100 mg sericin group, granulation tissue was more organized, filling most of the defect and showing early cartilage formation. The 150 mg group exhibited organized granulation tissue and significant dense bone formation beneath the fibrous tissue, effectively blocking the defect and severing the connection to the bone marrow. The 200 mg group also showed organized granulation tissue, advanced bone remodeling and the formation of Haversian systems at the repair site. Overall, the results indicated that oral sericin administration significantly enhanced tissue healing leading to optimal recovery from bone injuries in rats. This research highlighted sericin effects as a therapeutic agent in bone healing applications.

Keywords: Bone healing, Histopathology, Rat, Sericin

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Chemical castration using intra-testicular injection of carbon quantum dots synthesized from white onion (*Allium cepa*) juice in a rat model

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Carbon quantum dots (CQDs) can induce apoptosis, suppress proliferation, and target various types of cells selectively (1,2). The present study was aimed to evaluated chemical castration using intra-testicular injection of CQDs in rats. Fifteen male rats were divided into three groups including intact, intra-testicular injections of normal saline (0.50 mL), and CQDs (40 mg/kg in 0.50 mL normal saline). The CQDs were synthesized from white onion (*Allium cepa*) juice using one-step hydrothermal carbonization method (3). The rats were anesthetized using intra-peritoneal injection of 40.00 mg/kg ketamine and 5.00 mg/kg xylazine. Using hupodermic needle, normal saline and CQDs-containing normal saline were injected into each testicle. On day 60, the rats were anesthetized and castrated surgically. Epididymal sperm count, viability, motility, morphological abnormalities, and DNA damage were studied. Histopathological analysis was performed to evaluate the testicular histo-architecture. In comparison to the intact and normal saline groups, marked tubular depletion and atrophy, as well as germ cells degeneration were noted in CQDs group. No live spermatozoa were found in this group, and pronounced reduction in sperm concentration and escalation in morphological abnormalities were observed following CQDs injection. Non-metallic CQDs are non-toxic, yet their applications in the field of medicine are not widely documented. The cytotoxic effects of CQDs through the lysosomal damage and mitochondrial dysfunction, can induce apoptosis and/or necrosis. The results of this study showed that intra-testicular injection of CQDs could effectively induce infertility in male rats. Further mechanism-oriented studies using different doses of CQDs are recommended.

Key words: Castration, Carbon quantum dots, Fertility, Onion, Rat

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Section 11

Poultry Diseases

A review on post-antibiotic era: One health and herbal medicine in poultry

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Abstract

The fact is that the veterinary medicine and in particular avian medicine is considered as a preventive medicine. There is no doubt that predictable is preventable and let us to believe in-depth that prevention is better than cure. More interesting is that most of medicinal plants act as a preventive medicine and their use fit into all existing prevention strategies (1,2) with a promising source for the post-antibiotic era (3) in tackling of infections in both animal and human health (4-6). The history of medicine may have three phases including pre-antibiotic era (up to 1935), antibiotic era (1935-contiue), and post-antibiotic era. Undoubtedly, antibiotic era were golden age of discovery of the antibiotic and human being was happy enough to treat the challengeable diseases, mostly bacterial infections. Unfortunately, limitation on discovery of new drugs, increasing resistance of pathogenic agents to available drugs (7- 9) and drug residues in foods together with an increasing demand for antibiotic-free products have made it very clear that we have to move into post-antibiotic era. Moreover, increasing human being population on one hand and detrimental human activities on the other hand, have made pollution and climate change leading to the depletion of natural resources and more devastation of our environment. Therefore, there is a genuine understanding concerning health for all and one health approaches to address the global food safety challenges (7,8,10) starting since 2010. Medicinal plants play a distinguished role in poultry feed additives (11-13) to help promote productivity and performance (8,14), and preventive medication (15). It is also used for treatment of all poultry diseases (16-19), increasing poultry resistance to infections, improvement of poultry immune system (14,16,20) and finally enrichment of birds products (meat, egg) (11,16).

In conclusion, promotion of health for all & one-health system is not what we do, it is who we are. Centuries of use in traditional medicine practices can be used as testimony that the medicinal plants ingredients are effective, safe, and have less side effects. Medicinal plants have played an essential role in the development of human culture. WHO reported that 80% of the earth population rely on traditional medicine for their primary health care needs. Medicinal plants have potentials to play an essential role in the development of One-Health as a health for human being, animals and the environment.

Recommendations:

The scientists have an obligation to conduct scientific researches in- depth on the medicinal plants including their effects on blood profiles (14) in order to explore the medicinal properties of the plants, validate their use and overall commercialize traditional medicine (mechanism of action and practices) in modern medicine (19). There are some recommendations and future directions as follows:

• Dose-response trials to optimize supplementation levels.

• Analyses of active constituent pharmacokinetics and bioavailability in poultry tissues.

• Elucidation of mechanisms of action against target pathogen/s.
• Rigorously controlled long-term productivity studies including their side-effects.
• Systematic safety and toxicity evaluations in safety margin.
• Studies on withdrawal period to ensure customers with free-residual poultry products.
• Study on potential herb-herb and herb-drug interactions *in vitro* and *in vivo*.
• Cost-benefit for commercial formulation in different types of commercial poultry.
• Sensory and shelf-life impacts on final poultry products.

Keywords: Antibiotic, Health, Poultry

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Effect of extracts of thyme (*Thymus vulgaris*) on electrocardiographic parameters in experimentally ascitic broilers

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Abstract

The object of this study was to evaluate the effect of extracts of thyme (*Thymus vulgaris*) on electrocardiographic changes in broilers suffering from experimental ascites caused by cold stress. One hundred and twenty 1-day-old (Ross 308) male broiler chicks were randomly divided into 3 groups and 4 replicates (10 birds *per* replicate). The 1st and 2nd groups were fed with the basal diet, and 3rd group was fed with basal diet and 1.00% extracts of thyme. Temperature was gradually decreased in 2nd and 3rd groups to 30.00% of the standard program from 2nd week until the 6th week. At the end of 4, 5 and 6 weeks, four chicks from each group were randomly selected and necropsied after recording the electrocardiograms (ECGs). According to the ECGs analysis findings, heart rate in ascitic group (2nd) was lower than 1.00 % extracts of thyme + ascitic group (3rd) and control group. In ascitic group, four cases of arrhythmias (sinus bradycardia) and one case of atrioventricular block were observed. The mean ECG intervals and amplitudes of waves in ascitic broilers were significantly longer than those of the control group. The amplitudes of T and S waves in the 5th and 6th weeks in the 3rd group decreased compared to the 2nd group. The present study showed that the addition of 1.00 % extracts of thyme to broilers diet improved ECG parameters; so, 1.00 % extracts of thyme can be effective in preventing ascites syndrome due to cold condition.

Keywords: Ascites, Broiler, Electrocardiogram, *Thymus vulgaris*

Introduction

Ascites syndrome is one of the most common metabolic disorders in fast growing broiler chickens (1). Understanding the complexity and key factors in occurrence of ascites is vitally important for control of economic losses (2). Pulmonary hypertension is a cardiovascular disorder characterized by increased pulmonary artery pressure, ventricular hypertrophy, and ascites (1). It was shown that in chickens suffering from pulmonary hypertension, significant changes in electrocardiographic waves related to T and S waves can be seen from the 4th week onwards, which was quite evident in relation to the S wave in aVR, III and II derivations (3). Dilation and hypertrophy of the ventricles increase the voltage in S, R and T waves in broilers (4). It has been indicated that the use of medicinal plants in broiler chickens leads to an improvement in the efficiency of broiler production, weight gain, food conversion ratio improvement, acidity reduction, and health status and digestive system function improvement. Thyme (*Thymus vulgaris*) is an aromatic medicinal herb belonging to the mint family, growing in the form of thick, wild bushes on dry slopes and between boulders (5,6). Thymol and

carvacrol are among the important active substances in thyme essential oil, but other substances, such as flavonoids, terpenes, other phenolic compounds, spicy compounds and a number of other active substances can be seen in it (5). The object of this study was to evaluate the effect of extracts of thyme (*T. vulgaris*) on electrocardiographic changes in broilers suffering from experimental ascites caused by cold stress.

Materials and Methods

This study was conducted on one hundred and twenty 1-day-old (Ross 308) male broiler chickens, being kept in one room and randomly divided into 3 groups and 4 replicates (10 birds *per* replicate) until the age of 14 days. During this period, the temperature, food ration and lighting program were monitored according to the recommended protocol. The 1st and 2nd groups were fed with the basal diet, and 3rd group was fed with basal diet and 1.00% extracts of thyme. From the 3rd week, the 2nd and 3rd groups were moved to a separate room to reduce the temperature. Temperature was gradually decreased in 2nd and 3rd groups to 30.00% of the standard program from 2nd week until 6th week (1). At the end of 4, 5 and 6 weeks, four chicks from each group were randomly selected and necropsied after recording the electrocardiograms (ECGs).

Results

The ECGs analysis findings showed that heart rate in ascitic group (2nd) was lower than 1.00 % extracts of thyme + ascitic group (3rd) and control group. In ascitic group, four cases of arrhythmias (sinus bradycardia) and one case of atrioventricular block were observed. The mean ECG intervals and amplitudes of waves in ascitic broilers were longer than those of the control group ($p < 0.05$). The amplitudes of T and S waves in the 5th and 6th weeks in the 3rd group decreased compared to the 2nd group.

Discussion

It is well-documented that hypertrophy of the right ventricle of heart can produce recognizable findings in the ECG (3). In current study, some ECG parameters in broiler chickens were examined between the group received 1.00% extracts of thyme and the chickens that did not receive thyme. Changes in heart rate, wave height, time interval were among the desired parameters in this study. It has been shown that the dramatic variations could be seen in ECG of ascitic chickens (2). In general, according to the results obtained from this study, it can be said that there were changes in the ECG of broiler chickens suffering from ascites received thyme. The increase in the number of heart rate and decrease in amplitudes of T and S were among the most obvious changes. The present study showed that the addition of 1.00 % extracts of thyme (*T. vulgaris*) to broilers diet improved ECG parameters.

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Effect of Teucrium (*Teucrium polium* L.) and Thyme (*Thymus vulgaris* L.) on some blood metabolites in broiler chickens

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Abstract

Herbs with hypolipidemic properties can suggest as proper alternatives for antibiotics. This study was conducted to evaluate the effects of teucrium (*Teucrium polium* L.) and thyme (*Thymus vulgaris* L.) on some blood metabolites in broiler chickens. Two hundred one-day-old (Ross 308) male broilers were randomly allocated to four groups, five replications and 10 birds *per* each treatment. The birds were reared on litter for 28 days. Treated birds received feed containing teucrium and thyme seeds for 4 weeks. Teucrium seed (200 mg/kg), thyme seed (200 mg/kg) and blend of teucrium and thyme seeds (200+200 mg/kg) were added to diets. A treatment without any additive was considered as a control group. Serum total protein, globulin, albumin, glucose, total cholesterol (TC), triglycerides (TG), low-density lipoprotein (LDL) and high-density lipoprotein (HDL), liver enzymes activity including aspartate aminotransferase (AST), alanine transaminase (ALT) and gamma-glutamyltransferase (GGT) and heterophil/lymphocyte ratio were measured. Combination of teucrium and thyme seeds, significantly increased total protein, globulin, albumin, glucose and HDL, and decreased TC, LDL and TG compared to the control group. In addition, the lymphocyte and heterophil counts were markedly increased in teucrium and thyme seeds group compared to the control group. There was no significant difference among the groups regarding AST, ALT and GGT. The results of the present study showed that teucrium and thyme seeds in diet, particularly their combination, improve some blood metabolites level and decrease harmful fatty acids and they can be used as lipid-lowering compounds in broilers nutrition.

Keywords: Blood metabolites, Broiler, Teucrium, Thyme

Introduction

Teucrium (*Teucrium polium* L.) belongs to the *Lamiaceae* family and has several uses as a food and medicine. It has anti-oxidant and anti-microbial properties as well as anti-spasmodic effects. It is used in developing countries and widely applied for the treatment of different diseases, including liver problems, blood pressure, rheumatism, and parasitic diseases. It contains many anti-oxidant elements, as many active compounds have been diagnosed in it have inhibitory effects on the free radicals in body (1, 2). The thyme (*Thymus vulgaris*) and teucrium (*T. polium* L.) are rich in several functional compounds, such as carvacrol, thymol, lutein, and zeaxanthin, playing an important role in broilers health and growth performance. The use of thyme with prebiotics, such as mannan-oligosaccharides, in the feed formulation showed positive effects on the growth performance of broilers (3, 4).

To our knowledge, there are few studies investigated the effects of teucrium (*T. polium* L.) and thyme (*T.s vulgaris* L.) as a mixture on the health and some blood metabolites of broilers reared under commercial conditions. Therefore, the objective of this study was to examine the possible effects of thyme, teucrium and a combination of both on some blood metabolites in broiler chickens from day 1 to day 28 of age.

Materials and Methods

Birds. A total of 200 Ross 308 one-day-old male broiler chicks were provided by a local broiler breeder company, and randomly allocated into four dietary treatments. Each treatment was replicated five times with 10 birds *per* each. The birds were given 23 L: 1 D lighting program during each 24 hr period throughout the 28 days of trial.

Diets and sampling. Feed and water were provided *ad libitum* throughout the experiment. The diets were formulated to meet or exceed the National Research Council. A basal diet with no additives was considered as a control group, and three experimental treatments were formulated by supplementation of 200 mg/kg of two separate and blended teucrium and thyme to the diets. Treated birds received feed containing teucrium and thyme seeds for 4 weeks. Blood samples from five randomly selected birds *per* treatments were collected by wing vein puncture, and sera were harvested from clotted blood through centrifugation at 2000 g for 15 min. Serum samples were kept in – 24 ˚C until measuring related parameters, including total protein, globulin, albumin, glucose, total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), low-density lipoprotein (LDL), and liver enzymes activity including aspartate aminotransferase (AST), alanine transaminase (ALT) and gamma-glutamyltransferase (GGT). The heterophil/lymphocyte ratio was also recorded.

Results

Combination of teucrium and thyme seeds, increased total protein, globulin, albumin, glucose and HDL and reduced TC, LDL and TG compared to the control group ($p < 0.05$). In addition, the lymphocyte and heterophil counts were increased in teucrium and thyme seeds group compared to the control group ($p < 0.05$). There was no significant difference among the groups regarding AST, ALT and GGT.

Discussion

The current study aimed to know the possible effects of teucrium, thyme and a combination of both on some biochemical parameters in broiler chickens. In the current study, adding teucrium and thyme at a concentration of 200 mg/kg to the diets for 28 days led to a significant increase in the concentration of total protein, globulin, albumin, glucose, and HDL as well as heterophil/lymphocyte ratio and a significant decrease in the concentration of TC, LDL and TG in the blood serum of the birds of teucrium and thyme group. The results of the current study agreed with the results of the former reports (3, 2, 4), noticed a clear decrease in the concentration of TC, LDL and TG in the group of broiler received teucrium and thyme. In the current study, the treatment of birds with teucrium and thyme combination did not cause any significant change in the concentration of liver enzymes, while a decrease in the concentration of enzymes was observed when teucrium

and thyme groups were compared. The results of the current study agreed with the results of the previous studies (1, 5), reported a clear decrease in the concentration of these enzymes in the group of animals received teucrium, being attributed to the fact that the plant extract in the body prevents free radicals generation and this is due to the active compounds, of which flavonoids are the main active contents, improving the liver's performance in detoxification of exogenous substances. The results of the present study showed that teucrium and thyme seeds in diet, particularly their combination, improve some blood metabolites level and decrease harmful fatty acids and they can be used as lipid-lowering compounds in broilers nutrition.

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The effects of yarrow essential oil on *Escherichia coli* and *Lactobacillus microflora* in laying hens

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Abstract

Yarrow is utilized in traditional medicine to enhance digestive health, improve digestion and nutrient absorption, and promote overall physiological well-being in both humans and animals. This plant has been found to possess anti-bacterial properties, making it an alternative growth stimulant. It is a well-known fact that the presence of intestinal diseases, such as bacterial and intestinal flora imbalance can have a negative impact on nutrient intake and decrease the production performance of laying hens. To investigate the effectiveness of yarrow essential oil on gastrointestinal microflora, including *Escherichia coli* and *Lactobacillus* in laying hens, 80 laying hens were used in a completely randomized design. Experimental treatments include 1) Basic diet (control), 2) Basic ration containing 0.0125 mL of emulsifier, and 3, 4 and 5) Basic ration containing 50, 100 and 150 mg/kg of yarrow essential oil, respectively. The duration of the experiment was 8 weeks, with 2 weeks of adaptation and 6 weeks of treatment. At the end of the research, the fecal contents of each of the cages were sampled as a mixture for each treatment in three replicates to conduct microbiological investigations. Data were subjected to statistical analysis using SPSS version 23 software by comparison of averages with Duncan's method. Results showed that total *Coliform* and *E. coli* counts were significantly decreased and the lowest count was at 100 mg/kg of yarrow essential oil. Also, *Lactobacillus* population was increased by all treatments, but the significantly highest treatment group was the one with 150 mg/kg of yarrow essential oil.

Keywords: *Escherichia coli*, Essential oil, *Lactobacillus*, Layers, Microflora, Yarrow

Introduction

The decline in egg production and the decrease in egg quality towards the end of the laying period have significantly impacted the economic benefits, posing a major challenge to extend the laying period (1). With the limitations on the use of antibiotics as growth promoters for animals, there has been a growing interest in utilizing natural bioactive compounds like essential oils (EOs) to enhance the health and performance of poultry. Essential oils, derived from plant materials with aromatic oily liquids, have garnered attention due to their anti-bacterial properties, serving as natural alternatives to antibiotics in animal production. The main components of EOs, such as thymol and carvacrol, have the ability to disrupt the membrane structure and alter

its permeability by affecting the lipid part of the plasma membrane, thus exhibiting anti-bacterial activity (2). Research has demonstrated that the combined use of thymol and carvacrol can alleviate intestinal inflammation, impaired intestinal integrity, and mucosal barrier dysfunction caused by *Clostridium perfringens* challenge in broilers (3). Furthermore, EOs, including a combination of thymol and carvacrol or encapsulated cinnamaldehyde, can also modulate the intestinal microbial composition of birds (4). However, further studies are needed to explore the effects of EOs on the intestinal microbial population, mucosal barrier, and immune status of laying hens in the final stages of production.

The scientific name of yarrow is *Achillea millefolium*, a plant with a long history of use in traditional medicine. This plant is native to Europe and America and is abundantly found in Iran (5). The use of this plant in Chinese medicine dates back to 3000 years ago (6). Yarrow is known to reduce blood pressure (7) and blood sugar levels (8), as well as having anti-inflammatory effects (7). Its medicinal properties, such as its anti-histaminic and anti-cholinergic activities have been studied (9). Yarrow is used in the treatment of infectious fevers, typhoid, and diarrhea (5). The anti-microbial effects of various yarrow extracts have been investigated, showing that the methanol extract is more effective against severe Gram-positive bacteria (10). In another study, the long-term effects of yarrow consumption on some components of the metabolic syndrome have been examined (11). Yarrow contains several compounds being studied by Pozenya Kowsky and colleagues in human diet in 2003. This substance contains phenolic compounds, pectin, resin, ascorbic acid, and malic acid (12). The most important alkaloid present in the stem bark is berberine, which has significant effects.

In this current investigation, our hypothesis was that incorporating yarrow EO would have a beneficial impact on the gut microbiota of laying hens. Hence, the primary objective of this research was to examine how the addition of yarrow EO to the diet affects the microbial balance of *Escherichia coli*, a gastrointestinal pathogen, and beneficial intestinal bacteria like *Lactobacilli* in laying hens.

Materials and Methods

Animals. In this study, a total of 80 Hy-Line W-80 laying hens were utilized in a fully randomized design with 5 treatments, 4 replications, and 4 birds *per* replication. The feed ration, energy, protein levels, and consumption were tailored based on the guidelines outlined in the breeding and maintenance manual of the specific strain being used (W-80 Commercial Layers Management Guide). The laying hens were supplied with the necessary feed throughout the 8-week duration of the experiment, which included a 2-week adaptation period followed by 6 weeks of treatment.

Experimental treatments. In this research, experimental treatments included the following groups: 1. Basic ration (control), 2. Basic ration plus 0.0125 mL emulsifier mentioned as control^{+}, 3. Basic diet containing 50 mg/kg of yarrow EO, 4. Basic ration containing 100 mg/kg of yarrow EO, and 5. Basic ration containing 150 mg/kg of yarrow EO. In all treatment groups, the emulsifier was used to make the EO soluble at water.

The microbial population of the digestive tract. At the end of the research, the feces of the chickens of each treatment were collected separately for each treatment and transferred to the microbiology laboratory for further investigations. In this study, after counting the *Coliform* bacteria, *E. coli* as an indicator of Gram-negative pathogens and *Lactobacillus* as an indicator of beneficial intestinal bacteria were also counted.

Statistical analysis. All the data were first recorded in Excel software, and after classification, they were subjected to statistical analysis using SPSS version 23 for Windows (SPSS Inc., Chicago, USA), and *p* values less than 0.05 were considered as significant. Statistically significant differences in treatment means were evaluated using one-way analysis of variance (ANOVA) followed by Duncan's multiple comparison test. All data were expressed as means plus minus standard deviation.

Results

Total *Coliform*. Fecal samples were mixed for each group consisted of control, control^{+} and three treatment groups in three replicates and total *Coliform* was counted in a Violet Red Bile Agar medium culture and reported as CFU/g being shown in Table 1. Results showed low microbial activity of *Coliform* in the intestine of layer birds in groups treated by 50, 100 and 150 mg/kg yarrow EO compared to control and control^{+} groups ($p < 0.05$). Based on the population of *Coliform*, the pathogen bacteria in control^{+} group represented no significant difference ($p > 0.05$) compared to the control group, while in the treatment groups, intestine *Coliform* population was decreased to 470 CFU/g of feces at 50 mg/kg treatment group. The mean population was respectively 265 CFU/g and 305 CFU /g in 100 and 150 mg/kg treatment groups, with no significant difference among any of treated groups with yarrow EO ($p > 0.05$).

Table 1. Total *Coliform* count of all treatments (CFU/g).

Tests	**1 2 3**	**4 5 6**	**7 8 9**	**10 11 12**	**13 14 15**
	Control	**Control+**	**50 mg/kg**	**100 mg/kg**	**150 mg/kg**
Mean total *Coliform**	2.10×10^{3b}	2.10×10^{3b}	4.70×10^{2a}	2.60×10^{2a}	3.00×10^{2a}
Standard deviation	4.00×10^{2}	3.60×10^{2}	1.60×10^{2}	8.00×10	4.00×10
Variance	1.60×10^{5}	1.30×10^{5}	2.70×10^{4}	6.40×10^{3}	1.60×10^{3}

* Different letters indicate significant differences between groups in mean total *Coliform* row ($p < 0.05$).

***Escherichia coli*.** Tables 2 and 3 demonstrate the results of microbial evaluations of fecal samples for *E. coli* after six weeks. Accordingly, in 3 replicates *Coliform* bacteria were examined with IMViC tests (I is for indole test, M is for methyl red test, V is for Voges-Proskauer test, and C is for citrate test. The lower case "i" is merely for "in" as a citrate test requires *Coliform* samples to be placed in citrate) that should have been checked to confidently judge that it is *E. coli*. The results that were considered positive for the tests for *E. coli* are shown in Table 2.

Table 2. Microbial cultures and tests to select *Escherichia coli*.

Tests	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
	Control			**Control+**			**50 mg/kg**			**100 mg/kg**			**150 mg/kg**		
Total *Coliform*	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Indole	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Methyl red	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Voges-Proskauer	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Citrate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Escherichia coli	3/3 X	1/3 X	3/3 X	3/3 X	3/3 X	3/3 X	3/3 X	3/3 X	3/3 X	3/3 X	3/3 X	3/3 X	3/3 X	3/3 X	3/3 X

A significant decrease in the number of *E. coli* (CFU/g) was seen in samples with 50, 100 and 150 mg/kg treatments compared to the control and control^{+} groups ($p < 0.05$).

Even though, a reduced number of *E. coli* was observed in all treated groups compared to the control and control^{+} samples, but no significant difference was found between treatment levels ($p > 0.05$).

Measuring the *E. coli* population in all treatments indicated that yarrow EO administration resulted in the decrease in *E. coli* population from 50 to 100 mg/kg of treatment. In contrast, by increasing the level of treatment to 150 mg/kg an increase in *E. coli* population was seen that of course was less than 50 mg/kg treatment group and also there was no significant difference among them ($p > 0.05$). Mean *E. coli* population count for all groups is shown in Table 3.

Table 3. *Escherichia coli* population (CFU/g) derived from tests mentioned at Table 2.

Tests	**1 2 3**	**4 5 6**	**7 8 9**	**10 11 12**	**13 14 15**
	Control	**Control+**	**50 mg/kg**	**100 mg/kg**	**150 mg/kg**
Mean count*	1.70×10^{3b}	2.10×10^{3b}	4.70×10^{2a}	2.70×10^{2a}	3.00×10^{2a}
Standard deviation	1.00×10^{3}	3.60×10^{2}	1.60×10^{2}	8.00×10	4.00×10
Variance	1.00×10^{7}	1.30×10^{5}	2.70×10^{4}	6.40×10^{3}	1.60×10^{3}

* Different letters indicate significant differences between groups in mean count row ($p < 0.05$).

***Lactobacillus*.** The culture test in De Man–Rogosa–Sharpe agar medium showed increased microbial population of useful bacteria *Lactobacillus* in the 150 mg/kg treatment group compared to all other groups ($p < 0.05$). There was an increase in all groups compared to the previous group shown in Table 4; however, no significant differences were noted in this population between the groups ($p > 0.05$).

Table 4. *Lactobacillus* population from all groups (CFU/g).

Tests	**1 2 3**	**4 5 6**	**7 8 9**	**10 11 12**	**13 14 15**
	Control	**Control+**	**50 mg/kg**	**100 mg/kg**	**150 mg/kg**
Mean count*	6.10×10^{7b}	7.80×10^{7b}	9.40×10^{7b}	1.00×10^{8b}	3.60×10^{8a}
Standard deviation	1.00×10^{7}	2.10×10^{7}	1.70×10^{7}	2.50×10^{7}	2.40×10^{8}
Variance	3.40×10^{14}	4.40×10^{14}	2.90×10^{14}	6.20×10^{14}	5.70×10^{16}

* Different letters indicate significant differences between groups in mean count row ($p < 0.05$).

Discussion

In this study, it was aimed to determine that what effects did different amounts of yarrow EO have on the microbial population of the digestive tract in layer hens, and what are the fields of application of this EO in poultry farms and how will the results of use appear? Both the beneficial and pathogen bacteria were aimed to be investigated. It is a well-known fact that the presence of intestinal diseases, such as bacterial and viral infections, intestinal flora imbalance, and coccidiosis, can have a negative impact on nutrient intake and decrease the production performance of laying hens. To prevent intestinal diseases and enhance production performance, a variety of feed additives, particularly antibiotics, have been extensively utilized in the poultry industry for many years. The use of antibiotics in poultry feed has raised concerns about drug residues and the development of anti-microbial resistance (13). Following the complete ban on the use of antibiotics as feed

additives by the European Union in 2006, the search for suitable alternatives to antibiotics has become increasingly important. Common alternatives used as feed additives to improve performance and overall health include prebiotics, probiotics, organic acids, and plant additives. Plant additives, which are present in a wide range of plants, spices, and their derivatives, have been shown to have a positive impact on product quality, production performance, and animal health, and are considered safe in the food industry (14). Essential oils are derived from plant materials (flowers, herbs, leaves, roots, etc.) and are complex mixtures of various components, such as terpenes, aldehydes, esters, alcohols, and other chemical molecules. Essential oils have been incorporated into animal diets due to their anti-microbial, anti-bacterial, anti-oxidant, and digestive stimulant properties (15). In recent years, EOs have been considered as potential alternatives to antibiotics for animals. Numerous studies have been conducted on the use of EOs in broilers. Panda *et al*., in 2003 (16) suggested that the improved results obtained for eggshell quality variables can be partially attributed to the fact that thymol affects the metabolic activity of beneficial bacterial colonies in the gut of laying hens. For many years yarrow, also known as *A. millefolium* L., has been employed in traditional medicine to treat a wide array of ailments. Yarrow is reportedly used medicinally to address conditions affecting the circulatory, pulmonary, digestive, hepatobiliary, urinary, and reproductive systems. Furthermore, yarrow extracts have exhibited anti-microbial activity against a broad spectrum of bacteria, including *Streptococcus pneumoniae*, *C. perfringens*, *Candida albicans*, *Mycobacterium smegmatis*, *Acinetobacter loofii*, and *Candida krusei* (17).

The results of a research that investigated the effect of dry yarrow plant at the rate of 0.20% of the diet on the microbial population of ileum and feces of broiler chickens revealed that yarrow plant can reduce the *Coliform* population compared to the control group, although this reduction was not significant. It was also shown that the population of *Lactobacilli* increased in the group consumed this plant, but this increase was not significant either (18). The results of the present study were consistent with the above research in terms of improving the pathogenic and beneficial microbial population, but there were also differences in terms of the significance of the differences compared to the control group, which seems to be due to the difference in the form of herbal supplementation that we used EO form of yarrow and saw significant differences.

The results of another study also showed that it caused a non-significant decrease in the number of fecal *Coliform* in broiler chickens (19), which was similar to our research in low amounts of treatment. In that research, the amount of lactic acid bacteria was also examined, which did not show a significant difference and were similar to the present study, but they were different from our study in relation to the change in the number of *Lactobacilli*, which is most likely due to the type of herbal plant consumption, as well as the consumption amount and age of the poultry. This study showed a significant increase in number of *Lactobacilli* in the last group with the highest amount of treatment and also this study was conducted in older laying hens with a different microbial population and altered digestive system compared to the broiler chickens.

In a study on the population of *Coliform* and *lactobacillus* in the intestinal contents of broilers (20), it was observed that the addition of 1.50% and 3.00% of dry powder of yarrow herbal plant reduced the number of *Coliform* non-significantly, up to a concentration of 1.50%, and significantly in the concentration of 3%, which was similar to the results of our study, but in the present study, there was a significant reduction in all groups using the treatment, which was probably due to the use of EOs. In that study 20), they showed that *Lactobacilli*

had a non-significant increase in the groups under the influence of the treatments, which was exactly the same as the present study. Of course, in 150 mg/kg treatment, this increase showed itself in a significant way.

In conclusion, apart from the general observation of the beneficial effects on the gastrointestinal flora in all treatments, specifically the best anti-bacterial activity against *Coliform* and *E. coli* at 100 mg/kg and the most significantly growth enhancement of *Lactobacillus* population at 150 mg/kg of yarrow EO were obtained.

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Pennyroyal essential oil effects on *Escherichia coli* and *Lactobacillus* microflora in laying hens

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Abstract

Pennyroyal essential oil possess various useful functions such as antimicrobial, antioxidant and also aromatic and medicinal properties. Due to antimicrobial properties, it can cause increase in products shelf life. Researches demonstrate that pennyroyal essential oils have a strong antibacterial property against microorganisms. The objective of this study was to examine the impact of pennyroyal essential oil on the gastrointestinal microflora of laying hens, specifically focusing on *Escherichia coli* as pathogen and beneficial *Lactobacillus*. A total number of 80 laying hens were utilized in a fully randomized experimental design. Experimental treatments included: 1) basic diet (control), 2) basic ration containing 0.0125 mL of emulsifier 3) basic ration containing 50 mg/kg of pennyroyal essential oil, 4) basic ration containing 100 mg/kg of pennyroyal essential oil and 5) basic ration containing 150 mg/kg of pennyroyal essential oil. The study lasted for a period of 6 weeks, following a 2-week adaptation period. Upon completion of the research, faecal samples from each cage were collected and combined per treatment group for microbiological analysis. The relevant data were first registered in Excel software and after classification they were subjected to statistical analysis using SPSS software. Comparisons of averages were done with Duncan's method. Results indicated that lowest *coliform* and *E. coli* count with significantly difference was at 50 mg/kg of pennyroyal essential oil. *Lactobacillus* population showed an increase in 100 and 150 mg/kg treatments that only 150 mg/kg of pennyroyal essential oil had significantly high count compared to the others.

Keywords: *Escherichia coli*, Essential oil, *Lactobacillus*, Layers, Pennyroyal

Introduction

During the laying period, particularly towards the end, the intestinal function is compromised, leading to an imbalance in the immune system and disruption of the intestinal flora due to high-intensity production resulting in poor egg quality and performance (1). The impact of essential oils on reducing the colonization of *Escherichia coli*, *Clostridium perfringens*, and *Campylobacter jejuni* has been extensively studied in broilers (2). Furthermore, the beneficial effects of essential oils have been specifically documented in the poultry industry. The addition of essential oil (containing thymol) to the diet of broilers may enhance growth performance, increase the activity of intestinal and pancreatic digestive enzymes (3), as well as cellular and humoral immunity (4). Therefore, essential oils may positively impact gut health by maintaining gut integrity

and mucosal barrier functions, enhancing immune system activities, and regulating gut microbiota. It is determined that pennyroyal essential oils are aromatic and cyclohexenes and the original compounds of these oils is pulegone that has a specific mint aroma from an intense to balsamic and pungent aroma and the essential oil has an antioxidant property due to the existence of phenolic compounds such as phenolic acids, flavonoids, phenolic ditrepenes and tannins. The essential oil has also been useful for treatments in some diseases and disorders such as digestion, gallbladder and liver disorders, parasitic and infectious diseases, tuberculosis, and cholera (5). Antimicrobial properties of essential oils can be caused by some groups like menthone, pulegone and neo-menthone (6). This study assumed that the inclusion of pennyroyal essential oil had a positive effect on the intestinal microbial composition. Therefore, the aim of this study was to investigate the effects of pennyroyal essential oil in the diet on the population of *E. coli* bacteria as an indicator of pathogenic bacteria and *Lactobacillus* as an indicator of beneficial bacteria in the digestive system of laying hens.

Materials and Methods

Animals. The research involved 80 laying hens in a fully randomized design with 5 treatments, 4 replications, and 4 birds per replication. The feed ration, energy, protein levels and consumption were adjusted according to the guidelines outlined in the breeding and maintenance manual of the specific strain being used. Throughout the 8-week duration of the experiment, the laying hens were provided with the required feed including a 2-week adaptation period followed by 6 weeks of treatment.

Experimental treatments. The groups in this investigation were as follows: 1. Basic ration (control) 2. Basic diet plus 0.0125 mL emulsifier as control^{+} group 3. The basic diet containing 50 mg/kg diet of pennyroyal essential oil 4. The basic ration containing 100 mg/kg of ration of pennyroyal essential oil 5. The basic ration contains 150 mg/kg of ration of pennyroyal essential oil. Emulsifier was necessary in all treatment groups to ensure the essential oil could dissolve in water.

The microbiota residing in the gastrointestinal system. Upon completion of the investigation, the faecal samples from the chickens in each treatment group were collected separately and transported to the microbiology laboratory for further examination. The study involved the assessment and enumeration of *E. coli* bacteria as an indicator of Gram-negative pathogens, along with Lactobacillus as an indicator of beneficial intestinal bacteria.

Statistical analysis. The initial step involved recording all data in Excel software. Subsequently, the data underwent classification before undergoing statistical analysis with SPSS version 23 for Windows (SPSS Inc., Chicago, USA). Significance was determined by p values less than 0.05. Treatment means were compared using one-way analysis of variance (ANOVA) followed by Duncan's multiple comparison test to assess statistically significant differences. The data were presented as means ± standard deviation.

Results

Total *Coliforms*. At the end of the study all fecal samples were gathered from each of cages related to each group and mixed to prepare a mixed sample for each treatment. To make results reliable three replicates of each treatment sample had to be tested. The groups consisted of control, control+ and three levels of

treatment groups and in Violet Red Bile Agar medium culture total *coliform* was obtained and demonstrated in Table 1.

Table 2. Total *Coliform* Count of all treatments (CFU/g) (VRBA).

Tests	1 2 3	4 5 6	7 8 9	10 11 12	13 14 15
	Control	**Control+**	**50 mg/kg**	**100 mg/kg**	**150 mg/kg**
Mean total count*	2.1×10^{3c}	2.1×10^{3c}	5^{a}	6.3×10^{2b}	2.1×10^{2ab}
Std. Deviation	4×10^{2}	3.6×10^{2}	5	1.5×10^{2}	8.6×10
Variance	1.6×10^{5}	1.3×10^{5}	2.5×10	2.3×10^{4}	7.4×10^{3}

* Different letters indicate significant differences between groups in Mean Total Coliform ($p < 0.05$).

***Escherichia coli*.** Results of microbial evaluations for *E. coli* are shown in Table 3. The results of coliform counting tests were used for *E. coli* evaluation using confirmatory IMViC tests shown in Table 2.

Table 2. Microbial cultures and tests to select *E. coli*.

Tests	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	Control			**Control+**			**50 mg/kg**			**100 mg/kg**			**150 mg/kg**		
Total *Coliform*	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Indole	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
MR	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
VP	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Citrate	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E. coli	3/3 X	1/3 X	3/3 X	3/3 X	3/3 X	3/3 X	3/3 X	3/3 X	3/3 X	3/3 X	3/3 X	3/3 X	3/3 X	3/3 X	3/3 X

The results demonstrated that a significant decrease in the number of *E. coli* was seen in all samples treated with pennyroyal essential oil compared to control and control^{+} groups (Table 3). The lowest *E. coli* population was seen at 50 mg/kg treatment group and after that there was an increase in E. coli count in other two treatments in comparison with this treatment. The 100 and 150 mg/kg treatments showed 633.33 and 216.67 CFU of E. coli in each gram of feces, respectively, and despite the increase in *E. coli* count in last two treatment groups compared to 50 mg/kg treatment and there was no significant differences between treatment groups ($p > 0.05$).

Table 3. *E. coli* population (CFU/g) derived from tests mentioned at Table 2.

Tests	1 2 3	4 5 6	7 8 9	10 11 12	13 14 15
	Control	**Control+**	**50 mg/kg**	**100 mg/kg**	**150 mg/kg**
Mean Count*	1.7×10^{3b}	2.1×10^{3b}	5^{a}	6.5×10^{2a}	2.2×10^{2a}
Standard deviation	1×10^{3}	3.6×10^{2}	5	1.5×10^{2}	8.6×10
Variance	1×10^{7}	1.3×10^{5}	2.5×10	2.3×10^{4}	7.4×10^{3}

* Different letters indicate significant differences between groups in mean count row ($p < 0.05$).

***Lactobacillus*.** Counting *Lactobacillus* in De Man–Rogosa–Sharpe agar (MRSA) medium culture showed increased microbial population of beneficial bacteria *Lactobacillus* in the 150 mg/kg treatment group compared to control and all other treatment groups ($p < 0.05$). However, there were no significant variations observed within this demographic among the groups ($p > 0.05$). The results of *Lactobacillus* bacteria cultured in MRSA medium is presented in Table 4.

Table 4. *Lactobacillus* population from control and treatment groups (CFU/g) (MRSA)

Tests	1 2 3	4 5 6	7 8 9	10 11 12	13 14 15
	Control	Control+	50 mg/kg	100 mg/kg	150 mg/kg
Mean Count*	6.1×10^{7b}	7.8×10^{7b}	5×10^{7b}	1.36×10^{8b}	3.36×10^{8a}
Standard deviation	1×10^{7}	2.1×10^{7}	1.9×10^{7}	5.2×10^{6}	1.4×10^{8}
Variance	3.43×10^{14}	4.44×10^{14}	3.64×10^{14}	2.8×10^{13}	2.1×10^{16}

* Different letters indicate significant differences between groups in mean count row ($p < 0.05$).

Discussion

The aim of this study was to investigate the effects of pennyroyal essential oil in the diet on the population of *Escherichia coli* bacteria as an indicator of pathogenic bacteria and *Lactobacillus* as an indicator of beneficial bacteria in the digestive system of laying hens. It is widely recognized that the presence of intestinal diseases such as bacterial and viral infections, intestinal flora imbalance and coccidiosis can significantly reduce nutrient intake and impair the production performance of laying hens. To prevent intestinal diseases and improve production performance various feed additives, especially antibiotics, have been extensively used in the poultry industry for many years. The use of antibiotics in poultry feed has sparked concerns about drug residues and the development of antimicrobial resistance (7). Since the European Union completely banned the use of antibiotics as feed additives in 2006, the search for viable alternatives to antibiotics has become increasingly important. Common alternatives used as feed additives to enhance performance and overall health includes prebiotics, probiotics, organic acids and plant additives. Plant additives sourced from a wide range of plants, spices and their derivatives have been proven to positively impact product quality, production performance and animal health and are considered safe in the food industry (8). Panda et al found that the improved eggshell quality variables were partially due to thymol's impact on the metabolic activity of beneficial bacterial colonies in the gut of laying hens (9). This, in turn, positively influenced the absorption rate of minerals, particularly calcium and magnesium. Essential oils impact the function of the intestines by promoting the secretion of digestive fluids and enhancing the activity of enzymes (10). Studies on the impact of pennyroyal essential oil or its compounds on intestinal digestion in poultry have yielded conflicting results with some showing growth-promoting and antibacterial effects and others demonstrating positive effects on performance, antioxidant enzyme activities and digestive enzyme activity. Essential oils have been utilized as a substitute for antibiotics in the poultry industry due to its various biological properties including antimicrobial, antioxidant, disinfectant and antiparasitic activities. Furthermore, some studies suggested that herbal compounds could enhance the activity of digestive enzymes and nutrient absorption (8). Furthermore, certain plant-based feed additives have the potential to directly or indirectly impact the gut microflora as noted by Cowan (11). Various studies have demonstrated the positive effects of supplementing essential oil in the diet of laying hens on their intestinal flora as highlighted by (12).

It is known that pennyroyal is an herbal plant containing 1 - 2% essential oil of which the principal component is pulegone (60-90%) (13). Reportedly, minimum inhibitory concentration and minimum bactericidal concentration effects of pennyroyal essential oil on *E. coli* (laboratory strain) were 16 and 32, respectively, however, unfortunately it could not be compared to our findings. In another study (14), E. coli concentration (CFU/g) was measured at 4 levels of pennyroyal essential oil (100, 200, 300 and 400 ppm) and

the lowest count was at 100 ppm and then 400 ppm and the difference were significant in both groups compared to the control group and also between both of the treatment groups. Despite the difference in the type of herbal product which was essential oil in the present study and extract in the mentioned research, the results of our study were exactly the same as the findings of that study, the lowest count of *E. coli* with a significant difference was at 50 mg/kg treatment group and then at the 150 mg/kg treatment group. Also, there was no significant difference between 100 and 150 mg/kg treatment group like the research mentioned above. At the same research in boilers the *Lactobacillus* concentration was evaluated despite the increase in the number of *Lactobacillus* bacteria compared to the control group, this increase was not significant in any of the groups (14), while in the present study, it showed a significant increase in the last treatment group that was 150 mg/kg of pennyroyal essential oil that probably the results were due to the maximum amount of extract that was tested in that study and higher levels should be checked for it. We could not find any results for *coliforms* to compare to our study. It may be just like *E. coli* population mentioned above or like results from research (15) on rumen microbial population of sheep that showed no significant difference that due to the difference in the digestive system, it seems to be similar to the changes reported in the *E. coli* population.

In conclusion, from the results of this study, it was concluded that using 50 mg/kg pennyroyal have significant effects on pathogen bacteria and 150 mg/kg pennyroyal had significant effects on *Lactobacillus* population of laying hens.

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The Effect of *Achillea millefolium* and *Mentha pulegium* Essential Oils on *Bacillus* and *Bifidobacterium* microbiota in layers

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Abstract

This experiment was conducted to assess the efficacy of *Achillea millefolium* and *Mentha pulegium*, also known as yarrow and pennyroyal essential oils on gastrointestinal microflora specifically *Bacillus* and *Bifidobacterium* in layers. A total number of 128 laying hens were utilized within a completely randomized design framework. The experimental treatments consisted of the following: 1. A basic diet serving as the control group; 2. A basic diet supplemented with 0.0125 mL of emulsifier, referred to as control+; 3, 4 and 5. Basic diet plus 50, 100, 150 mg/kg of yarrow essential oil, respectively; 6, 7 and 8. Basic diet plus 50, 100, 150 mg/kg of pennyroyal essential oil. The energy and protein content of the food rations were adjusted in accordance with the specific strain guidelines. The treatments were lasted for 6 weeks. At the end, fecal samples from each cage were collected as a composite for each treatment group to facilitate microbiological analysis. The data were classified by excel and underwent statistical analysis utilizing SPSS software and means were compared using Duncan's method. Compared to control group, there was a reduction in the number of *Bacillus* bacteria in treated groups with Yarrow and Pennyroyal. However, no significant difference was found among the mentioned treatment groups. Also, a rise in the population of *Bifidobacterium* in the treatment groups compared to control and control^{+} samples and the count by yarrow essential oil was more than the count by pennyroyal essential oil.

Keywords: *Bacillus, Bifidobacterium*, Layers, Pennyroyal, Yarrow

Introduction

The reduction in egg production and the deterioration of egg quality towards the conclusion of the laying period have considerably affected the economic advantages presenting a significant obstacle to prolonging the laying duration (1). In light of the restrictions on the application of antibiotics as growth enhancers in livestock, there has been an increasing interest in the use of natural bioactive substances such as essential oils to improve the health and productivity of poultry. Essential oils which are extracted from plant materials and consist of aromatic oily liquids have attracted attention for their antibacterial properties positioning them as natural substitutes for antibiotics in animal husbandry (2). Nevertheless, additional research is required to investigate the impact of essential oils on the intestinal microbial community, mucosal barrier and immune function of layers during the later stages of production. The botanical designation of Yarrow is *Achillea millefolium*, a

plant recognized for its extensive application in traditional medicine. This species is indigenous to both Europe and America with a notable prevalence in Iran. Its utilization in Chinese medicine can be traced back approximately 3,000 years. Yarrow is reputed for its ability to lower blood pressure and blood sugar levels in addition to exhibiting anti-inflammatory properties. Research has been conducted on its medicinal attributes including its anti-histaminic and anti-cholinergic effects. Yarrow is employed in the management of infectious fevers, typhoid and diarrhea. Investigations into the antimicrobial properties of various Yarrow extracts have revealed that the methanol extract demonstrates superior efficacy against severe gram-positive bacteria. Furthermore, a study has explored the long-term impacts of Yarrow consumption on certain aspects of metabolic syndrome. Yarrow contains phenolic compounds, pectin, resin, ascorbic acid and malic acid. The genus Mentha is part of the Lamiaceae family and comprises approximately 25 species that are indigenous to Europe, North Africa, the Middle East including Asia Minor and the Near East (3). A widely recognized variety is Pennyroyal (*Mentha pulegium* L.) (4). *Mentha pulegium* L., commonly known as pennyroyal is a fragrant perennial herb found globally (5). The leaves of pennyroyal emit a potent mint-like aroma and have been historically utilized in traditional medicine for treating gastrointestinal issues serving as an astringent exhibiting antibacterial properties and for use in culinary applications (3, 6).

In the present study, we hypothesized that the inclusion of yarrow and pennyroyal essential oils would positively influence the gut microbiota of layers. Therefore, the main aim of this research was to investigate the effects of adding Yarrow and Pennyroyal essential oils to the diet on the microbial equilibrium of *Bacillus*, a gastrointestinal pathogen, as well as beneficial intestinal bacteria such as *Bifidobacterium* in laying hens.

Materials and Methods

Animals. In this research, a total number of 128 Hy-Line W-80 laying hens were employed within a completely randomized design featuring 8 treatments, 4 replications and 4 birds allocated per replication. The feed composition, energy content, protein levels and consumption rates were adjusted in accordance with the recommendations provided in the breeding and maintenance manual for the particular strain (W-80 Commercial Layers Management Guide). The laying hens received the requisite feed throughout the 8-week experimental period which comprised a 2-week adaptation phase followed by 6 weeks of treatment.

Experimental treatments. This research included experimental interventions organized into the subsequent groups: 1. Basic diet (control) 2. Basic diet plus 0.0125 mL emulsifier mentioned as control+, 3. Basic diet containing 50 mg/kg of yarrow essential oil 4. Basic diet containing 100 mg/kg of yarrow essential oil 5. Basic diet containing 150 mg/kg of yarrow essential oil, 6. The basic diet containing 50 mg/kg diet of pennyroyal essential oil (7). The basic diet containing 100 mg/kg of ration of pennyroyal essential oil and 8. The basic diet containing 150 mg/kg of ration of pennyroyal essential oil.

Microbial community present within the gastrointestinal tract. Upon completion of the research, the fecal samples from the chickens corresponding to each treatment were collected individually and sent to the microbiology laboratory for additional analysis. This study focused on examining and quantifying *Bacillus* bacteria as a marker for Gram-positive pathogens as well as *Bifidobacterium*, which served as an indicator of beneficial intestinal flora (7).

Statistical analysis. The data was first documented using Excel software. After classification, statistical analysis was performed utilizing SPSS version 23 for Windows (SPSS Inc., Chicago, USA), with a significance threshold established at p values below 0.05. Treatment means were evaluated through one-way analysis of variance (ANOVA) followed by Duncan's multiple comparison test to determine statistically significant differences. The data presentation comprised means along with standard deviation.

Results

***Bacillus*.** Figure 1 and Table 1 demonstrate the results of microbial evaluations after six weeks for *Bacillus* using *Bacillus cereus* selective agar base (BCSA) medium. Accordingly, a reduced number of *Bacillus* was observed in all yarrow treated groups compared to control samples. Also, there was a reduction in the number of *Bacillus* bacteria in treated groups with pennyroyal ($p < 0.05$). However, no significant difference was found ($p > 0.05$) among the treatment groups, a decrease in the number of *Bacillus* was seen in samples with yarrow by increasing the treatment dose from 50 to 150 mg/kg of yarrow essential oil and the group treated with 150 mg/kg of yarrow essential oil showed the lowest number of *bacillus* bacteria among three levels of yarrow treated groups. Also, the groups treated by pennyroyal essential oil showed the same trend of reduction in bacillus count as seen in yarrow treatments. The group treated by 150 mg/kg of pennyroyal essential oil showed the lowest mean *Bacillus* count and the 100 and 50 mg/kg treated groups had more *Bacillus* bacteria than 150 mg/kg treatment, although the difference among the treatments was not significant ($p > 0.05$). Comparing two different essential oils at the same values showed no significant difference among them ($p > 0.05$). Meanwhile 50 mg/kg of yarrow essential oil showed lower bacillus count in comparison with 50 mg/kg of pennyroyal essential oil ($p > 0.05$). This case also applied to the 150 mg/kg treatments and yarrow essential oil showed a stronger effect against *Bacillus*. In relation to 100 mg/kg treatment, the *Bacillus* population values were the same for both essential oils.

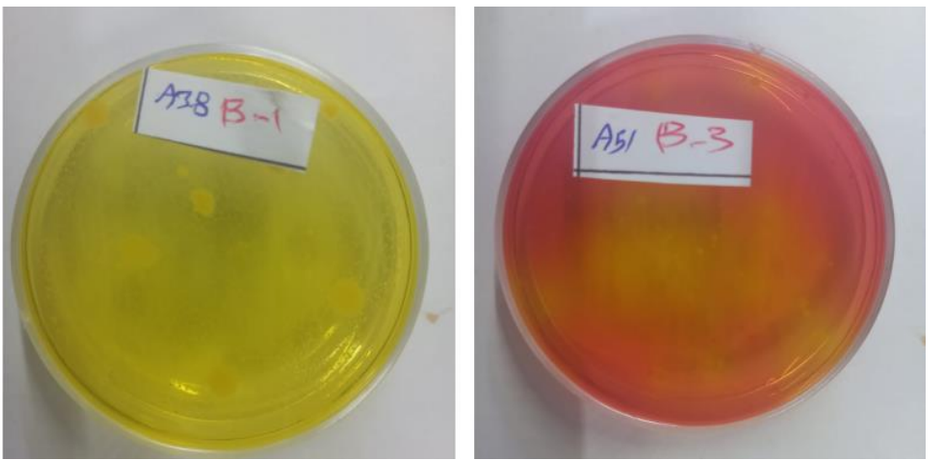

Fig. 1. Bacillus numeration in *Bacillus cereus* selective agar base (BCSA) medium.

Table 3. *Bacillus* Count of all treatments (CFU/gr) on BCSA.

Samples	Groups	Mean count	Std. deviation	Variance
1				
2	**Control**	3.6×10^{b}	3×10	9.3×10^{2}
3				
4				
5	**Control+**	3×10^{ab}	2×10	4×10^{2}
6				
7				
8	**50 mg/kg Yarrow**	2×10^{ab}	1.5×10	2.2×10^{2}
9				
10				
11	**100 mg/kg Yarrow**	1×10^{ab}	5.5	3.1×10
12				
13				
14	**150 mg/kg Yarrow**	4.6^{a}	3	9.3
15				
16				
17	**50 mg/kg Pennyroyal**	3×10^{ab}	1.3×10	1.7×10^{2}
18				
19				
20	**100 mg/kg Pennyroyal**	1×10^{ab}	4.5	2.1×10
21				
22				
23	**150 mg/kg Pennyroyal**	9.6^{ab}	6	3.6×10
24				

[ab] Different letters indicate significant differences between groups (in column) in mean count column ($p < 0.05$).

***Bifidobacterium*.** As shown in Figure 2 and Table 2, the reinforced clostridial agar (RCA) culture test indicated a rise in the population of beneficial bacteria *Bifidobacterium* in the treatment groups compared to control and control+ samples ($p < 0.05$). Accordingly, by increasing the amount of yarrow essential oil the count for *Bifidobacterium* was raised. These increased values were not significantly different from each other in different levels for yarrow essential oil. This increasing trend was also observed for three levels of pennyroyal essential oil treatment. These values were the same in two treatments of 100 and 150 mg/kg treatments. Comparison of the effects of two essential oils with each other showed that 150 mg/kg yarrow essential oil in relation to the increase of *Bifidobacterium* population had a greater but not significant effect than pennyroyal essential oil ($p > 0.05$). This increase in *Bifidobacterium* population also applied to the treatments with 100 mg/kg essential oils showed more *Bifidobacterium* count for yarrow essential oil treatment in comparison with 100 mg/kg pennyroyal essential oil but the difference was not significant ($p > 0.05$). In the case of the treatments that received 50 mg/kg of both of essential oils, it was similar to the two previously mentioned groups and despite the higher number of *Bifidobacterium* in yarrow essential oil treatment, this amount was not significantly different from pennyroyal essential oil treatment ($p > 0.05$).

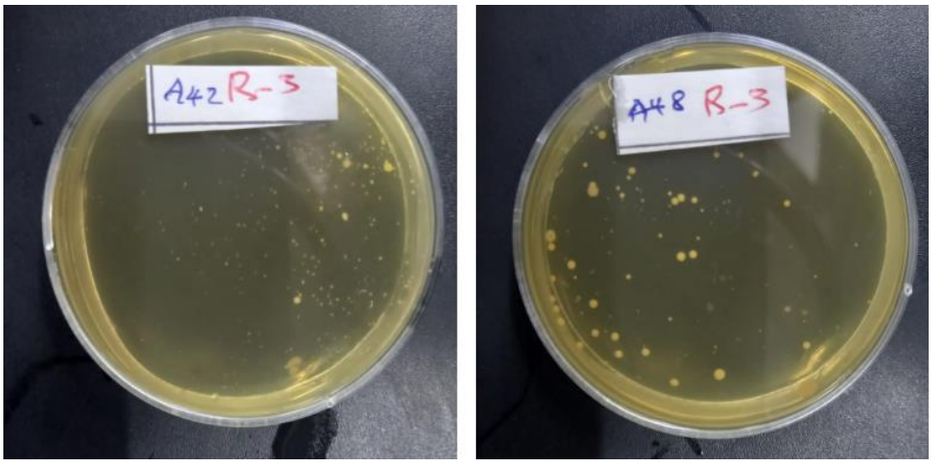

Fig. 2. *Bifidobacterium* numeration in reinforced clostridial agar (RCA) medium.

Table 2. *Bifidobacterium* population derived all groups (CFU/gr) (RCA).

Samples	Groups	Mean count	Std. Deviation	Variance
1 2 3	**Control**	7.1×10^{6d}	1×10^{6}	1×10^{12}
4 5 6	**Control+**	8×10^{6d}	2×10^{6}	4×10^{12}
7 8 9	**50 mg/kg Yarrow**	2.2×10^{7ac}	6.5×10^{6}	4.3×10^{13}
10 11 12	**100 mg/kg Yarrow**	2.5×10^{7ab}	6.5×10^{6}	4.3×10^{13}
13 14 15	**150 mg/kg Yarrow**	3×10^{7ab}	8.5×10^{6}	7.3×10^{13}
16 17 18	**50 mg/kg Pennyroyal**	1.4×10^{7cd}	2.6×10^{6}	7×10^{12}
19 20 21	**100 mg/kg Pennyroyal**	1.8×10^{7bc}	2.6×10^{6}	7×10^{12}
22 23 24	**150 mg/kg Pennyroyal**	1.8×10^{7bc}	4.5×10^{6}	2.1×10^{13}

* Different letters indicate significant differences between groups (in column) in mean count column ($p < 0.05$).

Discussion

This research aimed to investigate the effects of varying concentrations of yarrow and pennyroyal essential oils on the microbial population within the digestive tract of layer hens. Additionally, we sought to explore the potential applications of these essential oils in poultry farming and the anticipated outcomes of their usage. The study focused on both beneficial and pathogenic bacteria. It is widely recognized that intestinal diseases including bacterial and viral infections and imbalances in intestinal flora can adversely affect nutrient absorption and reduce the production efficiency of layers. To mitigate intestinal diseases and improve production performance, the poultry industry has long relied on a range of feed additives, particularly

antibiotics. The incorporation of antibiotics into poultry feed has generated apprehensions regarding the presence of drug residues and the emergence of antimicrobial resistance (10, 11). Since the European Union implemented a total prohibition on the use of antibiotics as feed additives in 2006, the quest for effective alternatives to antibiotics has gained significant importance (12). Prebiotics, probiotics, organic acids, and plant-based additives are frequently utilized as feed additives to enhance performance and overall well-being. These plant additives, derived from a diverse array of plants, spices and their derivatives, have demonstrated beneficial effects on product quality, production efficiency and animal health, and are regarded as safe within the food industry (12). Essential oils are extracted from various plant materials including flowers, herbs, leaves, and roots, and consist of intricate combinations of numerous components such as terpenes, aldehydes, esters, alcohols, and other chemical compounds. These oils have been integrated into animal feed due to their antimicrobial (13), antibacterial (14), antioxidant (15) and digestive stimulant (16) properties. Recently, essential oils have emerged as potential substitutes for antibiotics in animal husbandry. A variety of studies have explored the application of essential oils in layer chickens. Yarrow, scientifically referred to as *Achillea millefolium L.*, is a member of the Asteraceae family. This plant is widely recognized in traditional medicine for its ability to support digestive health, enhance digestion and nutrient absorption, and foster overall physiological wellness in both humans and animals. Researches have indicated that yarrow possesses antibacterial and antifungal characteristics positioning it as a potential alternative growth stimulant. It is frequently employed to alleviate various digestive disorders and allergic reactions. Numerous compounds including flavonoids, sesquiterpene lactones, and polyacetylenes have been extracted from various species of yarrow. Medicinally, yarrow is reported to be effective for conditions impacting the circulatory, pulmonary, digestive, hepatobiliary, urinary and reproductive systems. Additionally, extracts of yarrow have demonstrated antimicrobial properties against a wide spectrum of bacteria, such as *Streptococcus, Clostridium, Candida, Mycobacterium, Acinetobacter,* and *Candida*. Studies indicate that pennyroyal essential oils exhibit significant antibacterial activity against various microorganisms including *Salmonella*, *Listeria*, *Yersinia*, *Escherichia coli, Bacillus, Clostridium, Staphylococcus, Helicobacter, Pseudomonas, Klebsiella*, and *Brochothrix* (17).

Our findings were in consist with studies reported the effectiveness of the yarrow and pennyroyal essential oils on reduction of pathogen bacteria and also elevating beneficial bacteria population (18).

In some research it was indicated that usage of pennyroyal essential oil showed negative effects mainly on eggshell pigments, however, in our findings in three levels (50, 100 and 150 mg/kg) of these essential oils no negative effects were observed. In this research yarrow essential oil had better effects on bacterial population and showed less *Bacillus* and more *Bifidobacterium* compared to pennyroyal essential oil. Despite the results of a research by Rasouli et al. (2020) that was done in broiler farm, pennyroyal extract had no effects on *Lactobacillus* count as an indicator for beneficial microorganisms (19). Our findings showed a significant increase in *Bifidobacterium* count in intestinal flora in layers that may be due to the essential oil form used in our study or due to the different beneficial bacteria studied in our research.

In conclusion, the findings of this study suggested that yarrow and pennyroyal essential oils could improve the condition of intestinal microbial flora and reduce harmful bacteria and increase beneficial intestinal bacteria in layer hens. From the results of the present study, it was concluded that using 150 mg/kg yarrow essential oil and 100 mg/kg pennyroyal essential oil showed the best effects on Bifidobacterium count and

150 mg/kg of both essential oils had the greatest effect on *Bacillus* count in digestive tract in laying hens. Comparing two mentioned essential oils, yarrow had better effect in *Bacillus* count reduction and *Bifidobacterium* count elevation.

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Evaluation of the effects of dietary supplementation with Alliacin (alcoholic extracts of garlic, onion and leek) on performance of broiler chickens (Ross-308)

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Abstract

The use of growth stimulants in the poultry industry leads to an increase in the weight of broilers. Recently, the use of medicinal plants and their extracts in controlling poultry diseases and stimulating growth has received much attention due to its cost-effectiveness and minimal side effects. Alliacin is an herbal compound containing the alcoholic extracts of garlic, onion and leek**.** To evaluate the effect of Alliacin on broiler performance, Alliacin was added to a ratio of 1, 2 and 4 mL per L of drinking water in different experimental groups and weekly weight gain, end-of-period weight, feed conversion ratio and villi height and crypt depth of duodenum and jejunum were evaluated. The results of this study revealed that the groups receiving Alliacin weighed more than the control group during the rearing period. The mean weight gain in the group receiving Alliacin at 2 mL/L of drinking water was 240 g more than the control group. Also, the best feed conversion ratio (1.52 and 1.55) was belonged to the Alliacin group at 2 and 4 mL per L of drinking water, respectively. Histomorphometric evaluation of duodenum and jejunum showed that the groups receiving Alliacin had higher villi height and crypt depth than the control group, however, this difference was not significant. It could be concluded that dietary supplementation with Alliacin could improve weight gain, feed conversion ratio (2 mL per L of water)**,** villi height and crypt depth in duodenum and jejunum**.**

Keywords: Broiler chickens, Garlic, Leek, Onion, Performance

Introduction

The increase in demand for livestock products has resulted in the long-term and uncontrolled use of growth-promoting antibiotics. This can lead to the emergence of antibiotic resistance, which is a global threat to livestock and humans. The ban on the use of sub therapeutic levels of antibiotics in different countries and the growing awareness of consumers about antibiotic residues have intensified the efforts to find suitable alternatives to antibiotics in feed. The possible consequences of poultry farming without the use of growth-promoting antibiotics are poor growth, lower feed efficiency, increased disease and increased bird mortality (1). In recent years, a lot of research has been done on the use of medicinal plants or their compounds as growth stimulants. In this context, the use of Allium species with various types of valuable bioactive compounds such as organic sulfur compounds, polyphenols and saponins seems to be a suitable choice (2). Allium genus includes 550 species, few of which are important as medicinal plants, among them are onion

(*Allium cepa* L.), garlic (*Allium sativum* L.) and leek (*Allium ampleoprasam* var. *kurrat*) pointed out (1). Onions have many organic sulfur compounds including trans-S (1-propenyl) cysteine sulfoxide, S-methylcysteine sulfoxide, S-propyl cysteine sulfoxide, cyclolysine, flavonoids, phenolic acids, sterols including cholesterol, stigma sterol, bisitosterol, saponins, Bisterols, sugars and some volatile oil compounds, which are mainly sulphurous compounds. Most parts of the onion plant contain compounds with antibacterial, antiviral, anti-parasitic and antifungal properties (3). Leek and its leaves are a potential source of several bioactive or health-promoting compounds, including a large number of phytonutrients (4). Leek extract has been investigated for its antimicrobial, antioxidant, cytotoxic (5), blood sugar lowering (6) and blood lipid lowering (7) effects. Garlic has biologically active components such as sulfur-containing compounds including alliin, diallyl sulfides and allicin, which act as antibacterial, antifungal, anti-parasitic, antiviral, antioxidant, anti-thrombotic, anti-cancer and vasodilator (1). Aliacin is an herbal compound consisting of alcoholic extract of garlic, onion and leek, which was produced by the Research Institute of Medicinal Plants of Shahid Beheshti University of Tehran, which was tested according to the effects of each of its components so that it can be commercialized as a final product. In the present study, the synergistic effect of garlic, onion and leek in the form of Alliacin supplement was investigated on the weight performance of birds, food conversion ratio and intestinal tissue histomorphometry (including villi height and crypt depth).

Materials and Methods

Experimental design, birds and housing. 120 one-day-old male broiler chickens (Ross 308)® with almost the same weight were selected from one of the hatchery centers around Urmia city and randomly divided into four equal groups including control, treatment 1 (T1), treatment 2 and treatment. 3 (T3). During the rearing period (six weeks), temperature, light and humidity were adjusted according to the breeding catalog of the Ross 308 broiler strain. Also, the ration used during the breeding period was prepared and provided to the birds based on the age and weight of the birds. During the rearing period, Alliacin was prepared and added to the drinking water of T1, T2 and T3 groups, 1, 2 and 4 milliliters per liter, respectively.

Growth performance evaluation. At the end of each week, all the birds of each group were weighed and the food conversion ratio was calculated as below:

Food conversion ratio = amount of feed consumed (kg) / amount of body weight (kg)

Intestinal histomorphometric evaluation. In order to evaluate intestinal tissue histomorphometry, at the end of the rearing period, after slaughter, necropsy was performed and intestinal tissue samples were taken from duodenum and jejunum from 10 birds in each group. For histological evaluation, as previously described (8). Briefly, after tissue fixation in 10.00% buffered formalin, specimens were processed through paraffin embedding, cut into 6.00 μm sections and stained with Hematoxillin and Eosin technique. Then, the height of the villi and the depth of the crypts in the slides were examined using a light microscope graduated lenses.

Statistical analysis. The results were expressed as the mean ± standard error (Mean ± SE). Differences among the groups were assessed by one-way analysis of variance using SPSS Software Package for Windows (version 25.0, SPSS Inc., Armonk, USA). Statistical significance among the groups was determined by Tukey multiple comparison post-hoc test and the $p \leq 0.05$ were considered to be statistically significant.

Results

The comparison of the results of the average weight of broiler chickens (grams) during different weeks of the rearing period indicated that with increasing age, the difference in average weight between groups was increased. At the end of the sixth week, the highest weighting average (grams) was related to T2 (3118.57), T3 (3062.5), T1 (2920) and the control group (2877), respectively. No significant difference was observed among the groups in different weeks of the rearing period ($p > 0.05$) (Table 1). Also, comparison of food conversion ratio at the end of the rearing period in different groups showed that T2 and T3 groups had the lowest food conversion ratio (1.526, 1.555), and the control and T1 groups had the highest food conversion ratio of 1.651, 1.626, respectively.

Table 1. Comparison of the average weight of broilers (grams) at the end of each week of the rearing period (Mean ± SE).

Groups	1st week	2nd week	3rd week	4th week	5th week	6th week
Control	157.53±2.8	419.97±12.5	821±33.2	1389.75±74.7	2059.43±159.8	2877±272.4
T1	159.54±2.6	425.73±23.6	856.41±41.5	1458.15±68.8	2140±133.4	2920±261.9
T2	157.16±3	432.41±24.3	862.72±42	1505.01±81.9	2231.57±184.1	3118.57±330
T3	160±1.9	436.65±27.1	856.77±40.7	1487.86±64.2	2207.4±191.3	3062.5±334.5
***p*-value**	0.1005	0.5594	0.1393	0.0651	0.2147	0.4464

The comparison of Alliacin effect on villi height and crypt depth (Fig. 1) of duodenum and jejunum at the end of the rearing period in different groups is shown in Table 2. Comparison of the average height of villi and depth of crypt of duodenum and jejunum, at the end of the sixth week, showed that there was no significant difference between the groups receiving Alliacin and the control group, although the highest value of height of villi and depth of crypt of duodenum and jejunum was related to T2 group ($p > 0.05$).

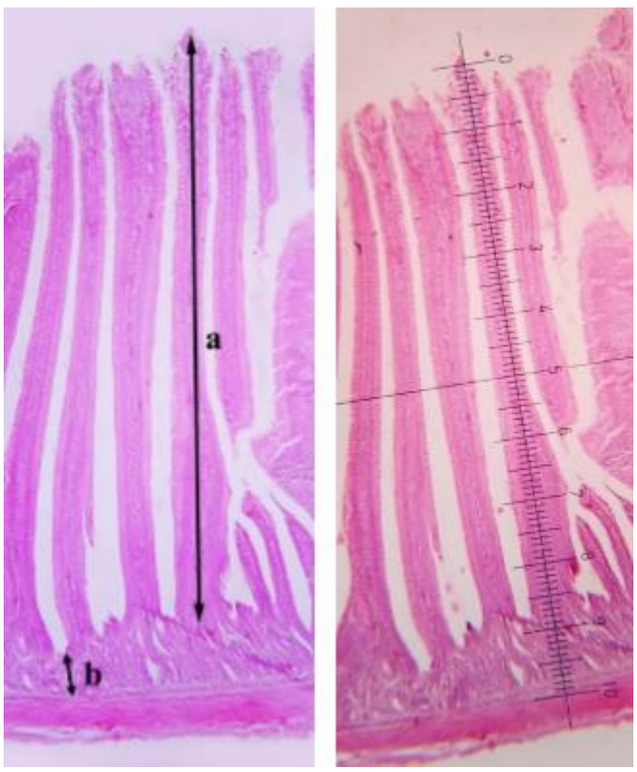

Fig. 1. Representative photomicrograph of a transverse section of the duodenum, a: Villi height, b: Crypt depth (Hematoxylin and Eosin stain, 400×).

Table 2. Comparison of average villi height and crypt depth of duodenum and jejunum (µm) in the studied groups (Mean ± SE).

Groups	Villi height		Crypt depth	
	Duodenum	**Jejunum**	**Duodenum**	**Jejunum**
Control	1676.97±156.3	797.26±58.3	137.75±6.9	119.25±8.3
T1	1755.32±224.3	800.99±39	141.3±7.04	122.4±6.9
T2	1947.99±191.4	886.42±71.5	147.75±9.5	125.2±7.9
T3	1940.56±106.4	879.19±93	139.25±5.6	125.1±5.7
***p*-value**	0.0616	0.0544	0.1201	0.4549

Discussion

Antibiotics have been used as growth promoters in animal diets for many years and there are several important reasons that limit their use. Issa and Omar eported that the use of antibiotics causes drug resistance in bacteria and drug residue in meat (1). In order to overcome the poor performance and increased susceptibility to diseases caused by the removal of antibiotics from the diet of birds, many efforts were made. In this regard, Saki and Tivey studied the use of plant-derived growth promoters (9). It has been demonstrated that alliums have antibacterial and antifungal activities as well as strong antioxidant activity and sulfur compounds and other phenolic compounds (10). Aji et al. showed that onions have positive effects on the growth of broiler chickens and the groups receiving rations containing onions had increased growth and improved food conversion ratio compared to groups receiving basic rations (11). It has been indicated that supplementing the diet of broiler chickens with onions at the rate of 30 mg per kilogram of body weight can be a good alternative to use antibiotics as growth stimulants. Consuming 30 g of fresh onion per kilogram of diet led to a significant increase in body weight compared to other groups (15 g of onion, 15 mg of virginiamycin and the control group) (3). AL-Homidan indicated that onion stimulates growth by increasing glucose delivery to tissues (thyroid-like activity) (12). Also, An et al. investigated the effect of onion extract on growth performance, carcass quality and blood factors of white dwarf broiler chickens which showed that the groups receiving onion extract had a higher body weight compared to the control group and the onion extract could be considered as a growth-stimulating factor and a suitable alternative to growth-stimulating antibiotics (13). Several reports have investigated the effect of garlic on feed efficiency and growth of broiler chickens. Ramakrishna et al. suggested that dietary supplementation with garlic increases the activity of pancreatic enzymes and provides better conditions for nutrient absorption (14). Eshairizadeh et al. reported that garlic powder added to the diet of broiler chickens had a significant effect on the percentage of carcass loss, however, did not change the efficiency and food intake, body weight, breast and thigh weight (15). It has been reported that broiler chickens fed with 100 ppm garlic diet showed a higher growth rate, food digestion, carcass characteristics and meat quality compared to the control group during the rearing period of 1 to 35 days (16). Also, Kumar et al. reported that dietary supplementation with garlic at 250 ppm significantly increased body weight in broilers during the 42-day rearing period (17). It has been reported that the use of 100 mg of garlic led to an improvement in body weight in chickens tested on days 7, 14, and 21, while the amount of food intake, feed conversion ratio and carcass quality were not changed. (11). Similarly, An et al. reported that chickens that received diets containing 0.3 and 0.5% garlic extract for 35 days showed an increase in body weight and daily weight gain compared to the control group (13). Al-Khalaifah et al. investigated the

effect of Egyptian leek leaf extract supplementation on the production and economic performance of Hubbard broiler chickens. The results showed that supplementing the diet with leek leaf extract led to an increase in body weight, improvement in carcass quality and a decrease in the percentage of carcass loss compared to the control group (18). In agreement with previous studies, the findings of this experiment showed that the use of Alliacin (combination of alcoholic extract of garlic, onion and leek) led to an improvement in weight gain and food conversion ratio compared to the control group during the rearing period. The average weight gains in the group receiving Alliacin at the rate of 2 mL per liter of drinking water was 240 g more than the control group, also the best food conversion ratio was related to the groups receiving Alliacin at the rate of 2 and 4 mL/L of drinking water (1.52 and 1.55, respectively).

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The assessment of *in vitro* timosil drug release through dialysis bag

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Understanding drug release in poultry's digestive system is crucial for effective disease treatment and feed efficiency optimization. Improper release can lead to side effects, like gastrointestinal irritation or toxicity (1). Understanding the mechanisms can help develop formulations minimizing adverse effects, ensuring the safety of final poultry products. The pH levels in the digestive tract significantly impact drug delivery and absorption (2). Designing appropriate drug delivery systems can improve the efficacy and bioavailability of medications in poultry production. This experiment evaluated the release of commercial drug in different parts of the poultry's digestive tract. The kinetics of drug release in phosphate-buffered saline at the specific pH (crop and proventriculus: 2.50-4.50; gizzard: 2.50-3.50; small intestine: 6.00-7.50; cecum: 6.00-7.00) at 41˚C were investigated (3). In time intervals of 30 to 480 min, 2 mL of the buffer was removed each time and 2 mL of the new buffer was replaced. The absorption of the samples at a wavelength of 250 nm was checked by a spectrophotometer. The results showed that the release rate of drug increased over time. During the investigation of the release in the phosphate buffer, it was found that about 72.00% of the drug was released in 8 hr (pH: 5.50). As a result, the release of Timosil drug in acidic pH had the lowest rate, indicating that the release of drug was more in the terminal parts of the digestive tract, where the absorption is higher.

Keywords: Digestive system, Drug release, Poultry, Timosil

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Effect of different levels of herbal medicine on growth performance, gastrointestinal tract traits and jejunal morphology of broilers

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Phytogenic feed additives are products being obtained from plants (1). The herbal products positively affect bird performance and health due to their anti-microbial, anti-oxidant, immune boosting, and gut manipulation properties (2,3). In this respect, this experiment was conducted to determine the effect of different levels of mixture of herbal medicine (HM), including *Curcuma longa*, *Artemisia aucheri* Boiss, and peppermint on growth performance, gastrointestinal tract organs weight and jejunal morphology of broilers from day 1 to day 42 of age. A total of 200 one-day-old chicks (Ross 308) were allocated in five treatments and four replicates (10 birds/pen in each), based on a completely randomized design. Dietary treatments included a corn-soybean meal basal diet (control) and treatments 2 to 5 including 3, 6, 9 and 12 kg/ton of HM, respectively. The results showed that inclusion of HM in the diet significantly improved body weight gain and feed conversion ratio compared to the control group from day 1 to day 42 of age. In relative terms, the weights of the gastrointestinal tract (with digesta), proventriculus, gizzard, liver and spleen were not affected by treatments. Also, the inclusion of HM did not affect the villus height, crypt depth and villus to crypt ratio of the jejunum. In conclusion, the inclusion of HM in the diet did not affect weight of the gastrointestinal tract organs and jejunal morphology, but improved growth performance of broilers from day 1 to day 42 of age.

Keywords: *Artemisia aucheri*, Broilers, Growth performance, Herbal medicine, Turmeric

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The impacts of a blend of various plants (Bioherbal and Bioherbal Plaus) used as feed supplements on production, egg characteristics, blood biochemistry, and immune parameters of laying hens

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Medicinal plants have shown a new approach for utilizing the phytogenic feed additives to improve production, health, and growth performance in the poultry industry (1). In this study, the effects of different phytogenic diets on egg production and quality, blood parameters and immune response of laying hens were investigated (2,3). The results of this study showed that no significant differences were observed between the treatments in terms of feed intake or egg production during the eight weeks of the experiment. The egg quality indicators did not show any significant difference by adding various phytogenic feed additives employed in this study in the 1st period of laying, while the yolk index was significantly different among the treatments. The finding of this study showed that the experimental treatments had no significant effect on blood hematological parameters of birds, while they caused a significant change in the means of biochemical attributes such as triglycerides (TG), cholesterol (CHO), albumin, total protein, and uric acid in the blood serum of laying hens. The bioherbal plus and mixture treatments caused the highest and lowest content of TG in the blood, respectively. Adding bioherbal to the diet of birds reduced blood CHO levels. The high-density lipoprotein levels did not show significant changes with the use of bioherbal/bioherbal plus mixtures alone or with minerals. The results showed that the content of immunoglobulin Y reached to the highest value in bioherbal/bioherbal plus mixtures treatment, while the lowest value was obtained in the bioherbal plus diet. This study provided good information about the use of some phytogenic feed additives with different proportions to strengthen the birds' immune system and maintain their health conditions.

Keywords: Cumin, Immunity, Laying hens, Performance, Production, Quality and quantity of eggs, Thyme

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Assessment of the effects of dietary supplementation with Alliacin (alcoholic extracts of garlic, onion and leek) on the immune system of broiler chickens (Ross-308)

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Allium genus includes 550 species, few of which are important as medicinal plants, among them are onion *(Allium cepa L.*), garlic (*Allium sativum L.*) and leek (*Allium ampleoprasam var. kurrat*) are pointed out (1). Several studies indicated the effect of garlic, onion and leek on the immune system (2,3). Alliacin is an herbal compound containing the alcoholic extracts of garlic, onion and leek. Alliacin was added to a ratio of 1, 2 and 4 mL per L of drinking water in different experimental groups to evaluate the effect of Alliacin on the immune system, antibody titer changes due to vaccination against avian influenza H9N2 and Newcastle disease as well as the weight of the thymus and bursa of Fabricius relative to body weight. The results of this study revealed that although in the groups receiving Alliacin, the weight of bursa of Fabricius and thymus at the end of the rearing period were higher than the control group, however, in the evaluation of bursa of Fabricius and thymus weight in relation to body weight, a significant difference was not observed ($p > 0.05$). Also, there was no significant difference in the avian influenza H9N2 and Newcastle disease antibody titers of the Alliacin-receiving groups with the control group ($p > 0.05$). It could be concluded that dietary supplementation with Alliacin hadsignificant effect on increasing serum antibody titers due to avian influenza (H9N2) and Newcastle disease vaccination and the weight of lymphatic organs (bursa of Fabricius and thymus).

Keywords: Broiler chickens, Garlic, Leek, Immune system, Onion

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Enhancing poultry health and productivity through lycopene extraction: a green approach using cloud point extraction

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Lycopene, a carotenoid found in tomatoes, watermelon and papaya, is a powerful antioxidant beneficial for human and animal health (1). In poultry nutrition, lycopene extracted from tomatoes enhances immune function, reduces oxidative stress, improves egg yolk color, meat quality and the stability of fats and proteins. These benefits lead to higher-quality eggs and poultry meat improving overall production efficiency (2). However, traditional methods for extracting lycopene which rely on solvents like hexane and ethanol present significant challenges. These solvents may leave harmful residues in poultry feed potentially affecting the health of birds and compromising the safety and quality of poultry products like eggs and meat (3). To address these concerns, we utilized the Cloud Point Extraction (CPE) method, a solvent-free technique that isolates lycopene using changes in solubility at specific temperatures. This method separates the solution into two phases concentrating lycopene in one phase. For this process, we used saponin derived from Acanthophyllum, with a concentration of 4 mg/mL which was extracted with an ultrasonic device, significantly reducing solvent use compared to conventional methods. In our experiments, we optimized the extraction process using 1 g of tomato powder and 1 to 5 mL of saponin. The CPE method effectively extracted a high amount of lycopene, providing a safe, natural alternative for poultry feed without introducing harmful chemicals. This eco-friendly approach enhances poultry health, improves product quality and ensures consumer safety by preventing toxic residues from entering the food chain.

Keywords: CPE, Green method, Lycopene, Poultry, Saponin

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Section 12

Aquatics

Immunomodulatory effect of *Allium sativum* and *Echinacea purpurea* to increase resistance of rainbow trout against *Yersinia ruckeri*

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Abstract

Some plants have antibacterial properties and enhance the immune system of fish. The objective of this study was to investigate the effect of *Allium sativum* and *Echinacea purpurea* extract on immune responses and resistance *Oncorhynchus mykiss* to *Yersinia ruckeri*. For this purpose, 1050 fishes (50 ± 5.6 g) were distributed in seven groups (with three replication) and fed with supplemented commercial diet for 30 days as follow: Commercial diet without plant extract (control), 0.5% and 1% *A. sativum*, 0.5% and 1% *E. purpurea*, a combination of 0.5% *A. sativum* and 0.5% *E. purpurea*, and a combination of 1% *A. sativum* and 1% *E. purpurea*. After treating period all groups were fed with commercial diet to next 15 days. On 0, 15, 30 and 45 days, blood and serum samples were prepared to measure lysozyme, and total immunoglobulin. A bacterial challenge were conducted to investigate resistance of treated fishes against *Y. ruckeri* on day 30. The results showed that *A. sativum* 1% enhanced immune parameters on days 15 and 30 beside control group significantly. Also this difference was significant for total immunoglobulin on 45th day. *A. sativum* 1% caused to decrease cumulative mortality of fishes after bacterial challenge significantly. Based on the results *A. sativum* extract %1 stimulated the immune system and increased resistance of rainbow trout against *Y. ruckeri*.

Keywords: *Allium sativum*, *Echinacea purpurea*, Immune response, Rainbow trout

Introduction

Rainbow trout is an important species in the aquaculture industry with increasing mortality rate affected by various environmental stresses such as prevalence of infectious diseases and the inadequate effect of synthetic drugs. Entric red mouth or yersiniosis is one of the most important bacterial diseases in cold water fish farming industry, which in the last few years has been causing the fish mortality in the country cold water fish farms (1). The most sensitive species to *Yersinia ruckeri* is rainbow trout, especially in fingerlings. The most important clinical symptoms observed in this disease include bleeding inside the mouth, jaws, fin base and internal organs, blood exophthalmia, and necrosis in the hematopoietic organs (2). In addition of drug treatment, which includes the use of chemical drugs such as antibiotics, prevention strategies that focus on enhancing fish immune responses are also common in preventing the yersiniosis (3, 4). Among all kinds of immunostimulants, plant-based immunity stimulants, have more advantages than others including failure to

create resistance in pathogens, availability, less risk to the environment and animals, and lower price. Since some of the medicinal plants have useful properties including stimulation and enhancing the immune system, hence, using them in fish farms increases production (5).

Garlic (*Allium sativa*) is one of the plants used in traditional medicine in ancient China, Rome and Egypt. It has been proved that garlic has immunomodulatory properties and is well capable of enhancing protection against pathogens. Hence, it can be concluded that garlic supplemented diets in fish enhance growth rate and improves the immune response in aquaculture. Garlic can also be used as an alternative to antibiotics or chemotherapeutic agents. Garlic contains various ingredients such as minerals and organic matter (terpenoid, enzyme, prostaglandin, and allicin) (6). The present study was designed to evaluate the effect of *Alium sativum* and *Echinacea purpurea* extracts on the immune system of rainbow trout and its resistance to yersiniosis.

Materials and Methods

Fish husbandry and Nutritional treatments. The number 1050 rainbow trout (mean weight 50±5.6 g) were purchased from one of the cold-water fish aquaculture field in Urmia, Iran. Water soluble essence of *A. sativum* and *E. purpurea* were prepared from Plant Essence Company (Gorgan-Iran). The extruded commercial food (GFT-2, Faradaneh Co., Shahrekord, Iran) was used in this study. The first group was control and fed exclusively with commercial food. Second and third groups were fed with commercial food containing, 0.5% (5 g/kg of food) and 1% (10 g/kg of food) *A. sativum* extract respectively. Fourth and fifth groups were fed with commercial food containing, 0.5% (5 g/kg of food) and 1% (10 g/kg of food) *E. purpurea* extract respectively. Sixth group were fed on commercial food with the combination of 0.5% *A. sativum* and 0.5% *E. purpurea* extract. Seventh group were fed with commercial food with the combination of 1% *A. sativum* and 1% *E. purpurea* extract. All treatments were fed as above diet for 30 days, and so all treatments were fed with just the control diet for next 15 days.

Sampling and preparing blood serum. Fifteen fish from each treatment were anesthetized by immersion in a clove powder (200 mg/L) solution and blood samples were collected from the caudal vein of fish (7) at the beginning of the experiment (day 0), and on days 15, 30, and 45.

Total immunoglobulin assay. Total immunoglobulin was measured by the method of Panigrahi et al. and Bradford (8, 9). About 0.5 mL of serum was mixed with 2.5 mL biuret reagent. The mixture was left at room temperature for 30 min. The samples, standard and blank were measured at 546 nm. The total immunoglobulin value was expressed as (mg/mL) calculated according to the following formula:
Total immunoglobulin (mg/mL) = total protein in serum sample - total protein treated with polyethylene glycol.

Determination of resistance to *Y. ruckeri* bacteria. After feeding period with diet containing *A. sativum* and *E. purpurea* extracts (the end of day 30 day), bacterial contamination was created with acute strain of *Y. ruckeri*. For bacterial challenge sixty fish from control group and each plant extract treatments (20 fish from each replication) were selected randomly and were anesthetized with 200 ppm of clove powder. Therefore, the fish were injected with 10 µL of bacterial suspension containing 6×10^8 CFU/mL by insulin syringe, intraperitonally (3) and were entered in 21 polyethylene tanks and monitored for next two weeks. Cumulative mortality of each injected fish group was recorded at the end of bacterial challenge period. During the bacterial challenge oxygen was supplied by aeration with an air pump and 50% of tanks water was exchanged daily (10).

Results

As shown in Table 1, there was no significantly different in total serum immunoglobulin activity of experimental treatments on day 0. However, on days 15 and 30 *A. sativum* 1% and *A. sativum* 1% + *E. purpurea* 1% treatments had high levels of serum total immunoglobulin activity, respectively, which was significantly different from control and other treatments ($p < 0.05$). Other experimental treatments were also significantly different from control group ($p < 0.05$). The control group had the lowest level of serum total immunoglobulin activity within study period except for day 0. At the end of the experiment (on day 45), by cutting out *A. sativum* and *E. purpurea* extracts from the fish diet, the level of serum total immunoglobulin activity was decreased in the treatments and the highest mean of serum total immunoglobulin was observed in *A. sativum* 1% treatment, which was significantly ($p < 0.05$) different from the control and other treatments except for treatment *A. sativum* 0.5% + *E. purpurea* 0.5% (Table 1).

Table 1. Total immunoglobulin amount (mg/mL) of fish serum in different treatments (mean ± SD).

Treatments	Days of sampling			
	Day 0	Day 15	Day 30	Day 45
Control	2.51±0.89^{a}	3.21±0.69^{c}	5.57±0.44^{c}	7.68±0.67^{c}
A. sativum 0.5%	2.61±0.78^{a}	8.21±0.54^{b}	13.57±0.32^{b}	9.24±0.76^{b}
A. sativum 1%	2.84±0.66^{a}	11.21±0.58^{a}	19.63±0.56^{a}	11.8±0.89^{a}
E. purpurea 0.5%	3.15±0.86^{a}	6.27±0.34^{b}	10.44±0.14^{b}	7.57±0.54^{c}
E. purpurea 1 %	2.83±0.67^{a}	7.19±0.52^{b}	11.35±0.45^{b}	9.58±0.98^{b}
A. sativum 0.5% + E. purpurea 0.5%	3.37±0.77^{a}	7.21±0.56^{b}	11.68±0.32^{b}	10.57±0.56^{ab}
A. sativum 1% + E. purpurea 1%	2.73 ±0.55^{a}	9.32±0.46^{ab}	17.75±0.26^{a}	9.69±0.77^{b}

Different superscript letters in each column indicate statistical significant difference at $p < 0.05$.

After the injection of bacteria, mortality rate were recorded for two weeks. Until day 2, the treatments did not show mortality. Gradually, symptoms such as darkening of body color, bleeding points around the mouth, the base of the fins and gills were appeared and eventually, the fish started to swim upside-down. At the end of two weeks, the lowest mortality rate was observed in *A. sativum* 1% extract and showed significant ($p < 0.05$) difference beside control group (Fig. 1).

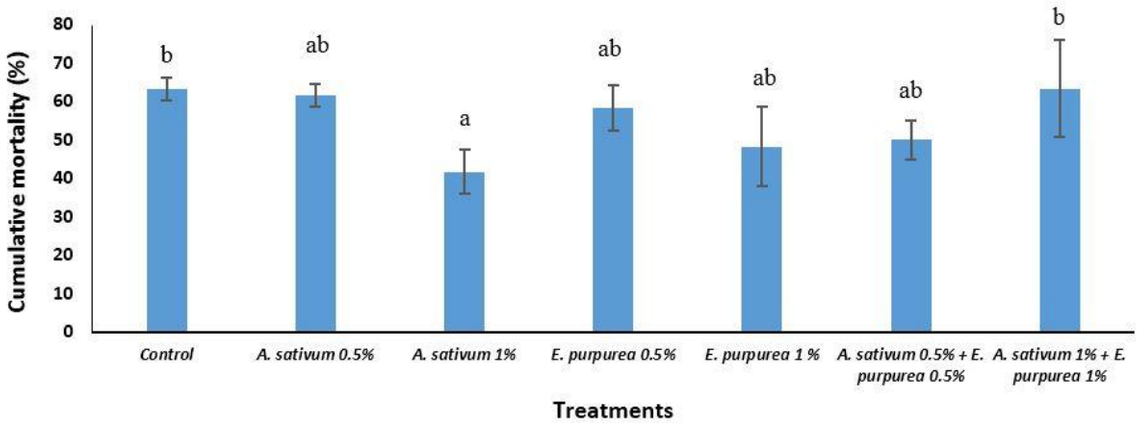

Fig. 1. Cumulative mortality of fish in every treatment at the end of bacterial challenge by *Yersinia ruckeri* (mean ± SD). The uppercase letters above columns indicate significant difference ($p < 0.05$).

Discussion

The non-specific immune system is more evolved than the specific immune system in fishand is responsible for many of its immune system functions. Nowadays, immunostimulant with plant origin have attracted more attention due to the availability, lower risk for the environment and animals and lower price (5). The results of present study showed that use of *A. sativum* and *E. purpurea* extracts increased the total immunoglobulin in fish fed on the extracts on days 15 and 30. Especially in fish fed on *A. sativum* 1% extract and fish fed on combine of *A. sativum* and *E. purpurea* 1% extracts) this increase was significant in comparison with other treatments ($p < 0.05$). The lowest level of activity of the above immunity indices was observed in treatment 1 (control). Guanghoug et al., have reported that combined extract of several traditional Chinese medicinal plants increased phagocytosis of macrophages, blood plasma protein content and serum globulin and lysozyme which led to an increase in the immunity level of carp (11).

Antibacterial properties of *A. sativum* refer to the organosulfur compound allicin ($C_6H_{10}S_2O$). Allicin is part of a defense mechanism against attacks by pests on the garlic plant and 1 mg of allicin is as effective as 15 standard units of penicillin (12). The main components of *E. purpurea* are caffeic acid and alkamides (13). There are remarkable evidences regarding the use of this plant in the activation of macrophages, the breakdown of polysaccharides and other factors affecting the immune system (9).

On day 45, with discontinuation of the extracts from the fish diet, the level of immunity indices was dropped, however, there was considerable discrepancy in some experimental groups. The total immunoglobulin activity in the treatment fed on *A. sativum* 1% extract was significantly different from other experimental treatments. These results showed that the effects of the extract after its discontinuation were similar to some studies on tilapia fish regarding the use of *A. sativum* and *E. purpurea* combination and observing its effects after discontinuation of medication (14).

In this study, to determine the effect of extracts, rainbow trout fish were injected by *Y. ruckeri* bacteria intraperitonally. Two days after injection, fish gradually showed symptoms such as darkening and bleeding points around the mouth, fins base, and gills and eventually there were signs of imbalance and upside down floating. However, the occurrence of these symptoms in *A. sativum* 1% treatment began with a delay day compared to other treatments and this treatment showed the lowest mortality rate at the end of bacterial challenge period (Figure 1). Therapeutic and antimicrobial effects of *A. sativum* are due to organophosphorus compounds including allicin and its antibiotic properties and stimulation of the immune system have been proven (15). The results of Sharif Rohani et al., have shown that mortality percentage in *E. purpurea* 1.5 g/kg treatment was significantly lower than other treatment groups of rainbow trout after challenge with *Streptococcus iniae* bacteria, ($p < 0.05$). *E. purpurea* extract has reported to stimulate immunity and resistance to bacterial infections in Grass Carp and to increase phagocyte, to transfer granulocyte in the blood and to protect against microbial contamination (16).

In conclusion, use of *A. sativum* and *E. purpurea* extracts, especially *A. sativum* 1% as a immunomodulatory agent in diet of rainbow trout enhanced the immunity responses and increased resistance of fish against *Y. ruckeri* bacteria. Therefore, it was suggested to use *A. sativum* 1% to enhance the non-specific immunity and to increase the readiness of fish and to reduce their mortality against bacterial pathogens.

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Investigating the effects of hydroalcoholic extracts of barberry (*Berberis vulgaris L.*) and sumac (*Rhus coriria L.*) on the activity of antioxidant enzymes in the liver of rainbow trout (*Oncorhynchus mykiss*)

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Abstract

There have been several studies on the use of medicinal plants to improve growth parameters, increase resistance to stress conditions and improve the immune system and increase the activity of digestive enzymes and antioxidants which have recommended the use of medicinal plants. This research was conducted with the aim of investigating the hydroalcoholic extracts of barberry and sumac on the activity of antioxidant enzymes in the liver of rainbow trout for a period of eight weeks. 360 pieces of rainbow trout with an average weight of 20±5 g were obtained from one of the rainbow trout breeding centers. Fish were randomly divided into six treatments including 100 and 200 mg of barberry extract per kg of food, 100 and 200 mg of sumac extract per kg of food, 100 mg of barberry extract and 100 mg of sumac extract as groups. The fifth and the control group were divided into subgroups. The results of investigating the activity of antioxidant enzymes showed that the antioxidant enzymes of the fish of different treatments were affected by the diets, therefore, there was a significant difference in the activity of antioxidant enzymes. Oxidation was observed in 100 and 200 mg sumac treatments and 200 mg barberry treatments. In total, the results of this research showed that the use of hydroalcoholic extract of sumac plant significantly improved the immune system of rainbow trout in commercial farming conditions.

Keywords: Antioxidant activity, Barberry, Rainbow trout, Sumac

Introduction

In recent years, the use of immune system stimulants in the feeding of aquatic animals in intensive fish farming systems in order to increase the power of the immune system and non-specific immune responses and to protect the body against unwanted factors and environmental stress through the promotion of phagocytic power, increasing the activity of lysozyme and raising the level of immunoglobulin. have become common and it seems that the use of substances that stimulate the immune system is a suitable nutritional solution for controlling aquatic diseases (1).

In the aquaculture industry, pathogenic factors are one of the factors that reduce production and considering the further development of non-specific immunity of fish compared to specific immunity and the

special place of immune stimulators in stimulating non-specific immunity, the use of this stimulator in aquatic animals is more preferable than warm-blooded animals (2). Among the various immune stimulants, plant-based immune stimulants have advantages such as availability, lower risk for the environment and animals and lower price (3).

Barberry is a thorny plant with the scientific name Berberis vulgaris and belongs to the Breberidacea family. This plant is mostly distributed in the temperate regions of the Northern Hemisphere. Barberry is one of the few plants whose roots, bark, stems, leaves, flowers and fruits are used for food, medicine, and industry (4).

Sumac with the scientific name *Rhus coriria* L belongs to the Anacardiaceae family with 250 species and its name is derived from the word Sumaqa which means red (5). Its fruit is small, hard, red or crimson in color, which turns brown after drying, has a sour and astringent taste, and after drying, it is usually consumed with meat and salad (6).

Materials and Methods

360 pieces of rainbow trout with an average weight of 20±5 g were purchased from one of the fish breeding and breeding centers in Urmia city and were transported in a special tank equipped with aerator to the breeding and breeding hall of the Faculty of Veterinary Medicine located in the university. They were transferred to Urmia. After seven days of adaptation and disinfection with salt solution, they were stored in the form of six treatments (each treatment has three repetitions) with a density of 20 pieces in each tank.

Food preparation and feeding. To feed the fish, commercial type food (FFT-2) produced by Faradane company (Tabriz, Iran) was used. After calculating and weighing the food, the hydroalcoholic extract of barberry and sumac plants were added to the food ration according to the food treatments. The fish were fed four times a day at 8, 11, 14, and 17 hr at 8, 11, 14, and 17 hr at the rate of 2.5% of body weight. Biometry was done every two weeks to determine the amount of daily food. Fish in six treatments including: Two treatments (one and two) of 100 and 200 mg of barberry extract per kg of food, two treatments (three and four) of 100 and 200 mg of sumac extract per kg of food and the combination of barberry extract and sumac They included a treatment (five) in the form of 100 mg of barberry extract and 100 mg of sumac extract and a control group (six). It should be noted that each treatment had three repetitions and each repetition had 20 fish and they were reared and fed with the aforementioned food treatments for eight weeks.

Sampling to measure liver antioxidant enzymes. At the beginning and at the end of the rearing period after the eighth week, after 24 hr of stopping food, three fish from each repetition were randomly selected from all treatments and after anesthetizing with clove powder solution, after dissection of the liver tissue of the fish. The fishes were separated and individually after washing with physiological serum, they were transferred into microtubes and the samples were kept in a freezer at – 80 ˚C until the measurement of the desired factors and the liver indices of the fishes were evaluated. (7).

Preparation of crude enzyme extract. The reduced pieces of liver tissue were homogenized in Tris-chlorhydric buffer and using a homogenizer. The resulting homogenates were centrifuged, the precipitate was discarded and the supernatant was used for protein measurement, enzyme activity and liver antioxidant tests (8).

Measurement of superoxide dismutase enzyme activity. To measure superoxide dismutase enzyme an indirect method was used based on the inhibition rate of nitrotetrazolium complex formation (9).

Measuring the activity of catalase enzyme. The general principles of the reaction are based on the decomposition of a substrate (hydrogen peroxide) by the enzyme catalase and the reduction of light absorption at a wavelength of 240 nm in 15 sec (9).

Measurement of Glutathione-Peroxidase (GPX) enzyme activity. Glutathione-peroxidase reaction mixture including 890 µL of potassium phosphate buffer (100 mM and 7-pH), EDTA (1 mM), NaN3 (1 mM), NADPH (0.2 mM), glutathione reductase (1 U/mL), reduced glutathione (mM1) and 10 µL of liver extract will be (9).

Measurement of malondialdehyde enzyme activity. Calorimetric method was used to measure malondialdehyde. The basis of this method is the reaction of malondialdehyde with thiobarbituric acid and extraction with normal butanol (10).

Statistical analysis. This research was done in the form of a completely randomized statistical design with six treatments and three repetitions per treatment. All data were evaluated using one-way ANOVA. If the analysis of variance was significant ($p < 0.05$), Tukey's test was used to compare the mean of different treatments. All statistical analyzes were done by statistical software SPSS ver. 20 done. Data were reported as "mean ± standard error" (Mean ± SE).

Results

Activity of superoxide dismutase enzyme. The amount of superoxide dismutase enzyme activity in fish in different treatments at the end of the period is shown in figure 1. The obtained results showed that the addition of sumac extract increases the amount of this enzyme and this stimulant increases the activity of superoxide dismutase more effectively than barberry, so that the highest amount of this enzyme was observed in the treatment of 200 mg of sumac. u/mg 11±1.70) which had a significant difference with the control group (u/mg 21.8±21.0), and fish fed on diets containing barberry 100 (u/mg 87.24) ±0.7) and 200 mg barberry (44.48±0.8 u/mg) showed ($p < 0.05$).

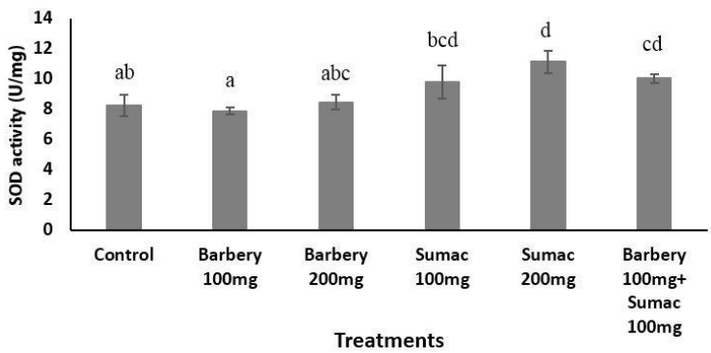

Fig. 1. Liver superoxide dismutase (SOD) enzyme activity of nutritional treatments at the end of the breeding period.

Catalase enzyme activity. Figure 2 shows that the activity of catalase enzyme in the treatment of 200 mg barberry (48.35±0.5 U/mg), 100 mg sumac (5±7.43 U/mg) and 200 mg Sumac gram (U/mg 0.5±5.06) shows a significant difference with the control group ($p < 0.05$). Among these treatments, the highest and the lowest

amount of catalase were observed in the treatment of 100 mg sumac (5±19.34 U/mg) and control (0.4±39.15 U/mg), respectively.

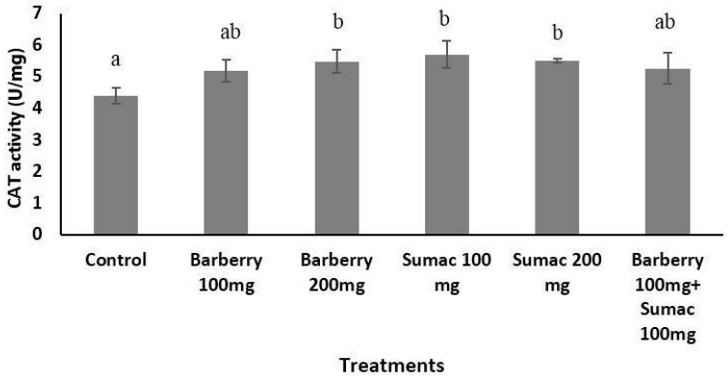

Fig. 2. Liver catalase (CAT) enzyme activity of nutritional treatments at the end of the breeding period.

Level of glutathione-peroxidase enzyme activity. The activity values of glutathione peroxidase enzyme in different treatments fed with barberry and sumac extract in figure 3 indicated that 200 mg of sumac extract (22.26 ± 0.75 U/mg) could significantly increase the amount of this enzyme. - Dari compared to control treatments (15±1.94 U/mg), 100 mg barberry (16±24.30 U/mg), 200 mg barberry (186±19.1 U/mg) and the combined group of sumac and barberry (44.36±1.1 U/mg) was increased and took the highest amount ($p < 0.05$). The lowest amount of this enzyme was observed in the fish of the control group.

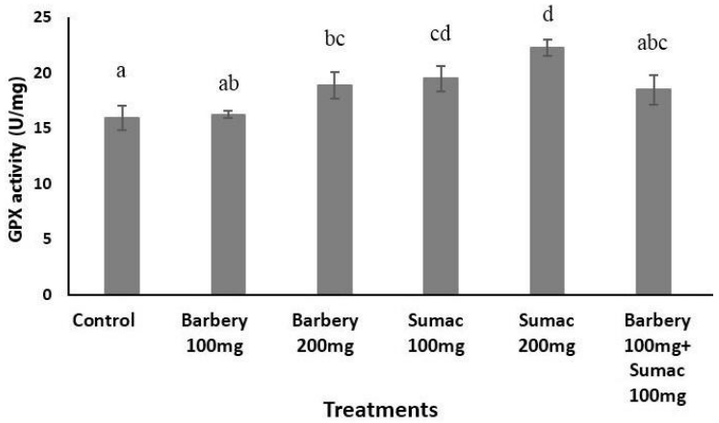

Fig. 3. Level of liver glutathione peroxidase (GPX) activity of nutritional treatments at the end of the breeding period.

Malondialdehyde enzyme activity level. The results obtained from the effect of two herbal extracts of barberry and sumac on the amount of malondialdehyde in rainbow trout are shown in figure 4. As can be seen, the addition of these two herbal stimulants increases the amount of this substance in the fish liver at the end of the period. The highest amount of malondialdehyde was observed in the treatment of 200 mg of sumac (U/mg 22.7±0.7), which showed a significant difference with the control treatments (U/mg 43.7±0.5), 200 mg of barberry (29.33±0.6 U/mg) and combined treatment of barberry and sumac (37.22±0.6 U/mg; $p < 0.05$). Also, the lowest amount of malondialdehyde produced was observed in the control and barberry 200 mg treatments. It seemd that increasing the amount of barberry decreased the amount of malondialdehyde.

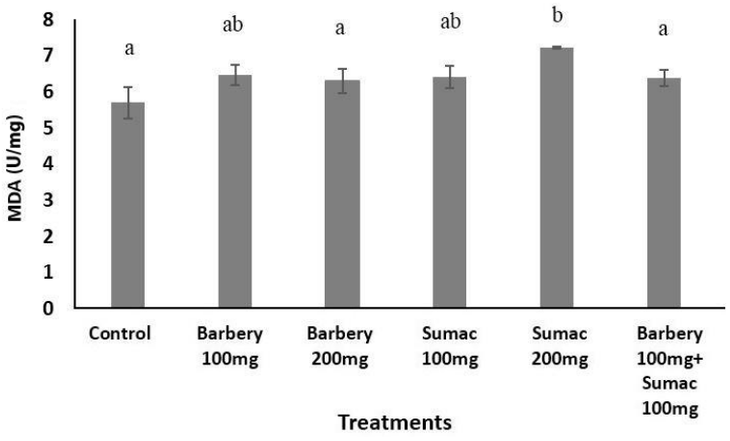

Fig. 4. Malondialdehyde (MDA) of the liver in nutritional treatments at the end of the breeding period.

Discussion

In a study, researchers stated that treatment with aloe vera extract increases the antioxidant activity of rainbow salmon. Also, a significant decrease in fat peroxidation products was observed with the decrease of malondialdehyde. Some studies also showed the beneficial effects of using aloe vera both in natural conditions and in laboratory conditions (11).

What was obtained from the results of this study was a confirmation of the studies of other researchers regarding the effects of plant compounds on the activity of antioxidant enzymes in fish. Sumac extract used in this research could improve the safety indicators. According to the obtained results, adding 200 mg of sumac extract to the diet improves the activity of antioxidant enzymes in fish liver. The use of this plant in the diet of rainbow trout had positive effects on the activity and safety of the fish.

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The potential of medicinal plants to fight parasite challenges in aquaculture

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Chemical drugs used in treatments can adversely affect not only diseased tissues or disease agents but also healthy organs (1). Chemical drugs used especially during mass control of parasites can harm not only the parasites but also other living things in the environment. Medicinal and aromatic plants offer an effective and sustainable alternative for the prevention and treatment of diseases without harming the environment thanks to their antimicrobial, antioxidant, anti-inflammatory and antiparasitic properties (2). With the increase in production volume in aquaculture, the use of chemical drugs, antiparasitic agents, feed additives and antibiotics to increase productivity has also been increased. However, using these chemicals prevents sustainability and causes negative environmental impacts. In particular, chemical treatments used to combat parasites, damage the environment and cause serious problems such as stress, tissue erosion, bacterial infections and osmotic imbalance in fish (3). In addition, pesticide residues pose a serious threat to food safety. In this context, medicinal and aromatic plants offer effective methods to combat parasites and prevent diseases with less damage to the environment. Their antimicrobial, antioxidant, anti-inflammatory and antiparasitic properties have the potential to reduce dependence on chemical interventions in aquaculture applications. This review aims to examine the efficacy of medicinal plants against parasites, their historical significance, mechanisms of action and challenges to their use in aquaculture.

Keywords: Aquaculture, Food safety, Parasites

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Phytogenics as a safe and promising tool to manage aflatoxicosis in aquatic animals

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Mycotoxins are secondary toxic metabolites of fungi that usually contaminate agricultural crops and their products (1). Aflatoxins are fungal toxins that are produced by *Aspergillus* species. Due to global climate change and warming, mycotoxin-producing fungi are frequent. Aflatoxin B_1 is the most important fungal toxin in terms of occurrence and toxicity. The toxin is classified as group I carcinogen by the International Agency for Research on Cancer. Aflatoxin B_1 causes liver damage, immune system suppression and affects animal growth and reproduction with subsequent reduction in livestock and aquatic animal performance. There are various strategies to control/ manage mycotoxin contamination of food/feed usually with limited efficacy (2). The use of medicinal plants is considered as safe, affordable and complementary biological method for the management of aflatoxins toxicity in animals. The phytogenics have anti-mutagenic, anti-microbial, anti-oxidant and anti-carcinogens properties that reduce genotoxic effects of mycotoxins (3). Medicinal plants improve body immune competence and increase animal resistance/resilience against stressful conditions.
Keywords: Aquatics, Fungal toxins, Pollution, Secondary metabolites

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