Shahid Bahonar University of Kerman
Agricultural Biotechnology Journal
Iranian Biotechnology Society
Print ISSN: 2228-6705 Online ISSN: 2228-6500

Study of the effect of *Boswellia serrata* in reducing the effects associated with induced arthritis in male albino rats

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Abstract

Objective

Arthritis is a chronic inflammatory disorder characterized by joint pain, swelling, and functional impairment. It is often associated with significant morbidity and a decline in the quality of life. The present study investigated the effects of different treatments on paw thickness, oxidative stress markers, and autoimmune/inflammatory parameters in an arthritis model.

Materials and methods

The study was conducted at Al-Qadisiyah University's Department of Life Sciences, using male Albino Rats. The rats were raised in special plastic cages and kept in a temperature range of 20-25°C. Arthritis was induced using Complete Freunds adjuvant, which was injected daily into the right foot pad for 14 days. Boswellia serrata extract was prepared by preparing aqueous extract from male frankincense. The rats were divided into three groups: Control group, Animals in which arthritis is induced by injecting Complete Freunds adjuvant into the foot pad, and Animals in which arthritis is induced for 14 days and then administered Boswellia serrata. The results were analyzed using SPSS statistical software, Anova test, and Least Significant Differences (LSD) to test the significance of the results.

Results

In the paw thickness evaluation, the Arthritis group showed a significant increase compared to the control group, which was partially alleviated by the Arthritis + Boswellia serrata treatments.

The control group maintained consistent measurements throughout, while the Arthritis group exhibited a partial recovery. the Arthritis + Boswellia serrata group had the most favorable outcomes. The reported LSD value of 0.219 indicates statistical significance of the differences between the groups. The analysis of oxidative stress and antioxidant markers revealed that the Arthritis group had the highest levels of the lipid peroxidation marker MDA, along with the lowest levels of the antioxidant enzymes SOD, GSH, and CAT, suggesting compromised antioxidant defenses. The Arthritis Arthritis + Boswellia serrata group showing the most advantageous outcomes. Regarding inflammation and autoimmune markers, the Arthritis group had significantly elevated levels of rheumatoid factor (RF), C-reactive protein (CRP), and anti-cyclic citrullinated peptide (ACCP), indicating substantial autoimmunity and inflammation. The Arthritis + Boswellia serrata group exhibited intermediate results. The statistical significance of the differences between the groups was supported by the reported LSD values.

Conclusions

The findings suggest that the Arthritis + Boswellia serrata treatments can provide beneficial effects in mitigating the alterations in paw thickness, oxidative stress, and autoimmune/inflammatory parameters observed in the arthritis model. The Arthritis + Boswellia serrata group often exhibited the most favorable outcomes among the treatment groups.

Keywords: albino rats, arthritis, *Boswellia serrata*, male

Paper Type: Research Paper.

Citation: Bashar AK, Kadhem WM (2025) Study of the effect of *Boswellia serrata* in reducing the effects associated with induced arthritis in male albino rats. *Agricultural Biotechnology Journal* 17 (1), 177-192.

Agricultural Biotechnology Journal 17 (1), 177-192. DOI: 10.22103/jab.2025.24637.1643

Received: December 01, 2024. Received in revised form: January 20, 2025.

Accepted: January 21, 2025. Published online: January 30, 2025.

Publisher: Faculty of Agriculture and Technology Institute of Plant Production, Shahid Bahonar University of Kerman-Iranian Biotechnology Society.

Introduction

Osteoarthritis (OA) is a prevalent and debilitating joint disorder characterized by the progressive degeneration of articular cartilage, accompanied by inflammation, pain, and impaired joint function. It is a leading cause of disability among the elderly population, with a global prevalence estimated to be around 303.1 million cases as of 2017 (Safiri et al. 2020). The burden of OA is expected to increase in the coming decades due to the aging population, obesity, and physical inactivity (Cross et al. 2014). Current pharmacological treatments for OA, such as nonsteroidal anti-inflammatory drugs (NSAIDs) and acetaminophen, often provide only partial relief and are associated with various adverse effects, including gastrointestinal, cardiovascular, and renal complications (Schjerning Olsen et al. 2011). Animal models, particularly those involving induced arthritis in rats, are commonly used to study the pathophysiology of this condition and evaluate potential therapeutic interventions. Inflammation and oxidative stress play pivotal roles in the progression of arthritis, making them important targets for therapeutic strategies. Moreover, the use of phytobiotics and medicinal plants as natural antimicrobial growth promoters in replacement to antibiotics has definitely many benefits for the progress of zootechnical efficiency parameters, suppression of specific diseases (Amirteymoori et al. 2021; Mohammadabadi et al. 2023), antimicrobial and antioxidants activities (Hajalizadeh et al. 2019; Jafari Ahmadabadi et al. 2023), hypocholesterolemic effects, digestive enzymes enhancement, and improvement of liver functions (Safaei et al. 2022; Shokri et al. 2023; Mohammadabadi et al. 2024). Researchers demonstrated that adding these plants to diet increased feed consumption, ratio of feed conversion and carcass yield, suppression of specific diseases, and antimicrobial and antioxidants activities (Vahabzadeh et al. 2020; Shokri et al. 2023). One such promising candidate is Boswellia serrata, a medicinal plant widely used in traditional systems of medicine, particularly in Ayurvedic medicine. Boswellia serrata, also known as Indian frankincense, has been traditionally used for the treatment of various inflammatory conditions, including arthritis, asthma, and inflammatory bowel diseases (Ammon 2016). The potential therapeutic effects of Boswellia serrata in OA are attributed to its rich content of bioactive compounds, primarily the boswellic acids, which possess potent anti-inflammatory and analgesic properties (Asteggiano et al. 2023). These compounds are thought to exert their beneficial effects by modulating various inflammatory pathways, such as the inhibition of 5-lipoxygenase and the suppression of proinflammatory cytokines (Siddiqui 2011). Similarly, a systematic review and meta-analysis by Yu et al. (2020) evaluated the clinical efficacy of Boswellia serrata in the treatment of OA. These researchers concluded that Boswellia serrata supplementation was associated with significant improvements in pain, physical function, and quality of life in patients with OA, with a favorable

safety profile compared to conventional NSAIDs. In other study Kim et al. (2023) demonstrated the promising potential of Boswellia serrata in reducing the effects associated with induced arthritis in male albino rats. The Boswellia serrata extract showed superior efficacy in improving joint function, reducing inflammation, and attenuating cartilage degeneration compared to the reference drug diclofenac sodium. Their findings highlight the importance of exploring natural products and herbal medicines as potential therapeutic options for the management of osteoarthritis and other joint disorders. Further research is warranted to elucidate the precise mechanisms of action, optimize the dosage and formulation, and investigate the safety and efficacy of Boswellia serrata in human clinical trials. Thus, the aim of this study was to investigate the effects of different treatments on paw thickness, oxidative stress markers, and autoimmune/inflammatory parameters in an arthritis model.

Materials and Methods

Experimental Animals: The study was conducted in the animal house of the Department of Life Sciences, Faculty of Education, Al-Qadisiyah University, for the period from 2/9/2023 to 5/3/2024 and used in this study male Albino Rats, which were purchased from the animal house of the Faculty of Veterinary Medicine, Al-Qadisiyah University, and were raised to obtain rats with similar ages and weights to be used in the experiment, their weights ranged between 200-250 grams and their ages between 3-4 months. The experimental animals were placed in special plastic cages prepared for this purpose, with metal mesh lids, equipped with a special water drinking system and furnished with sawdust, and the cages were kept clean and sterilized with disinfectants. The experimental animals were subjected to suitable laboratory conditions at a temperature of 20-25°C.

The Induce of Arthritis: Complete Freunds adjuvant was used to induce the development of arthritis, which was purchased by import from SCMA. After dissolving the daily dose of Complete Freunds adjuvant, each animal was injected daily into the right foot pad using an insulin syringe for 14 days and random blood samples were drawn to confirm the development of arthritis. The results showed an increase in the level of rheumatoid factor and Anti-CCP.

Preparation of Boswellia serrata extract: The aqueous extract of male frankincense (Boswellia serrata) was prepared as follows: 10 g of male frankincense was weighed, ground and placed in a 500 mL glass flask containing 200 mL distilled water, mixed with a Magnetic Stirrer for 15 minutes and the solution was left for 30 minutes to precipitate the fractions. The solution was filtered with tulle cloth, the precipitate was discarded and the filtrate was separated by centrifuge at a speed of 3000 rpm for 10 minutes to obtain a clear solution that was concentrated

Agricultural Biotechnology Journal; Print ISSN: 2228-6705, Electronic ISSN: 2228-6500

by rotary evaporator at a temperature of 45°C. The aqueous extract was dried after concentration by rotary evaporator by placing it in glass dishes (known weights) of 75 mL and placed in an electric oven at 40 °C to obtain the dry aqueous extract.

Design Experiment: In this experiment, 40 male rats were used and randomly divided into three groups with 10 rats per group. The animals were divided as follows:

C: Control group: They are fed with the serum solution during the duration of the experiment.

T1: Animals in which arthritis is induced by injecting Complete Freunds adjuvant into the foot pad of albino rats at a dose of 0.1 mL for 14 days.

T2: Group of animals in which arthritis is induced for 14 days and then administered Boswellia serrata at a dose of 50 mg/kgbw for 30 days.

Statistical Analysis: The results of the experiments were analyzed using SPSS statistical software. Anova test was used to compare the studied groups with the control group and Least Significant Differences (LSD) was calculated to test the significance of the results.

Results

Paw thickness: The study found that the Arthritis group showed a significant increase in their measured results (Table 1). The control group showed no significant changes, while the Arthritis group showed a partial recovery. while the Arthritic + Boswellia serrata group showed a significant improvement. The LSD value of 0.219 indicates statistical significance of the differences between the groups (Figure 1).

Table 1. Effect of *Boswellia serrata* extract on paw thickness in arthritis-induced male albino rats

Groups	Initial (mm)	14 days (mm)	44 days (mm)
C	6.825±0.165	6.825±0.165^{b}	6.825±0.165^{c}
Arthritis	6.986±0.223	14.86±0.164^{a}	12.166±0.253^{a}
Arthritis+ Boswellia serrata	6.862±0.170	14.62±0.210^{a}	11.651±0.058^{b}
LSD		**0.851**	**0.219**

* Different letters indicate significant differences between any two groups at p<0.05. C: Control, T1: CJ arthritis induction, T2: Induction of CJ arthritis with *Boswellia serrata* extract

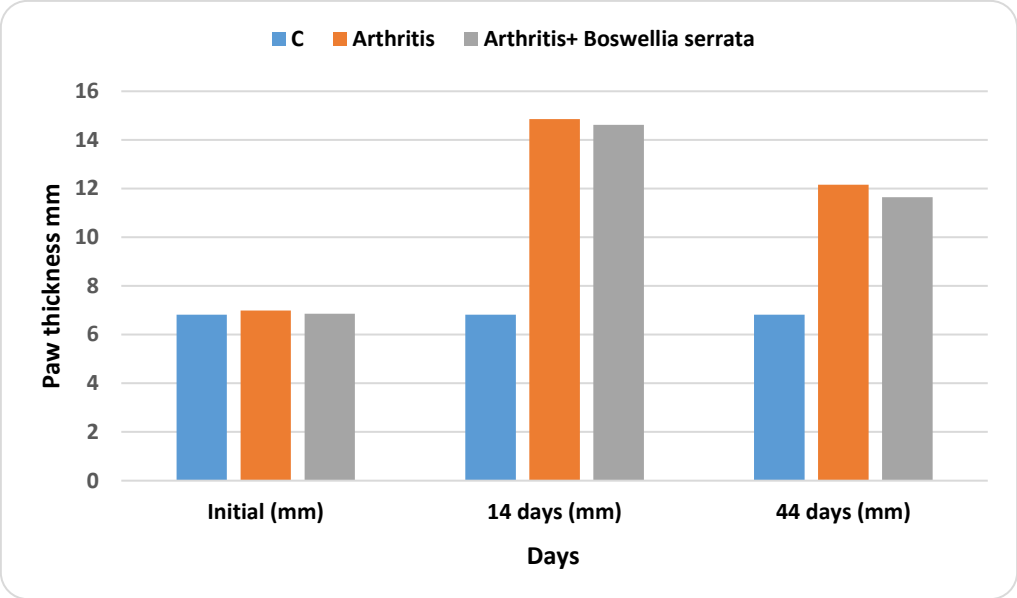

Figure 1. Effect of *Boswellia serrata* extract on paw thickness in arthritis-induced male albino rats

Antioxidants: The study analyzed the impact of various therapies on biochemical markers associated with oxidative stress and antioxidant status in an arthritic model (Table 2). The control group (C) had the lowest MDA level, while the arthritis group showed the highest MDA level. The Arthritis group had the highest SOD level, indicating compromised antioxidant defense. while the Arthritis + Boswellia serrata group had the highest SOD level. The Arthritis + Boswellia serrata group had the highest GSH level, while the control group had the highest GSH level. The Arthritis + Boswellia serrata group had the most advantageous outcomes. The study found that the Arthritis group had substantial elevations in oxidative stress, as evidenced by increased MDA levels, and a deterioration of the antioxidant system, as shown by reduced SOD, GSH, and CAT levels. The Arthritis + Boswellia serrata groups demonstrated enhancements in these parameters, with the Arthritis + Boswellia serrata group displaying the most advantageous outcomes (Figure 2).

Inflammatory parameters of arthritis: The study analyzed the effects of various therapies on inflammation and autoimmune markers in the arthritic model (Table 3). The categories included Arthritis, and Arthritis + Boswellia serrata. The Arthritis group showed significantly higher levels of rheumatoid factor (RF) and C-Reactive Protein (CRP), indicating substantial autoimmunity. The Arthritis + Boswellia serrata group had lower levels but higher CRP levels. The statistical significance of the differences between the groups was confirmed by the reported LSD values (Figure 3).

Table 2. Effect of *Boswellia serrata* extract on antioxidants in arthritis-induced male albino rats

Groups	MDA (µmole/mL)	SOD (U/mL)	GSH (µMole/mL)	CAT (U/mL)
C	1.629± 0.010^{F}	3.042± 0.009^{a}	3.268 ± 0.010^{a}	0.955±0.006^{a}
Arthritis	5.203 ± 0.211^{a}	0.954 ± 0.016^{d}	1.003 ± 0.025^{d}	0.247±0.009^{d}
Arthritis+ Boswellia serrata	4.269 ± 0.078^{b}	1.761 ± 0.020^{c}	1.347 ± 0.011^{E}	0.401±0.007^{c}
LSD	0.314	0.147	0.191	0.064

* Different letters indicate significant differences between any two groups at p<0.05. C: Control, T1: CJ arthritis induction, T2: Induction of CJ arthritis with *Boswellia serrata* extract

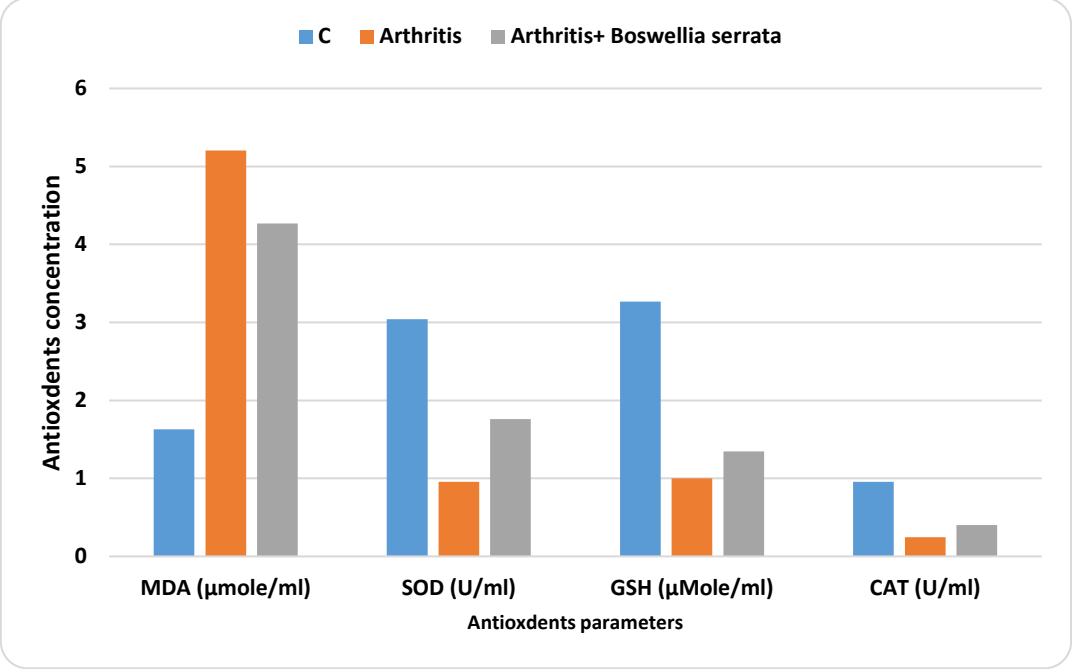

Figure 2. Effect of *Boswellia serrata* extract on antioxidants in arthritis-induced male albino rats

Discussion

This study sought to examine the impacts of various treatments, specifically the herbal supplement Boswellia serrata, on paw thickness, oxidative stress indicators, and autoimmune/inflammatory parameters in an arthritis-induced animal model. This study's findings offer significant insights into the possible therapeutic uses of these strategies for arthritis management.

Table 3. Effect of *Boswellia serrata* extract on inflammatory parameters of arthritis in arthritis-induced male albino rats

Groups	RF IU/mL	CRP mg/dL	ACCP U/mL
C	5.083±0.067^{c}	2.498±0.019^{d}	5.089±0.017^{c}
Arthritis	31.252±0.232^{a}	8.293±0.122^{a}	61.707±0.470^{a}
Arthritis+ Boswellia serrata	17.142±0.063^{b}	5.880±0.117^{b}	49.150±0.257^{b}
LSD	**0.417**	**0.109**	**1.487**

* Different letters indicate significant differences between any two groups at p<0.05. C: Control, T1: CJ arthritis induction, T2: Induction of CJ arthritis with *Boswellia serrata* extract

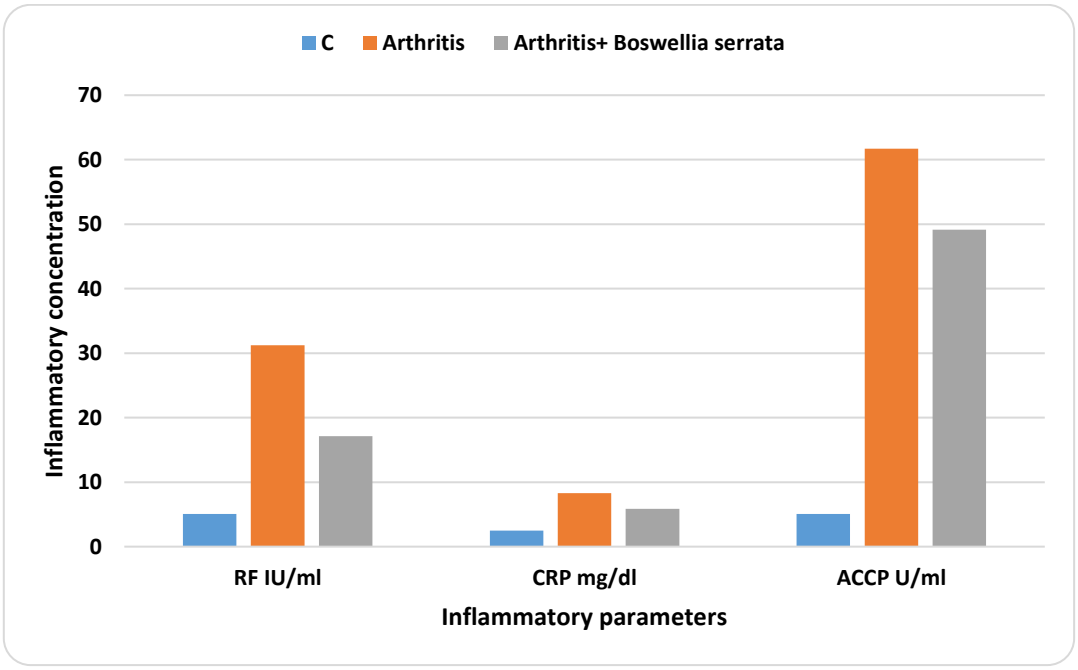

Figure 3. Effect of *Boswellia serrata* extract on Inflammatory parameters of arthritis in arthritis-induced male albino rats

Paw thickness: The findings demonstrated that the Arthritis group displayed a substantial increase in paw thickness relative to the control group, signifying the effective induction of an arthritic state (D'Amico et al. 2022; Srivastava et al. 2012). The augmentation of paw thickness is a definitive trait of arthritis, commonly ascribed to the buildup of inflammatory exudates, edema, and synovial hypertrophy (Knights et al. 2023; Schett & Firestein 2010). The study revealed that both the Arthritis + Boswellia serrata groups had a partial decrease in paw thickness relative to the Arthritis group. along with the supplementation of Boswellia serrata, a plant-derived compound with anti-inflammatory effects, effectively reduced the progression of the

Agricultural Biotechnology Journal; Print ISSN: 2228-6705, Electronic ISSN: 2228-6500

arthritic condition (Sengupta et al. 2008; Mohsenzadeh et al. 2023). The significant enhancement noted in the Arthritis + Boswellia serrata group may be ascribed to the direct anti-inflammatory actions, which primarily function by inhibiting the cyclooxygenase (COX) enzymes that facilitate the synthesis of pro-inflammatory mediators (Rainsford, 2009). The reported LSD value of 0.219 highlights the statistical significance of the changes in paw thickness between the groups, emphasizing the strength of the observed effects and the possible therapeutic implications of the therapies (Farhan et al. 2019).

Oxidative stress and antioxidant markers: The examination of oxidative stress and antioxidant metrics indicated that the Arthritis group demonstrated a notable elevation in malondialdehyde (MDA) levels, an indicator of lipid peroxidation, alongside a corresponding reduction in the activities of antioxidant enzymes including superoxide dismutase (SOD), glutathione (GSH), and catalase (CAT). The results align with the documented correlation between oxidative stress and the etiology of arthritis (Deligiannidou et al. 2021; Wruck et al. 2011). The noted elevation in MDA levels within the Arthritis group signifies heightened oxidative damage to lipids, potentially exacerbating joint inflammation and cartilage deterioration (Wang et al. 2022). The dysfunction of the antioxidant defence system, indicated by diminished levels of SOD, GSH, and CAT, implies a disparity between the production of reactive oxygen species and the body's capacity to neutralize them, resulting in heightened oxidative stress (Mateen et al. 2016; Umar et al. 2014). The Arthritis + Boswellia serrata cohorts demonstrated enhancements in oxidative stress and antioxidant metrics relative to the Arthritis cohort. The Arthritis + Boswellia serrata cohort had the most advantageous results, with the greatest concentrations of SOD, GSH, and CAT, alongside the lowest MDA levels across the therapy groups. These findings correspond with the increasing data indicating the significant antioxidant and anti-inflammatory capabilities of Boswellia serrata and its bioactive constituents, including boswellic acids (Poivre et al. 2017; Sengupta et al. 2010 and 2011). Boswellia serrata may modulate oxidative stress and antioxidant status through various mechanisms, such as inhibiting pro-inflammatory pathways, scavenging free radicals, and enhancing endogenous antioxidant defences (Bertocchi et al. 2018; Abdel-Tawab et al. 2011). These pathways may facilitate the noted enhancements in the Arthritis + Boswellia serrata groups, underscoring their potential as adjunct treatments for the management of arthritis-related oxidative stress.

Inflammatory and autoimmune markers: The examination of inflammatory and autoimmune markers clarifies the pathophysiological alterations in the arthritic model and the effects of various therapies. The Arthritis group demonstrated markedly higher levels of rheumatoid factor (RF), C-reactive protein (CRP), and anti-cyclic citrullinated peptide (ACCP)

in comparison to the control group. These markers are acknowledged as principal indicators of the autoimmune and inflammatory mechanisms implicated in the onset and advancement of rheumatoid arthritis (Aletaha et al. 2010; Sung & Tsai 2021). The elevated levels of RF and ACCP in the Arthritis group indicate the existence of autoantibodies directed against self-antigens, a characteristic feature of the autoimmune aspect of rheumatoid arthritis (Firestein & McInnes, 2017). The increased CRP levels indicate the systemic inflammatory response linked to the arthritic disease (Karimzadeh et al. 2018). The Arthritis + Boswellia serrata cohorts had decreased levels of inflammatory and autoimmune markers relative to the Arthritis group. The Arthritis + Boswellia serrata group exhibited enhancements in these parameters relative to the Arthritis group. The processes that may account for the beneficial effects of Boswellia serrata include the control of inflammatory cytokines, the suppression of nuclear factor-kappa B (NF-κB) signalling, and the reduction of autoimmune reactions (Ammon, 2016; Jäger et al. 2009). The statistical significance of the differences between the groups, as evidenced by the reported LSD values, highlights the trustworthiness of the observed effects and the possible therapeutic ramifications of the treatments in managing arthritis-related inflammation and autoimmune.

Conclusions: The results of this study offer significant insights into the diverse therapeutic potential of Boswellia serrata in relation to arthritis. The findings indicate that these therapies can successfully reduce changes in paw thickness, oxidative stress, and inflammatory/autoimmune markers noted in the arthritic model. The Arthritis + Boswellia serrata cohort frequently had the most advantageous results, indicating the possibility of this herbal supplement as an adjunct treatment for arthritis management. These findings have significant implications for the formulation of integrated therapy strategies that merge pharmacological approaches, such as NSAIDs, with adjunct medicines like Boswellia serrata to tackle the complex pathophysiology of arthritis. Additional clinical studies and translational research are necessary to confirm the effectiveness and safety of these therapies in human populations and to clarify the molecular processes underlying their therapeutic effects.

Author contributions: Adel Kateh Bashar: Methodology, Software, Formal analysis, Investigation, Data curation, Writing-original draft preparation, and Writing-review and editing. **Wejdan Matrood Kadhem:** Conceptualization, Methodology, Software, Validation, Formal analysis, Investigation, Resources, Data curation, Writing-original draft preparation, Writing-review and editing, Visualization, Supervision, Project administration, and Funding acquisition. All authors have read and agreed to the published version of the manuscript.

Data availability statement: Not applicable.

Acknowledgement: The authors thank all reviewers for their valuable comments in improving the quality of the article.

Ethical considerations: The authors avoided data fabrication, falsification, plagiarism, and misconduct.

Funding: The study was funded by the University of Al-Qadisiyah, Iraq.

Conflict of Interest: There is no conflict of Interest.

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مجله بیوتکنولوژی کشاورزی
شاپا چاپی: ۶۷۰۵-۲۲۲۸ شاپا الکترونیکی: ۶۵۰۰-۲۲۲۸
دانشگاه شهید باهنر کرمان
انجمن بیوتکنولوژی ایران

بررسی اثر *Boswellia serrata* در کاهش اثرات مرتبط با آرتریت القایی در موش‌های صحرایی نر آلبینو

عادل کته بشار

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تاریخ دریافت: ۱۴۰۳/۰۹/۱۰ تاریخ دریافت فایل اصلاح شده نهایی: ۱۴۰۳/۱۱/۰۱ تاریخ پذیرش: ۱۴۰۳/۱۱/۰۲

چکیده

هدف: آرتریت یک اختلال التهابی مزمن است که با درد مفاصل، تورم و اختلال عملکرد مشخص می‌شود. اغلب با عوارض قابل توجه و کاهش کیفیت زندگی همراه است. مطالعه حاضر اثرات درمان‌های مختلف را بر ضخامت پنجه، نشانگرهای استرس اکسیداتیو و پارامترهای خودایمنی/التهابی در مدل آرتریت بررسی کرد.

مواد و روش‌ها: این مطالعه در گروه علوم زیستی دانشگاه القادسیه با استفاده از موش‌های صحرایی نر آلبینو انجام شد. موش‌ها در قفس‌های پلاستیکی مخصوص پرورش داده شدند و در محدوده دمایی ۲۰-۲۵ درجه سانتی گراد نگهداری شدند. آرتریت با استفاده از ادجوانت کامل فروندز القا شد که روزانه به مدت ۱۴ روز به پد پای راست تزریق می‌شد. عصاره Boswellia serrata با تهیه عصاره آبی از کندر (frankincense) نر تهیه شد. موش ها به سه گروه تقسیم شدند: گروه کنترل، حیواناتی که در آن‌ها آرتریت با تزریق مکمل فروندز کامل به پد پا ایجاد شد، و حیواناتی که در آنها آرتریت به مدت ۱۴ روز القا شد و سپس Boswellia serrata تجویز شد. نتایج با استفاده از نرم افزار آماری SPSS، آزمون ANOVA و کمترین تفاوت معنی دار (LSD) برای آزمون معنی داری نتایج مورد تجزیه و تحلیل قرار گرفت.

نتایج: در ارزیابی ضخامت پنجه، گروه آرتریت نسبت به گروه کنترل، که تا حدی با تیمارهای آرتریت به علاوه Boswellia serrata کاهش یافته بود افزایش قابل توجهی نشان داد. گروه کنترل اندازه‌های ثابتی را در تمام طول آزمایش حفظ کردند، در حالی که گروه آرتریت بهبودی نسبی را نشان داد. گروه آرتریت به علاوه Boswellia serrata مطلوب‌ترین نتایج را داشتند. مقدار

LSD گزارش شده ۰/۲۱۹ نشان دهنده اهمیت آماری تفاوت بین گروه‌ها است. تجزیه و تحلیل استرس اکسیداتیو و نشانگرهای آنتی اکسیدانی نشان داد که گروه آرتریت دارای بالاترین سطوح نشانگر پراکسیداسیون لیپیدی MDA، همراه با پایین‌ترین سطوح آنزیم‌های آنتی اکسیدانیSOD، GSH و CAT است که نشان‌دهنده ضعف دفاعی آنتی‌اکسیدانی است. گروه آرتریت آرتریت به علاوه Boswellia serrata سودمندترین نتایج را نشان داد. با توجه به التهاب و نشانگرهای خودایمنی، گروه آرتریت سطوح فاکتور روماتوئید (RF)، پروتئین واکنشی C (CRP) و پپتید سیترولین دار ضد حلقوی (ACCP) را به طور قابل توجهی افزایش داد که نشان دهنده خودایمنی و التهاب قابل توجه است. گروه آرتریت به علاوه Boswellia serrata نتایج متوسطی را نشان داد. اهمیت آماری تفاوت بین گروه‌ها توسط مقادیر LSD گزارش شده پشتیبانی شد.

نتیجه‌گیری: یافته‌ها نشان می‌دهد که درمان‌های آرتریت به علاوه Boswellia serrata می‌توانند اثرات مفیدی در کاهش تغییرات ضخامت پنجه، استرس اکسیداتیو و پارامترهای خودایمنی/التهابی مشاهده‌شده در مدل آرتریت ارائه دهند. گروه آرتریت به علاوه Boswellia serrata اغلب مطلوب‌ترین نتایج را در میان گروه‌های درمان نشان می‌دهد.

واژه‌های کلیدی: آرتریت، آلبینو، موش صحرایی، نر، Boswellia serrata

نوع مقاله: پژوهشی.

استناد: بشار عادل کته، کادم وجدان مترود (۱۴۰۴) بررسی اثر *Boswellia serrata* در کاهش اثرات مرتبط با آرتریت القایی در موش‌های صحرایی نر آلبینو. *مجله بیوتکنولوژی کشاورزی*، ۱۷(۱)، ۱۷۷-۱۹۲.

Publisher: Faculty of Agriculture and Technology Institute of Plant Production, Shahid Bahonar University of Kerman-Iranian Biotechnology Society.