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Ameliorative effect of *Boswellia serrata* extract on experimentally induced arthritis in rats

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Abstract

Background: Rheumatoid arthritis is an inflammatory condition that damages the synovial joint lining, resulting in bone erosion, irreversible cartilage loss, and occasionally permanent disability.

Objectives: Observe the effect of *Boswellia serrata* powder on biological activities on rats with rheumatoid arthritis.

Material and Methods: 25 adult male Wistar rats, weighing 150±10 g, 90 days old, are used. The first regimen consisted of a standard management group (n=5). Each animal received 1 millilitre of Complete Freund's Adjuvant (CFA), which caused a relapse after 7 days and rheumatoid arthritis signs after 14 days. Normal control group and model control group obtained a base diet, while the other 3 rheumatoid arthritis groups obtained a base diet with 2.5, 5, and 7.5% *Boswellia serrata* powder daily for 28 days. The following parameters were measured: TNF-α and IL-6 malondialdehyde, antinuclear antibodies, rheumatoid factor, CRP, ESR.

Result: the resulrs demonstrated that the powdered *Boswellia serrata* becomes rich in boswellic acids, 11 keto beta boswellic acids (KBA), acetyl KBA, and antioxidants that impact the mobile defense apparatus. *Boswellia serrata* powder treatment resulted in serum TNF-α and IL-6 levels were markedly increased in arthritic animals (86.76 ± 2.01 pg/mL) and (43.74 ± 1.00 pg/mL) in comparison to the control group and significant ($p \leqslant 0.05$). MDA and SOD levels in arthritic group showed a marked elevation concentrations (4.95 ± 0.11 nmol/mL) and(44.16 ± 1.08 U/mL) compared to the control.

Conclusion: Results suggested that frankincense exhibits antioxidant, scavenging, and anti-inflammatory effects on rheumatoid arthritis in rats.

Keywords: Rheumatoid arthritis; *Boswellia serrata*; Malondialdehyde superoxide dismutase; Tumor necrosis factor-α

1. Introduction

Rheumatoid arthritis (RA) is an inflammatory disease characterized by severe inflammation of the joints and tissues . Autoantibodies against the fragment crystallizable region (Fc region) of immunoglobulin IgG, rheumatoid factor, and anticitrullinated protein antibodies (ACPA) are produced in this inflammatory disease and are used to categorize RA patients as either seropositive or seronegative (1). Although its precise etiology is still unknown, a few variables may raise the chance of developing RA. Thus, it is essential to reduce the incidence and consequences of arthritis to plan for future clinical and public health requirements (2). Poorly managed RA causes severe morbidity and mortality due to

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permanent bone and joint destruction, as well as extra-articular symptoms such as rheumatoid nodules, cardiovascular problems, pericarditis/pleuritis, and vasculitis (3). Nonsteroidal anti-inflammatory capsules (NSAIDs), corticosteroids, pathogenic anti-rheumatic drugs (DMARDs), and biochemical response modification are frequently used pharmaceutical drugs to prevent RA however they are compatible with (R. Kumar et al. 2019). are associated with many dangerous side effects. Environmental factors, genetic predisposition, or a mix of the two can increase the risk of RA. It is evident that immune cells, including neutrophils, macrophages, and lymphocytes, are crucial to the pathogenesis of RA (Shanmukhan et al. 2018). Macrophages and T cells that generate cytokines that promote inflammation and cell migration are seen in the synovium of RA patients. Macrophage tumour necrosis aspect-α (TNF-α), interleukin-1 (IL-1), and interleukin-6 (IL-6), as well as the cytokine interleukin-17 (IL-17) produced by CD4 + cells, are frequently involved in the inflammatory response and cartilage syndromes, causing syndromes in fly prosthesis synovial fluid that result in cartilage breakdown and breakdown of overgrown synovial tissue, or panus (Hitchon and El-Gabalawy 2004). Increased oxidative pressure or low antioxidant status is important within the pathogenesis of rheumatoid arthritis. 2020V. Kumar and others, 2016). In experimental arthritis, some antioxidants, such as SOD and vitamin E, show anti-inflammatory properties.(V. Kumar and others, 2016). This has changed with the discovery that SOD lila is lower in patients with arthritis and the administration of SOD through liposomes had a nice effect on preventing experimental arthritis.(Ugur et al. 2004; Karatas et al. 2003). One well-known medicinal plant that demonstrated drug action within animal model is boswellia (D'Amico et al. 2022). It is a member of the Burseraceae family, which is found in arid parts of East Africa and Asia. In the past, this family was utilised for both medicinal and aromatic purposes (Thulin 2020). Boswellia sp. are probable because of aromatic oils and gum resins in their structure, which can be attributed to olibanum incense(Najar et al. 2020) These goods are frequently employed in traditional Chinese and Arabic medical treatments for conditions like arthritis, fever, pain, and asthma (et al. 2019). The Boswellia gum resin, which is generated as a milky liquid from cracks in the bark of the stems, has a broad spectrum of bioactive ingredients, including monoterpenes, diterpenes and triterpenes, as well as being a major source of boswellic acids such as tetracyclic and pentacyclic triterpene acids (Abdel-Tawab, Werz, and Schubert-Zsilavecz 2011; Alluri et al. 2020).

2. Materials and Methods

2.1. Study design

The donation study was carried out in the Animal House of the College of Veterinary Medicine at Qasim Green University of AL for a considerable amount of time, from December 2025 to February 2026. Before the trial began, male mice were given time to become used to their new surroundings in animal housing. Animals were housed in polypropylene cages inside a well-ventilated room. Each cage should contain no more than five mice. Before the investigation, 5 mice were randomly housed in polycarbonate cages with smart humidity (forty-five±5%) and temperature (25±2°C) with a 12-hour soft/dark cycle for acclimation. Water and regular food were freely available to the animals. After training, mice orally treated for 28 dats as follows (Khalaj-Kondori et al. 2016).

2.2. Animal and study grouping

Animals will randomly have divided into five groups (n=5 per group) as follows:

- Normal control group (received distilled water).
- Arthritic control group (induced with Freund's adjuvant-induced, only) (6).
- *Boswellia serrata* extract group (100 mg/kg).
- *Boswellia serrata* extract group (200 mg/kg).
- Group (treated with Celecoxib 10 mg/kg) (6).

2.3. Preparation of *Boswellia serrata* extract

Boswellia serrata aqueous extract was made by weighing, grinding, and adding 10 g of male odour to a 500 mL glass flask with 200 mL of distilled water and a magnetic stirrer for 15 minutes. This was followed by a half-hour of different precipitations. The precipitate was disposed of after the aggregate was filtered using tulle fabric. After centrifuging the filtrate for 10 minutes at 3000 rpm, a clear solution was produced. A rotary evaporator operating at 45°C was used to concentrate the solution. The aqueous extract was concentrated by rotary evaporation and seventy-five mL of the acceptable weight was placed in glass containers and then heated to forty °C in an electrically powered oven and dried.

2.4. Assessment of Aqueous *Boswellia serrata* Extracts by GC/MS

Using a direct TG-5MS capillary column and a Trace GC-TSQ mass spectrometer (Thermo Scientific, Austin, TX, USA), phytochemicals in aqueous *Boswellia serrata* extracts were examined. The injection port and MS transfer line were kept

at 260°C. The temperature started at 50°C and raised to 250°C (by 5°C /min), which was held for 2 minutes. Then it was raised to the final temperature of 300°C (by 30°C /min), which was held for 2 minutes. The mass spectra were measured at 70 eV, with the carrier gas being helium at a constant flow rate of 1 ml/min. The mass fractionation parameters of the phytochemical components of *Boswellia serrata* were estimated using standard fact data from the WILEY 09 and NIST 14 mass spectrum databases.

2.5. Rheumatoid Arthritis Induction Stage

By injecting 0.1 ml of Complete Freund's Adjuvant (CFA) into the plantar fascia of the left hind paw, rats were exposed to rheumatoid arthritis. And after seven days, this approach was repeated. According to Kumar et al. (2019), standard protocols were followed. Baseline body weight, paw thickness, ankle diameter, and paw width parameters were achieved on day 0 .

2.6. Sample Collection And Preparation

All animals were sacrificed under ether anaesthesia twenty-four hours following the final treatment. Tests are performed on blood. Use non-heparinized tubes for direct coronary artery puncture, and let the blood settle at room temperature. Blood samples were centrifuged at 3000 rpm for 10 minutes to create pure serum, and then aliquots were kept at -18°C to measure cytokine levels [19].

2.7. Serum Cytokines Levels Detection

IL-6 and TNF-α (pg/mL) were simultaneously measured in the serum of every mouse in a 96-well microplate using the Milliplex rat cytokine/chemokine kit (MilliporeSigma, Germany) and the Bio-Plex xMAP assay.

2.8. Antioxidant Level Measurement In Serum

Using a commercial ELISA kit (MyBioSource.Com, USA), measured levels of MDA and SOD in the spleen at 450 nm were converted to investigate the absorbance of each marker in order to compare the synthesis of endogenous antioxidants in the spleen and thymus homogenates of all rats.

2.9. Statistical analysis

To assess pairwise comparisons between professionals, one-way analysis of variance (ANOVA) and Fisher's least significant difference (LSD) post hoc test were used to analyse all experimental data. Results are expressed as mean ± standard error of mean (SEM) per experimental system (n = eight per system). The threshold for statistical significance was established at $p < 0.05$, with denoting $p < 0.001$. All computations were carried out under the assumption of homogeneity of variance between groups using standard parameterization policies.

2.10. Ethical approval

College of veterinary medicine/ Department of physiology, biochemistry and pharmacology, this is to certify that the research has been reviewed and approved by the Research Ethics Committee of Al-Qasim Green University and documented in number qgec/41/2025, Date of Approval: 29/11/2025.

3. Results

3.1. Influence of pro inflammatory cytokines (TNF- α and IL-6)

Pro-inflammatory cytokines have a significant mechanistic role in the pathophysiology of rheumatoid arthritis, and current investigations have shown that TNF-α and IL-6 concentrations rise significantly after arthritis induction (Table 2). Serum TNF-α levels were markedly increased in arthritic animals (86.76 ± 2.01 pg/mL) compared to the control group (15.35 ± 0.17 pg/mL; $F = 482.546$, $p < 0.001$; LSD = 3.674). Treatment with BOS 100 mg/kg significantly reduced TNF-α to 45.86 ± 1.51 pg/mL, whereas BOS 200 mg/kg produced a further significant reduction to 25.95 ± 0.94 pg/mL. The celecoxib-treated group exhibited a mean TNF-α of 28.38 ± 0.97 pg/mL, with the LSD value (3.674) confirming that the difference between BOS 200 and celecoxib was statistically non-significant, suggesting equivalent cytokine-suppressive capacity.

Similarly, serum IL-6 concentrations were substantially elevated in the arthritic group (43.74 ± 1.00 pg/mL) compared to control animals (8.15 ± 0.09 pg/mL; $F = 431.814$, $p < 0.001$; LSD = 1.922). *Boswellia serrata* at 100 mg/kg significantly attenuated IL-6 levels (22.96 ± 0.82 pg/mL), and the 200 mg/kg dose produced a more pronounced suppression (13.76

± 0.50 pg/mL), which was statistically comparable to the value observed in the celecoxib group (14.98 ± 0.55 pg/mL). These findings collectively indicate that BOS 200 mg/kg exerts a potent anti-cytokine effect analogous to that of the standard anti-inflammatory drug.

Table 1 Effect on Pro-Inflammatory Cytokines: TNF-α and IL-6

Parameter	Control (n=8)	Arthritic (n=8)	BOS 100 mg/kg (n=8)	BOS 200 mg/kg (n=8)	Celecoxib (n=8)	LSD
TNF-α (pg/mL)	15.35 ± 0.17	86.76 ± 2.01	45.86 ± 1.51	25.95 ± 0.94	28.38 ± 0.97	3.674
IL-6 (pg/mL)	8.15 ± 0.09	43.74 ± 1.00	22.96 ± 0.82	13.76 ± 0.50	14.98 ± 0.55	1.922

Values are expressed as Mean ± SEM (n=8 per group). BOS = *Boswellia serrata* extract; LSD = Fisher's Least Significant Difference; *** $p < 0.001$ (one-way ANOVA). The arthritic column (highlighted) represents the disease control group.

3.2. Effect on Oxidative Stress Markers (MDA and SOD)

Oxidative stress represents a prominent contributor to joint tissue destruction in inflammatory arthritis. As presented in Table 3, one-way ANOVA revealed significant group differences in both MDA and SOD levels ($p < 0.001$). The arthritic group showed a marked elevation in malondialdehyde (MDA) concentrations (4.95 ± 0.11 nmol/mL) compared to control animals (1.17 ± 0.04 nmol/mL; $F = 301.721$, $p < 0.001$; LSD = 0.243). Administration of BOS 100 mg/kg led to a significant reduction in MDA to 2.81 ± 0.10 nmol/mL, while BOS 200 mg/kg produced a greater reduction (1.81 ± 0.07 nmol/mL), closely approaching that of the celecoxib group (1.93 ± 0.08 nmol/mL). The LSD value of 0.243 confirmed that the difference between BOS 200 and celecoxib was not statistically significant.

In contrast, superoxide dismutase (SOD) activity was substantially diminished in arthritic animals (44.16 ± 1.08 U/mL) relative to the healthy control group (99.49 ± 0.61 U/mL; $F = 504.739$, $p < 0.001$; LSD = 2.711). The present study showed that BOS 100 mg/kg treatment partially restored SOD activity (72.20 ± 1.06 U/mL), while BOS 200 mg/kg achieved a more marked recovery (87.85 ± 0.93 U/mL), which was comparable to the value observed with celecoxib (85.95 ± 0.97 U/mL). These results indicate that *Boswellia serrata* exerts notable antioxidant properties that are dose-dependent, with the higher dose approximating the protective effect of the standard drug.

Table 2 Effect on Oxidative Stress Biomarkers: MDA and SOD

Parameter	Control (n=8)	Arthritic (n=8)	BOS 100 mg/kg (n=8)	BOS 200 mg/kg (n=8)	Celecoxib (n=8)	LSD
MDA (nmol/mL)	1.17 ± 0.04	4.95 ± 0.11	2.81 ± 0.10	1.81 ± 0.07	1.93 ± 0.08	0.243
SOD (U/mL)	99.49 ± 0.61	44.16 ± 1.08	72.20 ± 1.06	87.85 ± 0.93	85.95 ± 0.97	2.711

Values are expressed as Mean ± SEM (n=8 per group). BOS = *Boswellia serrata* extract; LSD = Fisher's Least Significant Difference; *** $p < 0.001$ (one-way ANOVA). The arthritic column (highlighted) represents the disease control group.

3.3. Effect on Serological Inflammatory Markers (ESR, CRP, and RF)

Serological markers of systemic inflammation were significantly elevated following arthritis induction, as detailed in Table 4. Erythrocyte sedimentation rate (ESR) was substantially increased in the arthritic group (16.75 ± 0.75 mm/hr) compared to control animals (2.42 ± 0.05 mm/hr; $F = 142.362$, $p < 0.001$; LSD = 1.360). Treatment with BOS 100 mg/kg significantly lowered ESR to 8.12 ± 0.52 mm/hr, and BOS 200 mg/kg produced a further significant reduction (4.25 ± 0.37 mm/hr), which was lower than that observed in the celecoxib group (5.12 ± 0.40 mm/hr). The Fisher's LSD value (1.360) confirmed that this difference between BOS 200 and celecoxib was statistically significant, suggesting a superior anti-inflammatory effect of the higher Boswellia dose on systemic inflammatory responses.

C-reactive protein (CRP), a key acute-phase reactant, was markedly elevated in arthritic animals (19.51 ± 0.59 mg/L) relative to controls (1.12 ± 0.07 mg/L; $F = 392.039$, $p < 0.001$; LSD = 1.054). Both BOS doses significantly reduced CRP concentrations: BOS 100 mg/kg yielded 9.40 ± 0.42 mg/L, while BOS 200 mg/kg reduced levels to 3.66 ± 0.24 mg/L compared to 4.64 ± 0.29 mg/L in the celecoxib group. Based on the LSD value of 1.054, the difference between BOS 200 and celecoxib was statistically significant, indicating that the higher Boswellia dose conferred superior suppression of CRP.

Rheumatoid factor (RF) levels were significantly elevated in the arthritic group (19.89 ± 0.63 U/mL) compared to control animals (4.11 ± 0.08 U/mL; F = 249.946, p < 0.001; LSD = 1.142). BOS 100 mg/kg produced a significant reduction in RF (10.49 ± 0.47 U/mL), while BOS 200 mg/kg and celecoxib showed comparable reductions of 5.90 ± 0.24 U/mL and 6.80 ± 0.34 U/mL, respectively. The LSD test confirmed statistical similarity between BOS 200 and celecoxib groups for RF (LSD = 1.142), suggesting that the higher Boswellia dose achieves near-equivalent autoimmune-modulatory activity to the reference compound.

Table 3 Effect on Serological Inflammatory Markers: ESR, CRP, and R

Parameter	Control (n=8)	Arthritic (n=8)	BOS 100 mg/kg (n=8)	BOS 200 mg/kg (n=8)	Celecoxib (n=8)	LSD
ESR (mm/hr)	2.42 ± 0.05	16.75 ± 0.75	8.12 ± 0.52	4.25 ± 0.37	5.12 ± 0.40	1.360
CRP (mg/L)	1.12 ± 0.07	19.51 ± 0.59	9.40 ± 0.42	3.66 ± 0.24	4.64 ± 0.29	1.054
RF (U/mL)	4.11 ± 0.08	19.89 ± 0.63	10.49 ± 0.47	5.90 ± 0.24	6.80 ± 0.34	1.142

Values are expressed as Mean ± SEM (n=8 per group). BOS = *Boswellia serrata* extract; LSD = Fisher's Least Significant Difference; *** p < 0.001 (one-way ANOVA). The arthritic column (highlighted) represents the disease control group.

3.4. Histological changes

3.4.1. Control group

The manipulated rat's knee joint histopathological photomicrographs consistently display intact cartilage with normal synovium devoid of inflammatory cells and normal chondrocytes. Haematoxylin and eosin, single magnification, as shown in Figures (1).

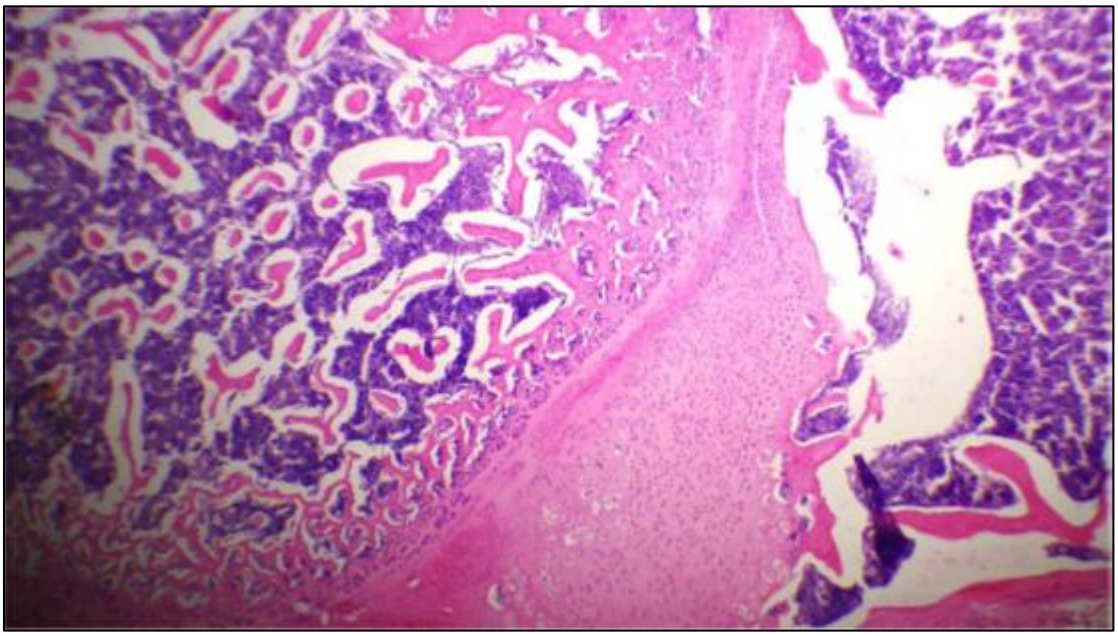

Figure 1 Light micrographs showing the histopathology of the control rat knee joint normal chondrocytes, normal cartilage, and a normal synovium devoid of inflammatory cells. Authentic magnification of hematoxylin and eosin (25 and 40 X)

3.4.2. Arthritic group

Hematoxylin and eosin, single magnification, histopathological photomicrograph of a rat knee joint caused by FCA, demonstrating synovial membrane with minor oedema and aberrant floor, as seen in Figures (2).

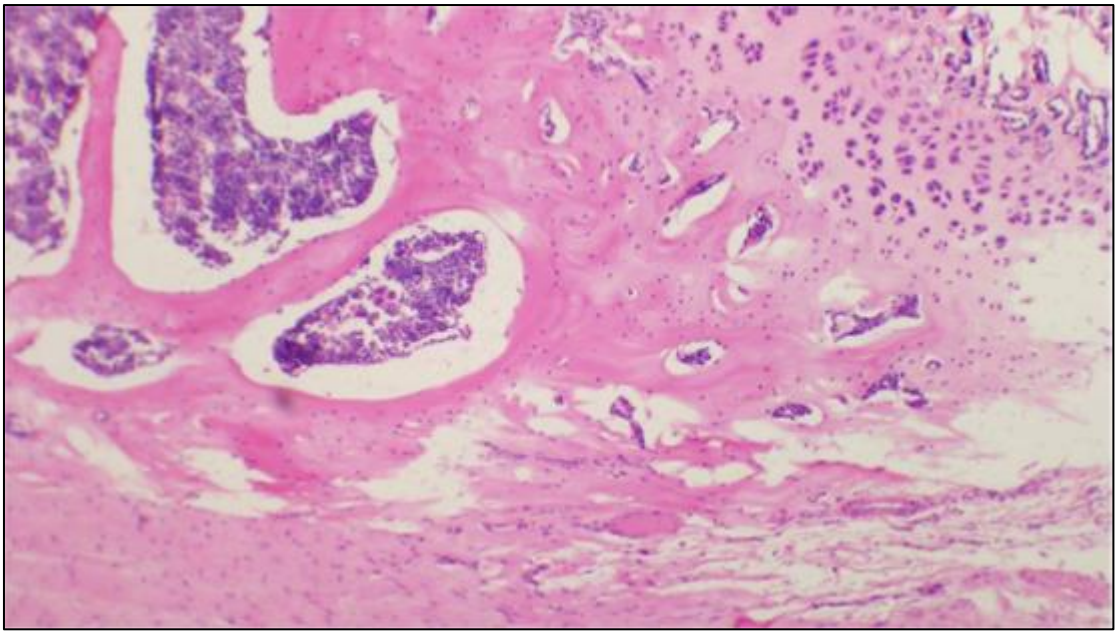

Figure 2 Rat knee joint histopathology photomicrograph, arthritis system It has an uneven surface and some puffiness. Eosin and hematoxylin, genuine magnification (25& 40 X)

3.5. *Boswellia serrata* (100 mg/kg. B.W)

Histopathological photomicrograph of *Boswellia serrata* knee joint articular cartilage (100 mg/kg). B.W) group has several intact chondrocytes and a synovial membrane with modest oedema. Haematoxylin and eosin, true magnification, as seen in images (3).

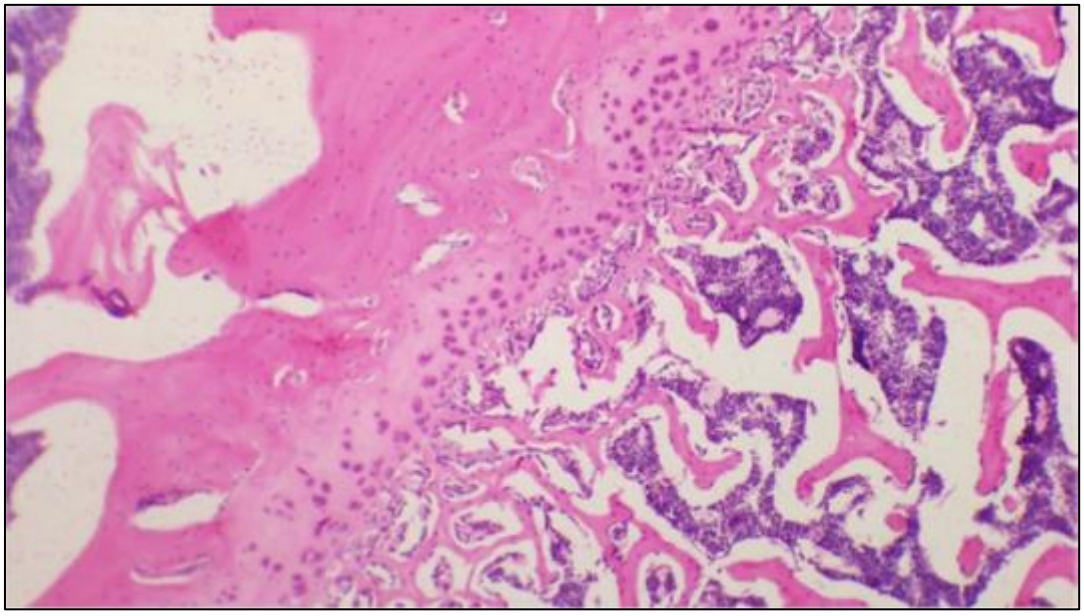

Figure 3 Rats on a 100 mg/kg B.W. regimen of *Boswellia serrata* exhibit moderate oedema and a synovial membrane with several undamaged chondrocytes. Rat knee histopathology of a *Boswellia serrata* (100 mg/kg B.W.) regimen. Original magnification of haematoxylin and eosin (25 and 40 X)

3.6. *Boswellia serrata* (200 mg/kg. B.W)

A histopathological photomicrograph of the articular cartilage from the knee joint of the *Boswellia serrata* (200 mg/kg B.W.) group reveals a normal base and a synovial membrane free of oedema. Images (4) illustrate haematoxylin and eosin at a single magnification.

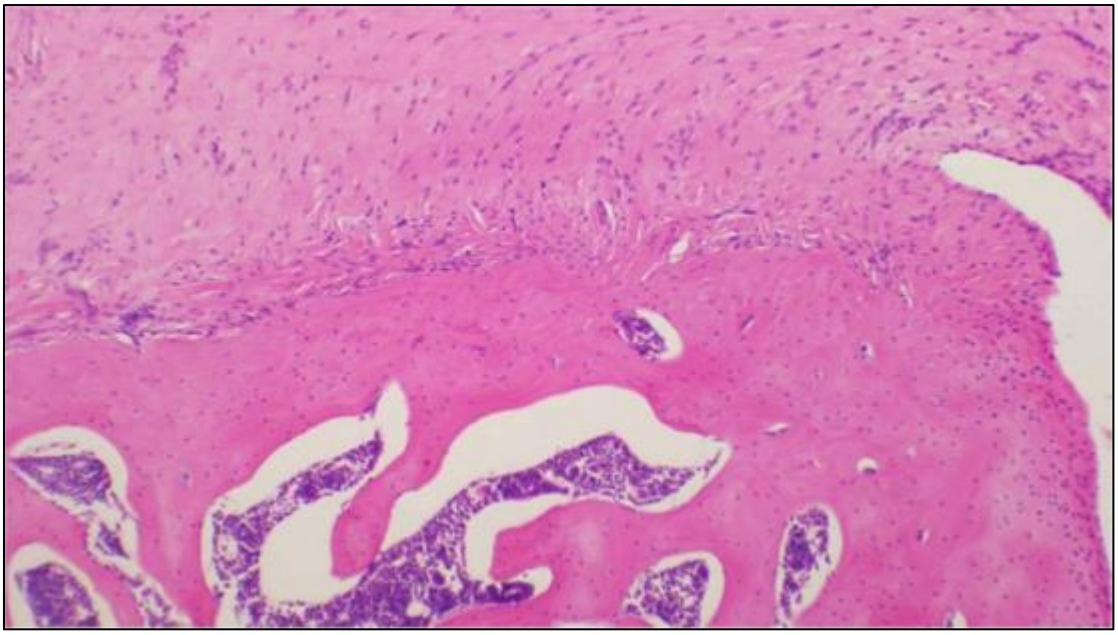

Figure 4 A photomicrograph of the histopathology of the rat knee joint in the *Boswellia serrata* (200 mg/kg B.W.) group demonstrates a normal surface and no oedema. Hematoxylin and eosin, single magnification (25& 40 X)

3.7. Celecoxib group

A histopathological photomicrograph of the knee joint in the celecoxib group demonstrates intact synovial membrane and chondrocytes. even in subchondral cartilage, whole chondrocytes. Images (5) show haematoxylin and eosin at a single magnification.

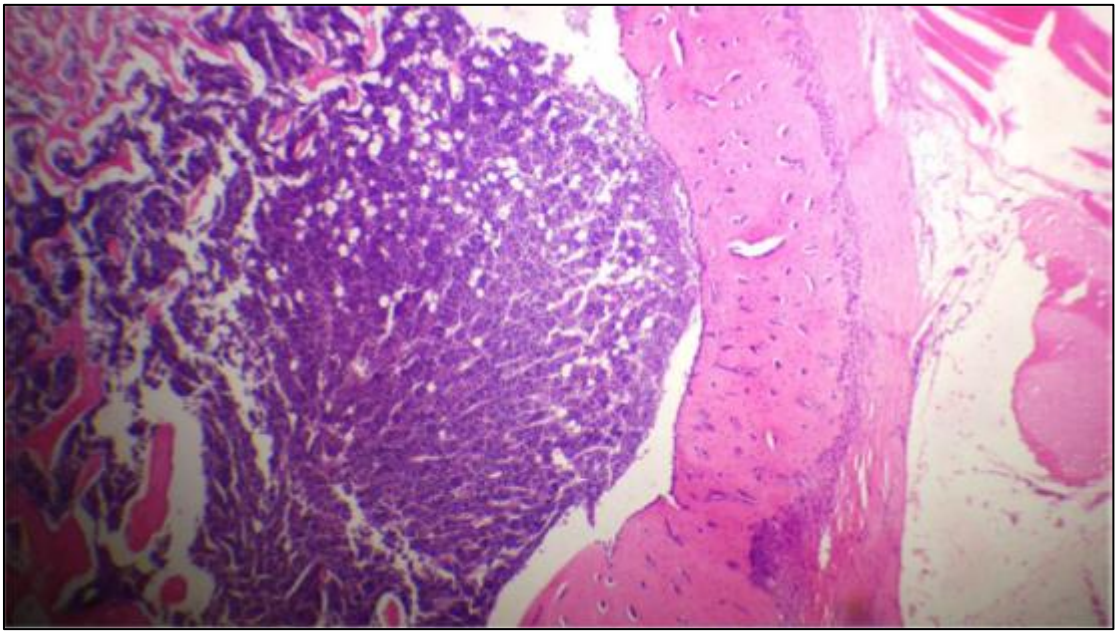

Figure 5 Histopathology photomicrograph of knee joint of Celecoxib group rat showing intact chondrocytes, intact synovial membrane. Also intact chondrocytes in subchondral cartilage. Hematoxylin and eosin, original magnification (25& 40 X)

4. Discussion

In an animal model of arthritis, this study looked at the impact of several therapies, including the natural supplement *Boswellia serrata*, on paw thickness, oxidative stress markers, and autoimmune/inflammatory parameters. 11 keto beta

boswell acids (KBA) and boswell acids, including acetyl BBA, are included in BSE. These compounds alter the amounts of different cytokines, which impact the mobile defence apparatus.(7) Previous investigations have defined it.(Siddiqui 2011; Pirotta 2010; R. Kumar et al. 2019). Angiogenesis, synovial enlargement, atherosclerosis, BSE cartilage deterioration, fibrous bone production, forehead formation, and findings that *Boswellia serrata* decreases cell infiltration in an inflammatory model of RA caused by carrageenan. (Rao, Bhaskar, and Ismail 2016). By inhibiting oxidative and inflammatory pathways, scavenging free radicals, and boosting endogenous antioxidant defences, *Boswellia serrata* can regulate oxidative pressure and antioxidant recognition (Bashar and Kadhem 2025). The increased CRP levels indicate the systemic inflammatory response linked to the arthritic disease (8). 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).

5. Conclusion and recommendation

Long-term symptom alleviation, decreased inflammation, stopped cartilage deterioration, and stimulated the production of the The synergy produced by mixing large quantities of *Boswellia serrata* extract is responsible for the nutrient combination's precise effectiveness, making it a potentially useful recipe. To ascertain long-term effectiveness and protection in a broader population, more research could be required. A number of important markers of progress can be proposed based on its findings. Next experiments and research should encompass long-term studies to evaluate the chronic effects and safety profile of *Boswellia serrata* extract, as well as investigate its potential interactions with management of Rheumatoid arthritis. Given the promising results observed at doses of 100-200 mg/kg BW, human clinical trials should be conducted focusing on this dosage range. The development of standardized extraction protocols is essential to ensure consistent extract potency.

Novelty statement

This study presents several novel contributions to the understanding of *Boswellia serrata* we demonstrate a comprehensive evaluation of the extract's effects on multiple aspects of arthritis The present study showed that BOS 100 mg/kg treatment partially restored SOD activity (72.20 ± 1.06 U/mL), while BOS 200 mg/kg achieved a more marked recovery (87.85 ± 0.93 U/mL), which was comparable to the value observed with celecoxib (85.95 ± 0.97 U/mL). BOS 100 mg/kg produced a significant reduction in RF (10.49 ± 0.47 U/mL), while BOS 200 mg/kg and celecoxib showed comparable reductions of 5.90 ± 0.24 U/mL and 6.80 ± 0.34 U/mL, respectively. The LSD test confirmed statistical similarity between BOS 200 and celecoxib groups for RF (LSD = 1.142), suggesting that the higher Boswellia dose achieves near-equivalent autoimmune-modulatory activity to the reference compound.

Limitation

While our sample size of 5 rats per group was determined through power analysis and aligns with the 3Rs principle, this represents a minimum threshold for statistical validity. This limitation particularly affects our ability to detect subtle differences, as evidenced in the *Boswellia serrata* treatment effects may have been masked by the small sample size. Future studies with larger sample sizes would provide more robust statistical power and potentially reveal effects that were not detectable in our current study. The dose range (100-200 mg/kg BW) was selected based on previous literature, but exploration of a wider dose range could help establish a more precise therapeutic window. Additionally, pharmacokinetic studies would provide valuable information about bioavailability and metabolism of active compounds

Compliance with ethical standards

Acknowledgments

- This manuscript is original and has not been published or submitted to any other journal.
- All authors contributed significantly to the research and have given their complete approval to the publication.
- All authors agree to comply with the copyright transfer form.

Disclosure of conflict of interest

The authors declare that there are no conflicts of interest in this study. The authors also declare that this study was not conducted using any funds or financial support from any individual or organization.

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