Anti-inflammatory Effects of Boric Acid in Treating Knee Osteoarthritis: Biochemical and Histopathological Evaluation in Rat Models

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Abstract

Objective: This study aimed to examine the anti-inflammatory properties of boric acid (BA) in treating knee osteoarthritis (KOA) in rats, evaluating its biochemical and histopathological therapeutic effects.

Methods: The KOA rat model was induced by injecting monosodium iodoacetate into the knee joint. Random assignment was performed for the experimental groups as follows: group-1 (control), group-2 (KOA control), group-3 (BA: 4 mg/kg, orally), group-4 (BA: 10 mg/kg, orally), group-5 (BA: 4 mg/kg, intra-articularly), and group-6 (BA: 10 mg/kg, intra-articularly). The rats received 100 µL of BA intra-articularly on days 1, 7, 14, and 21 or 1 mL orally once a day (5 days/week) for 4 weeks. Serum levels of interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and matrix metalloproteinase-13 (MMP-13) were measured. Histopathological and immunohistochemical analyses were performed on knee joint samples using specific antibodies for IL-1β, TNF-α, MMP13, and nitric oxide synthase-2 (NOS-2).

Results. Group-2 exhibited higher serum levels of IL-1β, TNF-α, and MMP-13 than group-1 (P<0.05). However, these levels were lower in all treatment groups compared to group-2, with statistically significant reductions observed in groups-4, 5, and 6. Histopathologically, group-2 displayed joint space narrowing, cartilage degeneration, and deep fissures. Groups-5 and 6 demonstrated significant joint space enlargement, articular cartilage tissue regeneration, and immunostaining patterns similar to those in group-1. Immunohistochemically, group-2 showed significant increases in IL-1β, TNF-α, MMP-13, and NOS-2 expression. However, all treatment groups exhibited reductions in these expression levels compared to group-2, with statistically significant decreases observed in groups-5 and 6 (P<0.01).

Conclusions. BA shows potential efficacy in reducing inflammation in experimental KOA models in rats. It may be a promising therapeutic agent for KOA, warranting further clinical studies for validation.

Key Points

1. This study represents the first evidence demonstrating the effectiveness of boric acid (BA) in alleviating symptoms of knee osteoarthritis (KOA) using an experimental rat model.
2. Specifically, BA effectively reduces the levels of inflammatory cytokines, including metalloproteinase-13 (MMP-13), tumor necrosis factor-alpha (TNF-α), and interleukin-1beta (IL-1β), both in the serum and knee joint samples of the experimental KOA rat model.
3. The application of BA through intra-articular (i.a.) administration has been shown to significantly decrease proinflammatory cytokines, as evidenced by histopathological, immunohistochemical, and biochemical analyses, indicating its potential as an alternative treatment option for KOA.

Introduction

Osteoarthritis (OA) is a widespread condition that is expected to become more prevalent due to the aging population and the rise in obesity rates. Risk factors for OA include aging, trauma, uneven distribution of
The relationship between weight gain and cellular components in OA has been a subject of scientific interest. Both aging and obesity independently contribute to joint inflammation [1]. Knee osteoarthritis (KOA) is a common chronic degenerative disease affecting approximately 22.9% of individuals over 40 years old [2]. It significantly impacts quality of life, leading to functional limitations, disabilities, and musculoskeletal disorders [3]. OA is characterized by the deterioration of hyaline articular cartilage, inflammation of the synovial membrane, ligament changes, sclerotic alterations, osteophyte formation, and meniscal degeneration and fibrosis of the infrapatellar fat pad [4].

Synovial inflammation in osteoarthritis (OA) is characterized by angiogenesis and hyperplasia leading to effusion [5]. At the molecular level, both local and systemic proinflammatory activity in OA can be attributed to tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6). These cytokines can also increase the levels of C-reactive protein (CRP), a systemic marker of inflammation [6]. Joint tissue can be activated by lipid metabolites, reactive oxygen species (ROS), and advanced glycation end products, which in turn trigger the release of matrix metalloproteinases (MMPs) into the joint space [7]. Cells in the joint tissue, including synovial macrophages, chondrocytes, and subchondral bone cells, can produce these mediators, exacerbating the inflammatory response in OA. Other sources of inflammation in the knee joint include the meniscus and the infrapatellar adipose tissue [8]. Additionally, the local production of inflammatory mediators can be triggered by cellular stress caused by damaged proteins generated by proteolytic enzymes [9]. Therefore, informing patients about the nature of the disease and the benefits of weight loss in symptom prevention is crucial in the treatment of knee osteoarthritis (KOA). While nonsteroidal anti-inflammatory drugs (NSAIDs) can be used for analgesic purposes, their usage is limited due to contraindications. Topical NSAIDs and intra-articular (i.a.) glucocorticoid injections may provide pain relief. However, there is no consensus on the effectiveness of complementary treatments such as chondroitin or glycosaminoglycan supplementation [1].

Boric acid (BA), also known as boracic acid, hydrogen borate, or orthoboric acid, is a derivative of a weak monobasic acid. It is a solid, colorless powder or crystal with a chemical formula of H₃BO₃. BA possesses chemical properties such as a density of 1.5172 g/cm³, a molecular mass of 61.83 g/mol, and a melting point of 170.9°C [10]. Salts of BA can be naturally found in fruits, plants, and seawater, and their applications are widespread. BA exhibits antiseptic and pesticidal effects and is utilized as a flame suppressor, neutron absorber, and precursor for other chemicals [11]. In the field of medicine, BA finds applications in the treatment of various cancer types, arthritis, coronary heart disease, and osteoporosis. The use of boron, a constituent of BA, has demonstrated positive effects in mitigating arsenic-induced changes in the gene expression rates of IFN-γ, TNF-α, IL-1β, NFκB, DNA damage, and proinflammatory cytokines [12]. Furthermore, BA is employed in the treatment of non-Candida albicans candidiasis and otitis externa and the prevention of athlete's foot. Human and experimental studies have indicated that BA and its salts exhibit bioactive properties, alleviate arthritic symptoms, and positively influence bone growth and central nervous system function. These therapeutic effects may be mediated by different forms of boron within biomolecules [13]. While there is a considerable body of research on the relationship between BA and inflammation, the specific association between BA levels and inflammation in knee osteoarthritis (KOA) has not been explored in the literature. Therefore, it is hypothesized that BA
treatment may help reduce inflammation in KOA patients. This study aims to investigate the impact of BA on inflammation in the treatment of KOA in rats and evaluate its therapeutic efficacy through biochemical and histopathological analyses.

Materials and Methods

Chemicals

ELISA kits for interleukin-1 beta (IL-1β), tumor necrosis factor-alpha (TNF-α), and matrix metalloproteinase-13 (MMP-13) were obtained from Elabscience® (USA). Primary antibodies against IL-1β, TNF-α, MMP-13, and nitric oxide synthase-2 (NOS-2) were purchased from Santa Cruz Biotechnology Inc. (USA). Paraffin with a noncaking solidification point of approximately 56–58°C and Entellan New Rapid Mounting Medium were obtained from Merck (Germany). Ketamine HCl, xylazine HCl, the IVIEV DAB detection kit, and antibody diluent were purchased from Prizer company (Turkey), Bayer company (Turkey), Vertana company (USA), and Abcam company (UK), respectively. All other reagents, including monosodium iodoacetate (MIA), methanol, xylene, ethanol, hematoxylin-eosin, and formaldehyde, were purchased from Sigma–Aldrich (USA).

Experimental Animals

The experimental animals were provided by the Atatürk University Laboratory Animal Facility. The study received ethical approval from the Local Ethical Committee for Animal Experiments (Atatürk University, Turkey) on 02/07/2020, with approval number 93722986-000-E.2000165282. Forty-two male Wistar (albino) rats, aged 12 to 16 weeks and weighing 250 to 300 g, were obtained from the Medical Experimental Application and Research Centre (Atatürk University, Turkey). The rats were housed in a temperature-controlled room maintained at 23 ± 2°C. A light and dark regime was followed, with lights on at 8:00 a.m. and lights off at 8:00 p.m.

Animal model of induced KOA

Prior to the experimental days, all the animals underwent an overnight fasting period while having unrestricted access to water. Monosodium iodoacetate (MIA) was dissolved in a 0.9% sodium chloride (NaCl) solution. To induce the experimental knee osteoarthritis (KOA) rat model, the MIA solution (1.5 mg/50 µL per animal) was injected intra-articularly (i.a.) into the right patella of the rats using a 30 G needle. The injection procedure was performed under a combination of ketamine and xylazine anesthesia [14].

Experimental groups and procedures

The rats were randomly assigned to six groups to initiate the treatment. Both oral and intra-articular routes of boron treatment were employed in this study to evaluate the effects of metabolization and local administration on KOA.

Group-1 (control; n = 7): Rats that received no treatment.
**Group-2** (disease control; KOA + 0.9% NaCl solution; n = 7): Rats with induced KOA in the right patellar ligament, receiving injections of 0.9% NaCl solution (100 µL per animal) on days 1, 7, 14, and 21.

**Group-3** (4 mg/kg/day BA orally + KOA; n = 7): Rats with induced KOA exposed to 4 mg/kg/day BA through oral gavage for four weeks (5 days per week) [15].

**Group-4** (10 mg/kg/day BA orally + KOA; n = 7): Rats with induced KOA exposed to 10 mg/kg/day BA through oral gavage for four weeks (5 days per week) [16].

**Group-5** (4 mg/kg BA i.a.+ KOA; n = 7): Rats with induced KOA exposed to 4 mg/kg BA through intra-articular injection (50 µL per animal) on days 1, 7, 14, and 21 [15].

**Group-6** (10 mg/kg BA i.a.+ KOA; n = 7): Rats with induced KOA exposed to 10 mg/kg BA through intra-articular injection (100 µL per animal) on days 1, 7, 14, and 21 [17].

At the end of the study, the rats were euthanized by decapitation under combined anesthesia of ketamine HCl and 2% xylazine HCl (90 mg/kg and 10 mg/kg, respectively) following the completion of all treatment protocols. Blood samples were collected from the abdominal aorta, and serum samples were obtained by centrifugation at 3000 rpm for approximately 15 min. The samples were then stored at -80°C until they were assayed using ELISA. The knee joints, articular capsule, and patella were removed and placed in tubes containing 10% formaldehyde solution to study the inflammatory effect. Histopathological examinations using H&E staining and immunohistochemistry with specific antibodies for MMP-13, TNF-α, IL-1β, and NOS-2 were performed at the Histological Lab of the Medical Faculty, Atatürk University, Turkey.

### Serum Level of IL-1β, TNF-α and MMP-13 by ELISA Method

Serum levels of IL-1β, TNF-α, and MMP-13 were measured using ELISA kits obtained from Elabscience® (USA) following the manufacturer's instructions. The results were expressed as concentration percentages in units of pg/mL and ng/mL.

### Histopathological Procedure

At the end of the study, knee joint tissues from each group of rats were excised and immersed in a 10% buffered formaldehyde solution for 48 h. Following fixation, the tissues were transferred to a 10% EDTA solution for decalcification over a period of four weeks. Subsequently, the tissues were embedded in paraffin blocks. This process involved dehydration through a series of graded alcohol solutions or xylene. The paraffin blocks were then sectioned into slices with a thickness of 4–5 µm using a microtome. To visualize the tissue structure, the sections were stained with hematoxylin-eosin and subjected to histological examination under a light microscope.

### Immunohistochemical Procedures

Immunohistochemical (IHC) staining was conducted to assess the expression levels of inflammation markers, including IL-1β, TNF-α, MMP13, and NOS-2, in the knee joints of the experimental groups. Slices
of paraffin blocks, measuring 5 µm in thickness, were used for the IHC staining procedure.

Primary antibodies against IL-1β (dilution 1/50), TNF-α (dilution 1/50), MMP13 (dilution 1/50), and NOS-2 (dilution 1/50) were appropriately diluted according to the manufacturer's recommendations (Santa Cruz Biotechnology). The Ventana Benchmark Ultra System (AZ, USA) was employed for IHC staining. Subsequently, the sections were examined and photographed using a Nikon Eclipse E600 light microscope (Japan).

To evaluate the intensity of immunohistochemical staining for IL-1β, TNF-α, MMP13, and NOS-2 proteins in the control and experimental groups, a semiquantitative method called the immunoreactivity scoring system (IRS) was utilized[18]. The scoring was conducted based on two factors: the percentage of positively stained cells and the intensity of the stain.

The scoring criteria were as follows: Percentage of positively stained cells:

- no positive cell = 0
- < %10 = 1
- %11–50 = 2
- %51–80 = 3
- >%81 = 4

Intensity of the stain;

- none = 0
- Slightly stain = 1
- moderately stain = 2
- heavily stain = 3

In this method, the IRS was determined by multiplying the score for the positive staining cell ratio (ranging from 0 to 4) by the staining intensity score (ranging from 0 to 3). The maximum value resulting from this multiplication is 12.

**Statistical Evaluations**

The reference study reported a large effect size of $d = 1.22$. Power analysis ($f = 0.9$) indicated that a minimum of 42 rats (with a minimum of 7 rats per group) would yield 80% power with 95% confidence. Consequently, a total of 42 rats were included in the study. The collected data were analyzed using IBM SPSS Statistics 21 software (IBM Corp, Armonk, NY, USA). The results are presented as the means ± standard deviations. Initially, the normality of the variables assessed in the results was examined using the Shapiro–Wilk test. When the assumptions for parametric tests were not met, the Kruskal–Wallis analysis of variance (followed by the Mann–Whitney U test with post hoc Bonferroni correction) was utilized to compare independent group differences. Statistical significance was defined as $P < 0.05$. 
Results

**Determination of Serum Levels of MMP-13, TNF-α, and IL-1β**

Statistically significant differences were observed in the serum IL-1β levels among the groups. Group-2 (1519.14 ± 199.36 pg/mL) showed a significant increase compared to group-1 (741.07 ± 79.14 pg/mL) \( (P = 0.046) \). In contrast, all treatment groups exhibited decreased IL-1β levels compared to group-2. The decrease was statistically significant in groups 4, 5, and 6 \( (P = 0.029, P = 0.001, \text{and } P = 0.01, \text{respectively}) \). Group-5 showed the most significant decrease \( (P = 0.001) \) (Fig. 1A).

Significant differences were also observed in the serum TNF-α levels. Group-2 (3127.51 ± 231.90 pg/mL) showed a significant increase compared to group-1 (1597.40 ± 286.72 pg/mL) \( (P = 0.001) \). Conversely, all treated groups exhibited decreased TNF-α levels compared to group-2. The decrease was statistically significant in groups 5 and 6 \( (P = 0.001) \) (Fig. 1B).

Furthermore, the serum MMP-13 levels showed statistically significant differences among the groups. Group-2 (17.55 ± 1.18 ng/mL) exhibited a significant increase compared to group-1 (12.18 ± 0.93 ng/mL) \( (P = 0.032) \). Although all treated groups showed decreased MMP-13 levels compared to group-2, the decrease was statistically significant in groups 4, 5, and 6 \( (P = 0.013, P = 0.001, \text{and } P = 0.005, \text{respectively}) \) (Fig. 1C).

**Histological Results**

In the KOA group, the disappearance of the joint space was observed, with some areas showing contact between the articular surfaces, whereas a clear joint space was present in the control group (Fig. 2A). Significant cartilage degeneration, wear, and deep cracks were evident on the articular surface. The cartilage also exhibited irregular thinning and tissue loss. Superficial fibrillation was observed due to the accumulation of collagen fibers in place of dead chondrocytes and loss of cartilage matrix. Nucleated phantom chondrocytes were observed in the deeper regions of the articular cartilage (Fig. 2B). In groups 3 and 4, a significant enlargement of the joint space was observed. However, there were no deep cracks or tissue loss in the cartilage. The formation of new cartilage resembling natural cartilage was minimal, and the articular cartilage remained thin (Figs. 2C and 2D). In groups 5 and 6, the joint space increased, and there was significant healing of the cartilage tissue. No degeneration was observed in the articular cartilage, which exhibited a smooth surface, and new chondrocytes were formed (Figs. 2E and 2F).

**Immunohistochemical Results**

In group-1 (control) sections, there were sporadic and weak immune-positive stains for IL-1β, TNF-α, MMP-13, and NOS-2 markers of inflammation. However, in the MIA-induced osteoarthritis and treatment groups, the expression of these proteins varied. Group-2 exhibited diffuse and intense staining for the four proteins. In the oral treatment groups (groups 3 and 4), both the intensity and extent of staining
decreased. In the groups receiving intra-articular BA (groups 5 and 6), immune-positivity was minimal (Fig. 3).

The IRS values of IL-1β were significantly higher in group-2 than in group-1 \( (P = 0.001) \). However, all treatment groups showed significantly lower IRS values for IL-1β than group-2 \( (P = 0.012, P = 0.01, P = 0.004, \text{and } P = 0.003) \), respectively) (Fig. 4A). Similarly, the IRS values of NOS-2 were significantly higher in group-2 than in group-1 \( (P = 0.001) \), but all treatment groups exhibited significantly lower IRS values than group-2 \( (P = 0.02, P = 0.01, P = 0.002, \text{and } P = 0.002) \). The IRS value of TNF-α was significantly higher in group-2 than in group-1 \( (P = 0.001) \). The oral treatment groups (group 3 and group 4) showed significantly lower IRS values for TNF-α than group 1 \( (P = 0.023 \text{ and } P = 0.02) \), but this decrease was more pronounced in group 5 and group 6 \( (P = 0.001 \text{ and } P = 0.001) \) (Fig. 4C). The IRS value of MMP-13 was significantly higher in group-2 than in group-1 \( (P = 0.001) \). The oral treatment groups (group 3 and group 4) exhibited significantly lower IRS values for MMP-13 than group 1 \( (P = 0.011 \text{ and } P = 0.011) \), but this decrease was more pronounced in group 5 and group 6 \( (P = 0.001 \text{ and } P = 0.001) \) (Fig. 4D).

**Discussion**

KOA is the most common joint disease and is characterized by the degeneration of cartilage in synovial membrane-covered joints. It manifests with symptoms such as joint pain, stiffness, limited range of motion, crepitation, local tenderness, and varying levels of inflammation. In addition to affecting joint cartilage, OA can also impact the subchondral bone, synovium, ligaments, capsule, and surrounding muscle tissue \([4, 19]\). OA primarily affects the knee joint and is a slow-progressing chronic disorder. While various risk factors have been associated with OA, the exact pathophysiological process and underlying mechanisms remain incompletely understood. Consequently, there is no singular mechanism to explain the development of OA. It is a metabolically dynamic and active condition characterized by both destructive and reparative processes influenced by a range of mechanical and biochemical factors \([20]\).

However, this delicate balance is disrupted in favor of tissue destruction \([21]\). Chondrocytes undergo a series of reactions, leading to abnormal changes in the extracellular matrix (ECM) and compromised articular cartilage homeostasis \([22]\). When subjected to stress, chondrocytes release proinflammatory cytokines such as IL-1β, IL-6, and TNF-α, which stimulate the synthesis of MMPs, inflammatory chemokines, and cytokines \([22–24]\).

TNF-alpha, along with IL-1β, is considered a crucial inflammatory cytokine involved in the pathophysiological processes of OA \([25]\). The same joint cells that express IL-1β also secrete TNF-α, leading to elevated levels observed in subchondral bone, synovial fluid, cartilage, and synovial membrane \([26]\). The imbalance between matrix formation and degradation in knee osteoarthritis (KOA) results in tissue damage. This catalytic process leads to morphological, biochemical, and molecular changes, including reduced matrix synthesis, increased proteinase activity, and chondrocyte apoptosis. The synthesis and release of cytokines such as TNF-α, IL-1β, and IL-6 by the synovium and chondrocytes
contribute to cartilage destruction [27]. Moreover, the synthesis of metalloproteinases, inflammatory chemokines, and reactive oxygen species (ROS) plays a significant role in cartilage degradation and inflammation in KOA [28]. The inflammatory process in KOA involves various interleukins, including TNFs, IL-1β, IL-6, IL-15, IL-17, and IL-18. These inflammatory agents exacerbate KOA by activating relevant pathways and promoting the release of inflammatory mediators [29–31]. In a previous study, we demonstrated increased levels of MMP-13, TNF-α, and IL-1β, both in the serum and knee joints of KOA rats [32]. Similarly, in the current study, we observed higher levels of these proinflammatory cytokines in both the serum and knee joints of experimental KOA rats.

Boron, a trace element, plays various roles in cellular functions, including cell membrane function, mineral and hormone metabolism, and enzyme activity. It has been demonstrated to have beneficial effects at dietary levels, such as promoting bone growth, supporting central nervous system function, alleviating arthritic symptoms, facilitating hormone action, and reducing the risk of certain cancers [33, 34]. Boron compounds have also exhibited anti-inflammatory and antioxidant properties, contributing to cancer prevention, disease control, wound healing, reduction of genotoxicity, and modulation of mitochondrial membrane activity [35, 36]. Moreover, boron species have shown importance in bone formation and mineralization. As an effective treatment option for arthritis, boron promotes calcium incorporation into bone, joints, and cartilage, leading to significant improvement in bone development observed in 95% of patients [37]. Furthermore, it influences various metabolic activities in bone by interacting with minerals essential for bone metabolism, such as magnesium, vitamin D, and calcium [37].

Inflammasome activation triggers the release of IL-1β at high levels from monocytes and macrophages during inflammatory processes. Inflammatory sites, infections, or cell activation can lead to an increase in extracellular calcium levels. A study conducted by Rossol et al. demonstrated that elevated extracellular calcium activates the NLRP3 inflammasome by stimulating calcium-sensing receptors coupled with G-proteins [38]. In the context of bone degradation and porous bones, boron plays a beneficial role in counteracting this deterioration by ensuring the effective performance of calcium and magnesium levels [39, 40]. This element is believed to enhance osteoblastic activity through the influx of calcium [39]. Calcium fructoborate, a form of boron, has been shown to regulate inflammation and enhance bone health and strength by reducing the level of C-reactive protein in the bloodstream [41]. Boron also acts as a stimulator for the synthesis and release of TNF-α. TNF-α, in turn, stimulates angiogenesis and the expression of genes that encode various mediators of inflammation [15].

BA is believed to exert a modulatory effect on cellular calcium, which contributes to alleviating or preventing pathological conditions triggered by inflammatory stress [42]. Other studies have indicated that BA can attenuate cellular damage induced by inflammation without significantly impairing protective defenses [43]. Furthermore, Türkez et al. demonstrated that the application of boron to glioblastoma cells reduced the secretion of inflammatory factors and levels of IL-1α and TNF-α [44]. Boron, recognized for its antimicrobial properties, regulates the production of extracellular matrix, which plays a crucial role in wound healing by enhancing the release of proteins, collagen, and proteoglycans [45]. Additionally, a study involving 20 patients with osteoarthritis showed that an appropriate dosage of boron administered
over an 8-week period improved bone disease [46]. Another investigation exploring the impact of boron on DNA damage induced by arsenic revealed that doses of 10–20 mg/kg boron suppressed proinflammatory cytokines (IFN-γ, IL-1β, TNF-α, and NFκB), attributing this effect to the antioxidant activity of boron through the inhibition of ROS [12].

Therefore, boron is known for its anti-inflammatory properties and its potential to ameliorate inflammation. Since inflammation plays a significant role in the pathogenesis of KOA, we hypothesized that BA could be beneficial in treating KOA. Consistent with our hypothesis, this study employed an experimental rat model of KOA in which KOA rats were treated with BA orally or via intra-articular administration. Following BA administration, the effects of BA on inflammation were assessed through histological and biochemical evaluation of inflammatory cytokines.

In this study, the efficacy of BA in reducing inflammation in KOA was evaluated by measuring the levels of IL-1β, TNF-α, and MMP13 in serum samples using ELISA. Immunohistochemical analysis was also performed to assess the expression of IL-1β, TNF-α, and MMP13 in rat knee joint tissue samples. The results showed that BA administration led to a decrease in IL-1β, TNF-α, and MMP13 levels compared to the KOA group, with the most significant decrease observed in the i.a. BA administration group (Fig. 1). Similarly, a reduction in the expression of these cytokines was observed with the use of BA compared to the KOA group, with the greatest decrease observed in the i.a. BA group (Fig. 4).

Furthermore, TNF-α and IL-1β induce the production of inducible nitric oxide synthase (iNOS), which contributes to bone loss [47]. Nitric oxide (NO) is involved in inflammation by upregulating TNF-α and MMPs [48]. The inflammatory process also involves the release of reactive oxygen species (ROS) from the inflamed area [49]. ROS-induced oxidative stress is a critical mechanism in the development and progression of OA [50, 51], and it plays a significant role in inflammation and cartilage degradation in OA [52]. IL-1 stimulates ROS production in chondrocytes, leading to DNA damage, as well as oxidation of proteins, lipids, and carbohydrates, thereby causing oxidative damage to cartilage cellular proteins [53, 54]. Additionally, ROS act as secondary messengers in cartilage degradation, inhibiting cell migration and matrix synthesis. Growth factors can contribute to the degradation of matrix components by activating MMPs and inducing cell death [21, 47]. Boron, by reducing intracellular levels of reactive oxygen species (ROS) and calcium ions (Ca2+), is believed to enhance antioxidant capacity [35]. Administration of boron has been shown to increase glutathione reserves and reduce oxidative stress [43]. In a study involving mice, boron administration was found to alleviate oxidative stress induced by organophosphates, resulting in improved antioxidant defense mechanisms and enzyme activity [55]. The study also revealed that boron enhanced the antioxidant defense mechanism and restored various organs in mice [55].

Immunohistochemical evaluation of NOS-2 expression using rat knee joint tissue samples in this study indicated higher levels of NOS-2 in knee joints of experimental KOA rats. However, the use of BA led to a decrease in NOS-2 expression compared to KOA, with the most significant decrease observed with intra-articular (i.a.) application of BA (Fig. 4).
Conclusion

This study, which focused on the effects of BA treatment on inflammation-related patellar tendon injury and KOA, demonstrated its efficacy. Biochemical and immunohistochemical analyses revealed that BA effectively mitigated inflammation, an important pathway for tissue repair in KOA. Moreover, histopathological examinations demonstrated that BA increased joint space and significantly improved cartilage tissue. These findings highlight the potential of BA as a promising therapeutic agent for the treatment of KOA based on its beneficial effects on inflammation and tissue repair.

Abbreviations

BA, $\text{H}_3\text{BO}_3$: Boric acid
CRP: C-reactive protein
IHC: Immunohistochemical
IL-1$\beta$: Interleukin-1$\beta$
IRS: Immuno reactive score
i.a.: Intraarticular
iNOS: Inducible NO synthase
KOA: Knee osteoarthritis
MMP-13: Matrix metalloproteinase-13
MIA: Monosodium iodoacetate
NOS-2: Nitric oxide synthase-2
OA: Osteoarthritis
po: Orally
ROS: Reactive oxygen species
TNF-α: Tumor necrosis factor-α

Declarations

Competing Interests
None.

**Ethics Approval**

This study has been approved by the Ethics Committee of the Faculty of Medicine (Atatürk University).

**Data Availability**

All data are presented in the manuscript.

**Authors Contributors**

The study was designed by GG, FDM, and KG. GG, KG, and SYT carried out the experimental studies. Laboratory studies were performed by GG, FDM, and SYT. Histopathology was performed by TD. Data analysis and interpretation of results were performed by GG, FDM, KG, and TD. The paper was drafted by GG, KG, AMA, SYT, and FDM. AMA edited the English. All authors approved the final version of the manuscript.

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None

**References**


**Figures**
Figure 1

A. Serum IL-1β concentrations in the experimental groups.

B. Serum TNF-α concentrations in the experimental groups.

C. Serum MMP-13 concentrations in the experimental groups.

(Results are given as the mean ± standard deviation, n=7; #: P <0.05, ##: P <0.001 statistically significant difference from group-1, *: P <0.05, **: P <0.01, ***: P <0.001, statistically significant difference from group-2; Group-1: control, Group-2: Diseased control (KOA + 0.9% of NaCl solution), Group-3: 4 mg/kg/day BA po + KOA, Group-4: 10 mg/kg/day BA po + KOA, Group-5: 4 mg/kg/day BA i.a. + KOA, Group-6: 10 mg/kg/day BA i.a. + KOA)
Figure 2

Histological images of the knee joints [a. Group 1 (control), b. Group 2 (Diseased control; KOA + 0.9% of NaCl solution), c. Group 3 (4 mg/kg/day BA po + KOA), d. Group 4 (10 mg/kg/day BA po + KOA), e. Group 5 (4 mg/kg/day BA i.a. + KOA), f. Group 6 (10 mg/kg/day BA i.a. + KOA), c; Normal chondrocytes, js; Joint space, F; Fibrillation, Arrow Head; Tissue loss in articular cartilage, Arrow; Deep cracks in articular cartilage, gc; Ghost chondrocytes, nfc; Newly formed chondrocytes, Dye; H-E, Bars; 100m.].
Figure 3

Light microscopic images showing immunohistochemically IL-1β, NOS-2, TNF-α and MMP13 expression of knee joints. Cells showing immunoreactivity and giving a positive reaction are seen as brown in immunohistochemical staining. (A. Group-1 (control), B. Diseased control; KOA + 0.9% of NaCl solution), C. Group-3 (4 mg/kg/day BA po + KOA), D. Group-4 (10 mg/kg/day BA po + KOA), E. Group-5 (4 mg/kg/day BA i.a. + KOA), F. Group-6 (10 mg/kg/day BA i.a. + KOA). Magnification; 50 m.
Figure 4

A. IRS score of IL-1β expression in the experimental groups.
B. IRS score of NOS-2 expression in the experimental groups
C. IRS score of TNF-α expression in the experimental groups
D. IRS score of MMP-13 expression in the experimental groups

(Results are given as the mean ± standard deviation, n=7; Results are given as the mean ± standard deviation, n=7; #: P <0.05, ##: P <0.001 statistically significant difference from group-1,*: P <0.05, **: P <0.01, ***: P <0.001, statistically significant difference from group-2; A. Group-1 (control), B. - (Diseased control; KOA + 0.9% of NaCl solution) , C. Group-3 (4 mg/kg/day BA po + KOA), D. Group-4 (10 mg/kg/day BA po + KOA), E. Group-5 (4 mg/kg/day BA i.a. + KOA), F. Group-6 (10 mg/kg/day BA i.a. + KOA). Magnification; 50 m.)