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

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Abstract

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. A 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 activity of 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, MMP-13, and nitric oxide synthase-2 (NOS-2). Group-2 exhibited higher serum IL-1β and TNF-α levels and MMP-13 activity than group-1 ($P < 0.05$). However, IL-1β and TNF-α levels and MMP-13 activity were lower in all treatment groups than in 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 ($P < 0.01$). BA shows potential efficacy in reducing inflammation in experimental KOA model in rats. It may be a promising therapeutic agent for KOA, warranting further clinical studies for validation.

Keywords Boric acid · Knee osteoarthritis · Anti-inflammatory effect · Rat model · Biochemical evaluation · Histopathological analysis

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.

Extended author information available on the last page of the article

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 joint load, and obesity. 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 intrapatellar fat pad [4].

Synovial inflammation in 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 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 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 H_3BO_3. BA possesses chemical properties such as a density of 1.5172 g/cm^3, 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]. Recently, boron has garnered significant attention due to its impactful role in nutrition and biochemistry. Boron compounds, notably BA, have been subject to research for their potential applications in the pharmaceutical field. Several studies have explored the antioxidant, hepatoprotective, antibacterial, and anti-inflammatory properties of boron compounds [12]. In the medical field, BA is utilized for treating various conditions, such as different types of cancer, arthritis, coronary heart disease, and osteoporosis. A noteworthy application includes its use as an antiseptic, particularly in managing various infections, including vaginal infections [13]. Furthermore, extensive research has focused on boron supplements, particularly in the context of osteoporosis treatment, a debilitating condition characterized by weakened and fragile bones [14]. Scientific investigations have also explored the anti-inflammatory potential of boron, suggesting its relevance in managing specific medical conditions associated with the inflammatory response [15]. His multifaceted research underscores the diverse roles of boron, linking its essential function as a nutrient with its promising potential as a therapeutic agent. 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 [16]. 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 [17]. In summary, it is well established that boron is an essential nutrient vital for numerous physiological processes within the human body. However, compounds derived from boron, such as BA and boron supplements, have garnered significant attention for their potential therapeutic applications. Whether boron is classified as a nutrient or a pharmaceutical agent depends on the specific context and purpose of its use within various medical and pharmaceutical domains. Ongoing research continues to unveil the multifaceted roles that boron compounds may assume in the realms of health and medicine. Despite a substantial body of research investigating the connection between BA and inflammation, the precise correlation between BA levels and inflammation in KOA remains unexplored 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 IL-1β, TNF-α, and 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 (Turkey), Bayer (Turkey), Vertana (USA), and Abcam (UK), respectively. All other reagents, including monosodium iodoacetate (MIA), methanol, xylene, ethanol, hematoxylin–eosin (H&E), 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 14 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. Additionally, the rats were provided with a standard diet (Purina 5001 Rodent laboratory chow, Purina,USA) and access to fresh water.

Animal Model of Induced KOA

Monosodium iodoacetate (MIA) was dissolved in 0.9% sodium chloride (NaCl) solution. To induce the experimental KOA rat model, the MIA solution (1.5 mg/50 µL per animal) was injected 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 [18].

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 received 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 were exposed to 4 mg/kg/day BA through oral gavage for four weeks (5 days per week) [19].
Group-4 (10 mg/kg/day BA orally + KOA; $n = 7$): Rats with induced KOA were exposed to 10 mg/kg/day BA through oral gavage for four weeks (5 days per week) [20].
Group 5 (4 mg/kg BA i.a. + KOA; $n = 7$): Rats with induced KOA were exposed to 4 mg/kg BA through i.a. injection (50 µL per animal) on days 1, 7, 14, and 21 [19].
Group-6 (10 mg/kg BA i.a. + KOA; $n = 7$): Rats with induced KOA were exposed to 10 mg/kg BA through i.a. injection (100 µL per animal) on days 1, 7, 14, and 21 [21].

At the end of the study, on the day prior to the experimental procedures, all the animals underwent an overnight fasting period with free access to water. Following this, the rats were humanely euthanized through decapitation under the influence of combined anesthesia involving ketamine HCl and 2% xylazine HCl, administered at doses of 90 mg/kg and 10 mg/kg, respectively. 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 Levels of IL-1β, TNF-α and MMP-13 by ELISA

Serum levels of IL-1β, TNF-α, and MMP-13 activity 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. A process involving a series of graded alcohol solutions and xylene was applied to the tissues. Subsequently, the tissues were embedded in paraffin blocks. 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

Immunohistochemistry (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), MMP-13 (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, MMP-13, and NOS-2 proteins in the control and experimental groups, a semiquantitative method called the immunoreactivity scoring system (IRS) was utilized [22]. 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 $= 1$
- moderate staining $= 2$
- 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 Analyses

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 $\pm$ 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 ($P = 0.029$, $P = 0.001$, and $P = 0.01$, 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 activities 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 activities compared to group-2, the decrease was statistically significant in groups-4, 5, and 6 ($P = 0.013$, $P = 0.001$, and $P = 0.005$, respectively) (Fig. 1C).

Histological Results

In the group-2, 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 group-1 (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
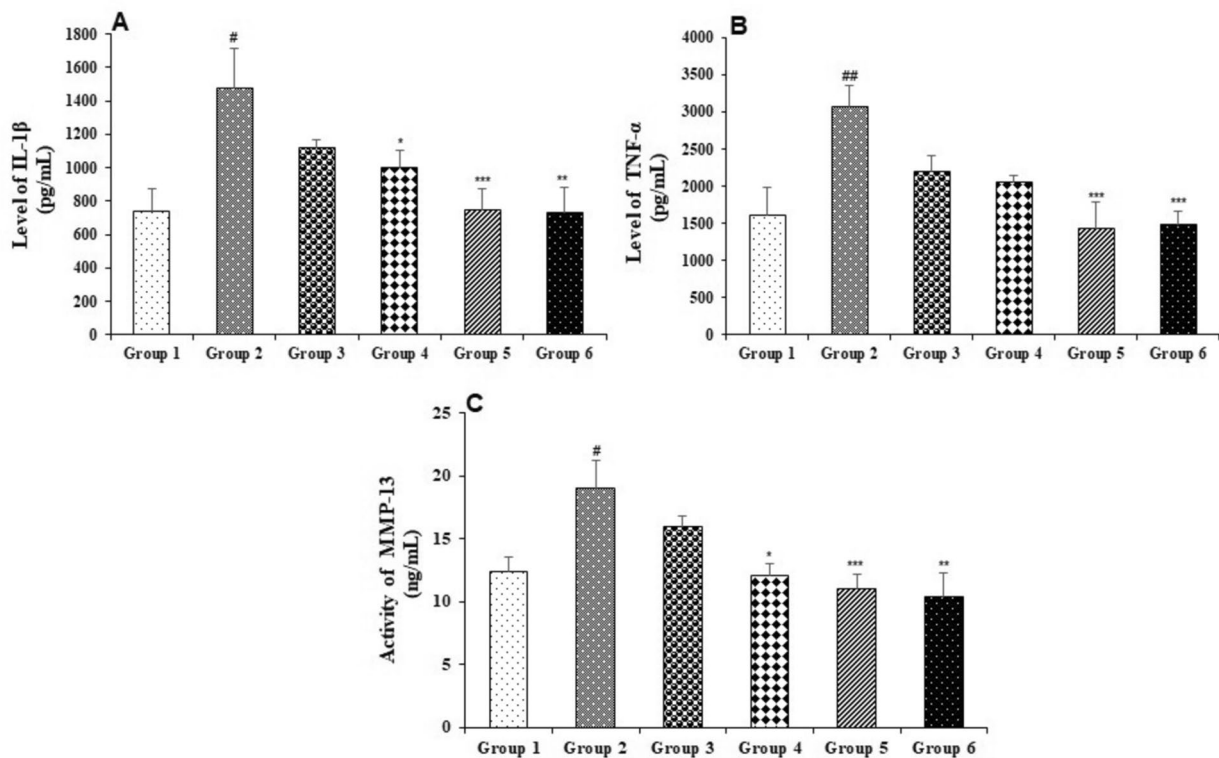
Fig. 1 A. Serum IL-1β levels in the experimental groups. B. Serum TNF-α levels in the experimental groups. C. Serum MMP-13 activities in the experimental groups. (Results are given as the mean ± standard deviation, $n = 7$; #: $P < 0.05$, ##: $P < 0.001$ statistically significant difference from group-1,*: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$, 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)

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 (Fig. 2C and D). 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 (Fig. 2E and F).

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 i.a BA (groups-5 and 6), immunopositivity 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 ($P = 0.012$, $P = 0.01$, $P = 0.004$, 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 ($P = 0.02$, $P = 0.01$, $P = 0.002$, and $P = 0.002$, respectively) (Fig. 4B). 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 ($P = 0.023$ and $P = 0.02$, respectively), but this decrease was more pronounced in group-5 and group-6 ($P = 0.001$ and $P = 0.001$, respectively) (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 ($P = 0.011$ and $P = 0.011$, respectively), but this decrease was more pronounced in group-5 and group-6 ($P = 0.001$ and $P = 0.001$, respectively) (Fig. 4D).
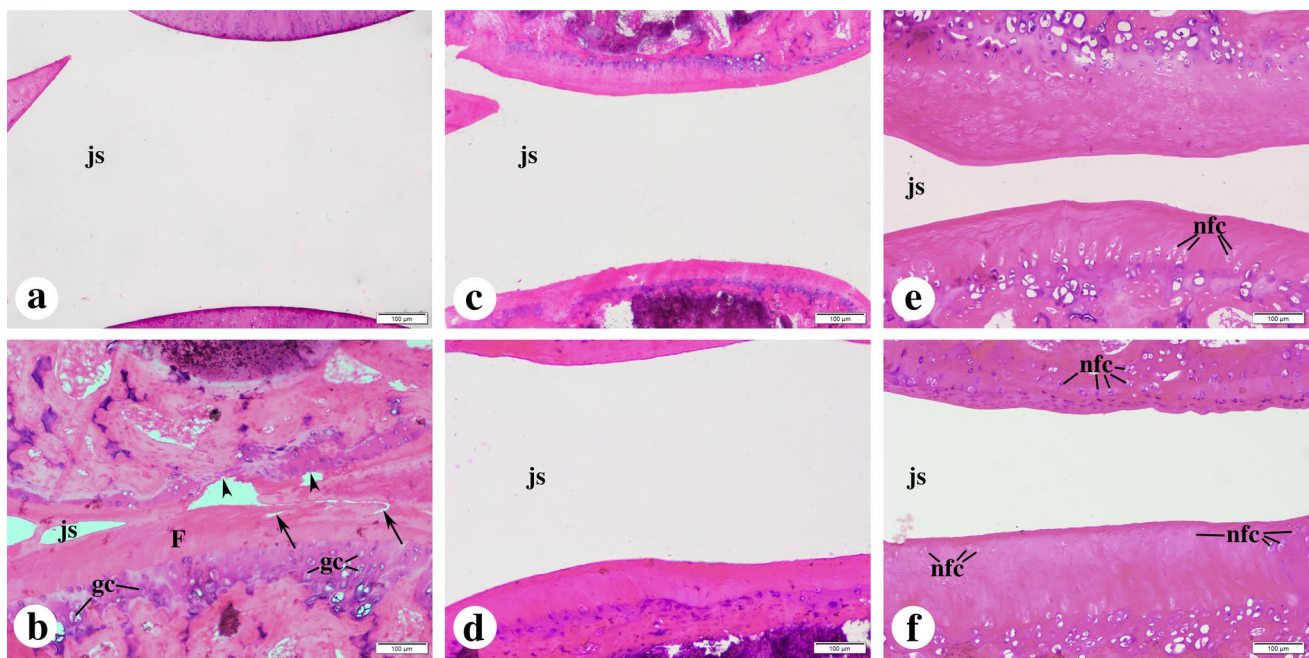
Fig. 2 Histological images of the knee joints [**a**. Group-1 (control), **b**. Group-2 (Diseased control; KOA + 0.9% of NaCl solution), **c**. Group-3 (4 mg/kg/day BA po + KOA), **d**. Group-4 (10 mg/kg/day BA po + KOA), **e**. Group-5 (4 mg/kg/day BA i.a. + KOA), **f**. Group-6 (10 mg/kg/day BA i.a. + KOA), **c**; Normal chondrocytes, **js;** Joint space, **F**; Fibrillation, **Arrow Head**; Tissue loss in articular cartilage, **Arrow**; Deep cracks in articlar cartilage, **gc**; Ghost chondrocytes, **nfc**; Newly formed chondrocytes, **Stain**; H&E, **Bars**; 100 μ.]

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, 23]. 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 [24].

However, this delicate balance is disrupted in favor of tissue destruction [25]. Joint chondrocytes undergo a series of reactions, leading to abnormal changes in the extracellular matrix (ECM) and compromised articular cartilage homeostasis[26]. 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 [26–28].

TNF-α, along with IL-1β, is considered a crucial inflammatory cytokine involved in the pathophysiological processes of OA [29]. 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 [30]. The imbalance between matrix formation and degradation in 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 [31]. Moreover, the synthesis of metalloproteinases, inflammatory chemokines, and ROS plays a significant role in cartilage degradation and inflammation in KOA [32]. 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 [33–35]. In a previous study, we demonstrated increased levels of MMP-13, TNF-α, and IL-1β in both the serum and knee joints of KOA rats [36]. Similarly, in the current study, we observed higher levels of these proinflammatory cytokines in both the serum and knee joints of experimental KOA rats.
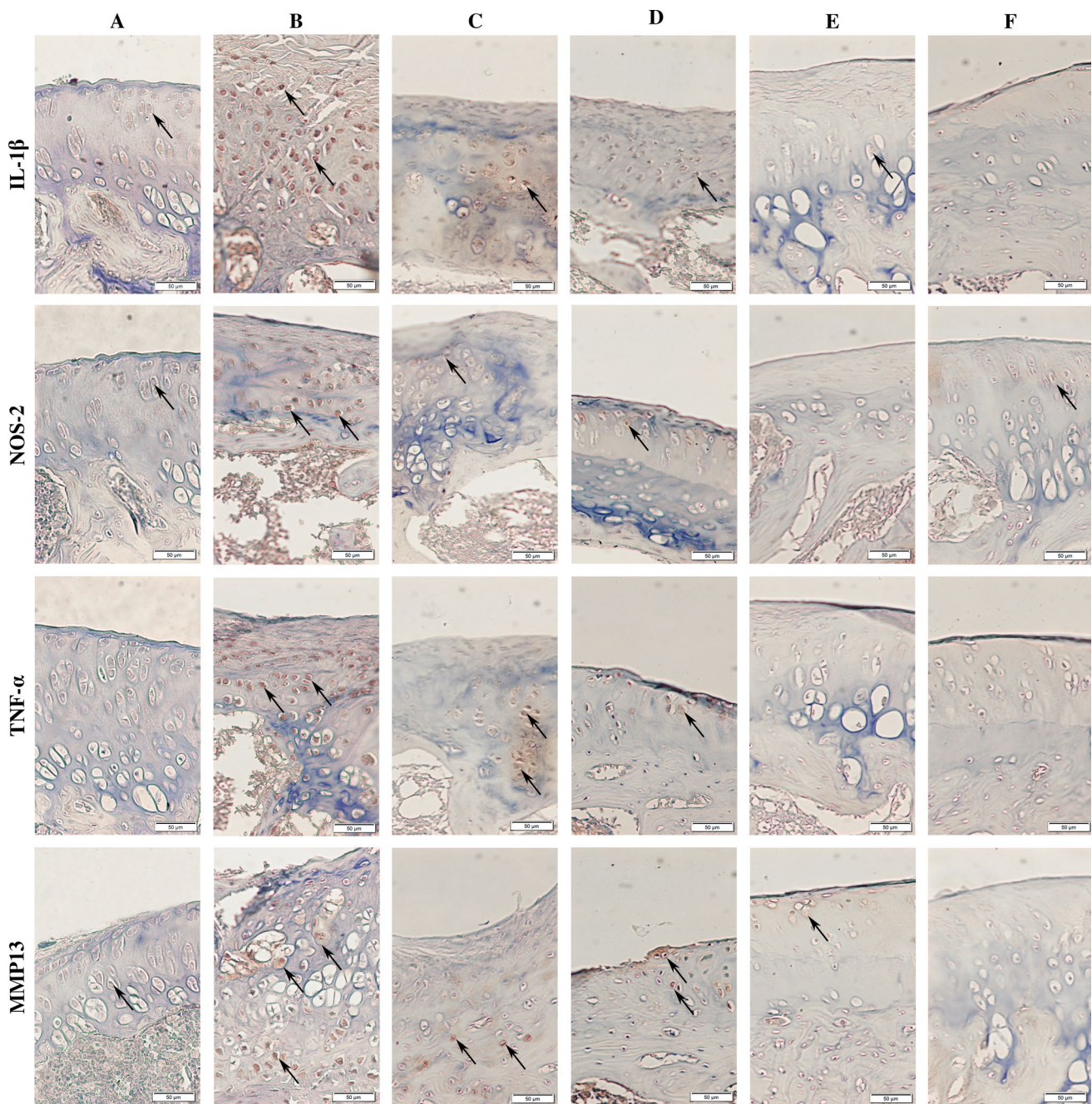
Fig. 3 Light microscopy images showing immunohistochemistry IL-1β, NOS-2, TNF-α and MMP13 expression in knee joints. Cells showing immunoreactivity and giving a positive reaction are seen as brown in immunohistochemistry staining. (**A.** Group-1 (control), **B.** Group-2 (diseased control; KOA+0.9% 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). **Bars**; 50 μ

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 [37, 38]. 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 [39, 40]. Moreover, boron species have shown importance in bone formation and mineralization. As an effective treatment option for arthritis, boron promotes
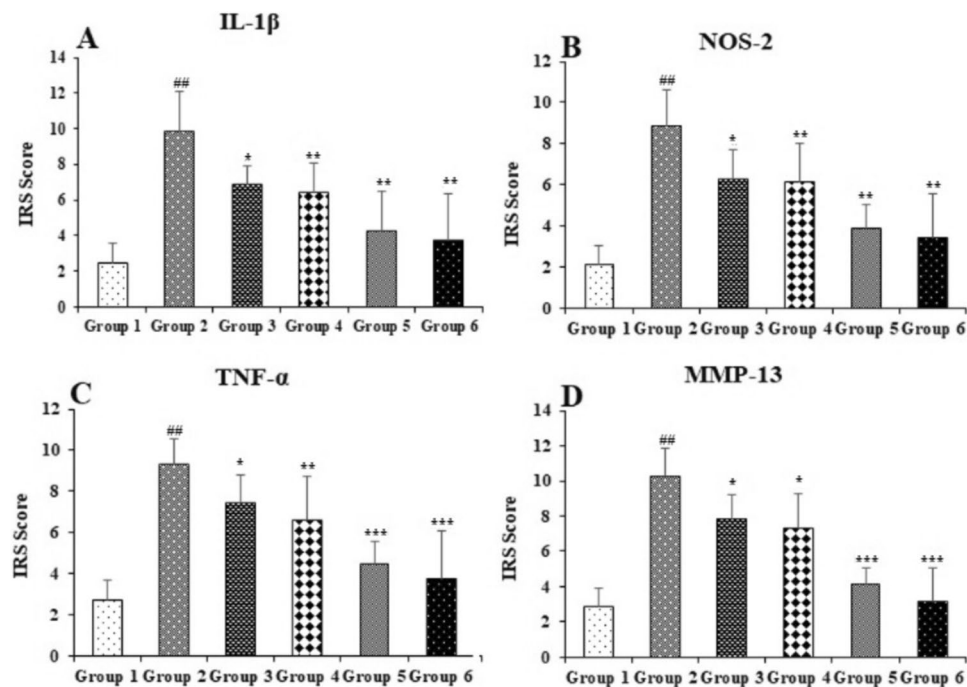
Fig. 4 **A**. IRS score of IL-1β expression in the experimental groups. **B**. IRS score of NOS-2 expression in the experimental groups. **C**. IRS score of TNF-α expression in the experimental groups. **D**. IRS score of MMP-13 expression in the experimental groups. (Results are given as the mean ± standard deviation, $n = 7$; #: $P < 0.05$, ##: $P < 0.001$ 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.** 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)

calcium incorporation into bone, joints, and cartilage, leading to significant improvement in bone development observed in 95% of patients [41]. Furthermore, it influences various metabolic activities in bone by interacting with minerals essential for bone metabolism, such as magnesium, vitamin D, and calcium [41].

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 [42]. 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 [43, 44]. This element is believed to enhance osteoblastic activity through the influx of calcium [43]. 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 [45]. 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 [19].

BA is believed to exert a modulatory effect on cellular calcium, which contributes to alleviating or preventing pathological conditions triggered by inflammatory stress [46]. Other studies have indicated that BA can attenuate cellular damage induced by inflammation without significantly impairing protective defenses [47]. Furthermore, Türkez et al. demonstrated that the application of boron to glioblastoma cells reduced the secretion of inflammatory factors and the levels of IL-1α and TNF-α [48]. Boron, recognized for its antimicrobial properties, regulates the production of ECM, which plays a crucial role in wound healing by enhancing the release of proteins, collagen, and proteoglycans [49]. Additionally, a study involving 20 patients with osteoarthritis showed that an appropriate dosage of boron administered over an 8-week period improved bone disease [50]. 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 [16].

Previous studies have established a link between gut microbiota and OA. Boer et al. observed an association between gut microbiota and OA-related knee pain, particularly noting that a higher relative abundance of *Streptococcus* spp. correlated with increased knee pain, potentially due to localized joint

inflammation [51]. Tsai et al. demonstrated that maintaining the integrity of the intestinal barrier could delay OA progression [52]. Huang et al. reported a connection between serum and synovial lipopolysaccharide (LPS) levels, originating from gut microbiota, and the severity of KOA and associated inflammation [53]. It is worth noting that LPS can activate innate immune cells such as macrophages and neutrophils, prompting the synthesis of pro-inflammatory factors such as IL-1β, TNF-α, MMPs, and free radicals, resulting in significant secondary inflammation in joint tissues. Furthermore, researchers have identified a specific pathway through which LPS contributes to OA progression [54]

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 i.a. 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 MMP-13 activity 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 levels, and MMP-13 activity 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 [55]. Nitric oxide (NO) is involved in inflammation by upregulating TNF-α and MMPs [56]. The inflammatory process also involves the release of ROS from the inflamed area [57]. ROS-induced oxidative stress is a critical mechanism in the development and progression of OA [58, 59], and it plays a significant role in inflammation and cartilage degradation in OA [60]. IL-1β 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 [61, 62]. 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 [25, 55]. Boron, by reducing intracellular levels of ROS and calcium ions (Ca^{2+}), is believed to enhance antioxidant capacity [39]. Administration of boron has been shown to increase glutathione reserves and

reduce oxidative stress [47]. 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 [63] The study also revealed that boron enhanced the antioxidant defense mechanism and restored various organs in mice [63]

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 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. Furthermore, histopathological assessments revealed that BA widened the joint space and notably enhanced cartilage tissue. Considering the link between gut microbiota levels and the severity of KOA and inflammation, it is conceivable that BA, with its anti-inflammatory properties, functions as a microbiome inhibitor. 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, H_3BO_3: Boric acid; CRP: C-reactive protein; IHC: Immunohistochemistry; IL-1β: Interleukin-1β; IRS: Immunoreactive 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

Ethics Approval This study was approved by the Ethics Committee of the Faculty of Medicine (Atatürk University).

Competing Interests None.

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