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Histopathological and Immunohistochemical Investigation on Effects of Boric Acid Used in Treatment of Rats with Knee Osteoartritis on Kidney Tissues

Kübra Asena TERİM KAPAKİN[1] İsmail BOLAT[1,*] Esra MANAVOĞLU KİRMAN[1] Gülşah GÜNDOĞDU[2] Köksal GÜNDOĞDU[3] Fatma DEMİRKAYA MİLOĞLU[4] Şeymanur YILMAZ TAŞÇI[4]

[1] *Atatürk University, Faculty of Veterinary Medicine, Department of Pathology, Erzurum, Türkiye*
[2] *Pamukkale University, Faculty of Medicine, Department of Physiology, Denizli, Türkiye*
[3] *Denizli State Hospital Orthopedics and Traumatology, Denizli, Türkiye*
[4] *Atatürk University, Faculty of Pharmacy, Department of Analytical Chemistry, Erzurum, Türkiye*

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ABSTRACT Osteoarthritis (OA) is a disease that often occurs in the knee joints and is characterized by disruption of cartilage homeostasis. Due to the systemic inflammation it creates, it affects not only the joint area, but also many tissues and organs. In this study, the damage caused by systemic inflammatory reactions due to OA in kidney tissue and the protective effect of boric acid were investigated. Wistar albino rats were used in the study. An experimental knee OA model was created by intraarticular injection of monosodium iodoacetate (MIA) in rats. It was formed from 4 groups as Control, OA, OA + 4 mg Boric Acid, OA + 10 mg Boric Acid to work. At the end of the study, kidney tissues were taken from the rats and TNF-α, IL-1β, NOS2 and MMP13 analyzes were performed by histopathological and immunohistochemical methods. Histopathological examinations revealed severe degenerative and necrotic changes in tubular epithelial cells in the OA groups, and these changes decreased in the boric acid-administered group depending on the dose. In immunohistochemical analyzes, it was determined that systemic inflammatory reactions occurring in OA application decreased in a dose-dependent manner with boric acid application. In conclusion; It was determined that kidney tissues were damaged due to systemic inflammatory reactions in rats with OA and boric acid had a protective effect against this damage.

Keywords: *Boric acid, Histopathology, Kidney, Immunohistochemistry, Osteoarthritis.*

ÖZ

Diz Osteoartritli Sıçanların Tedavisinde Kullanılan Borik Asitin Böbrek Dokuları Üzerindeki Etkilerinin Histopatolojik ve İmmünohistokimyasal Olarak Araştırılması

Osteoartrit (OA) sıklıkla diz eklemlerinde ortaya çıkan ve kıkırdak homeostazının bozulması ile karakterize bir hastalıktır. Yarattığı sistemik enflamasyon nedeniyle sadece eklem bölgesini değil, birçok doku ve organı da etkilemektedir. Bu çalışmada, OA'ya bağlı sistemik enflamatuar reaksiyonların böbrek dokusunda oluşturduğu hasar ve borik asidin koruyucu etkisi araştırılmıştır. Çalışmada Wistar albino sıçanlar kullanıldı. Sıçanlarda monosodyum iyodoasetatın (MIA) intraartiküler enjeksiyonu ile deneysel diz OA modeli oluşturuldu. Çalışma; Kontrol, OA, OA + 4 mg Borik Asit, OA + 10 mg Borik Asit olmak üzere 4 gruptan oluşturuldu. Çalışma sonunda sıçanlardan böbrek dokuları alınarak histopatolojik ve immünohistokimyasal yöntemlerle TNF-α, IL-1β, NOS2 ve MMP13 analizleri yapıldı. Histopatolojik incelemelerde OA gruplarında tübüler epitel hücrelerinde şiddetli dejeneratif ve nekrotik değişiklikler görülmüş, bu değişiklikler borik asit uygulanan grupta doza bağlı olarak azalmıştır. İmmünohistokimyasal analizlerde ise OA uygulamasında ortaya çıkan sistemik enflamatuar reaksiyonların borik asit uygulaması ile doza bağımlı bir şekilde azaldığı tespit edildi. Sonuç olarak; OA'li sıçanlarda sistemik enflamatuar reaksiyonlar nedeniyle böbrek dokularının hasar gördüğü ve borik asidin bu hasara karşı koruyucu etkisi olduğu tespit edildi.

Anahtar Kelimeler: *Borik asit, Böbrek, Histopatoloji, İmmünohistokimya, Osteoartrit.*

✉ *Corresponding author: ismail.bolat@atauni.edu.tr

INTRODUCTION

Osteoarthritis (OA) is a joint disease that occurs with the disruption of the balance between tissue destruction and regeneration, resulting in degeneration of cartilage homeostasis. It is known that this type of degeneration around the connective tissue is caused by many various factors, including mechanical stress and biochemical changes (Allen et al. 2022). The articular cartilage covers the ends of the femur and tibia, acting as a cushion and absorbing the physical impact of the joint area. When exposed to mechanical and chemical stress, it wears out and causes severely impaired functional change (Ni et al. 2022).

The pathophysiology of OA includes local inflammation induced mainly by proinflammatory cytokines such as interleukin 1β (IL1-β) and tumor necrosis factor α (TNF-α), and expression of inducible nitric oxide synthase (iNOS) and up regulation of matrix metalloproteinases (MMPs) leads (Goldring and Goldring 2004). For this reason, it is known that degenerative changes develop in many tissues and organs in the body in OA (Selig et al. 2022).

Clinical manifestations of osteoarthritis limit daily activities. Later, it may lead to other disorders such as sleep disturbance, depression, disability and deterioration in quality of life (Allen et al. 2022). Regeneration is not possible in cartilage tissue and this is almost non-existent in the elderly. OA treatment focuses on partial relief of symptoms and pain caused by inflammation, without a definitive solution. The most commonly used drug groups in OA treatment arenon-steroid anti-inflammatory drug (NSAIDs). Analgesic and anti-inflammatory drugs alleviate the clinical symptoms of OA. However, it should not be forgotten that NSAIDs also have different side effects (Selig et al. 2022).

In a worldwide study; While the number was 247.51 million in 1990, it increased to 527.81 million in 2019, an increase of 113.25% (Long et al. 2022).As the estimates of the WHO, 25% of people over the age of 65 experience pain and loss of function due to osteoarthritis. Every age group is affected by this disease, but this number increases at the age of 50 for men and over 40 for women (Bodur et al. 2011). According to the data in Türkiye, it has been reported that the rate of OA patients over the age of 50 is 14.8%, 22.5% in women and 8% in men (Bilge et al. 2018).

Monosodium iodoacetate (MIA) is known to experimentally induce OA symptoms in animals (Fonsi et al. 2019). It contributes to the formation of OA by disrupting energy and cellular metabolism in joints and chondrocytes (Chien et al. 2016). With MIA, inflammatory reactions increase in cartilage tissue and initiate pain by causing significant damage to nerve cells (Kuyinu et al. 2016).

Boron (B) is not found in its basic form in nature and is bound to oxygen, where 98.4% is boric acid and 1.6% is borate (Yildirim et al. 2022). Boric acid (H_3BO_3) dissolved form of boron and is the most common form of this form. Boron is mostly found in tissues and fluids as boric acid. Boric acid is excreted from the kidneys very quickly after being taken into the body. It is kept in the feces with brain, bone, kidney, testes and liver tissue, muscle, prostate, adrenals and body fluids such as plasma, semen, milk, saliva until it is excreted (Dilmani et al. 2023).

It is known that boric acid has a key feature in the body, especially in hormonal balance and mineralisation reactions (Estevez-Fregoso et al. 2023).

In addition, it has been shown that it shows serious protective activity in DNA in cells thanks to its antioxidant activity, especially in oxidative stress cases (Kar et al. 2020).

It has also been shown to have effective anti-inflammatory, anticoagulant, hypolipidemic, antiosteoporotic, and antineoplastic effects in animals both in vitro and in vivo (Haveric et al. 2020). When we look at the field of health, boric acid is used as a boron compound in brain tumors, wound and burn treatments, ointments, mouthwashes, eye drops and lens solutions as an antiseptic (Kuru and Yarat 2017).

Boron is involved in the metabolism of vitamin D and calcium. It has been reported that it is effective in magnesium and calcium metabolism, strengthens the bone framework and reduces pain in arthritis (Söğüt and Acar 2020). Studies have shown that boron has a positive effect on progesterone, estrogen, testosterone, thyroid hormone, steroid and insulin levels (Desordi et al. 2017). Among the tasks of boron at the cellular level; to assist cell membrane functions, to support metabolic activities and the wall structure in organisms with cell walls (Güneş et al. 2017).

Boron increases the level of glutathione in cells and subsequently prevents both oxidative stress and oxidative stress-induced oxidative damage. Boric acid is known to induce inflammatory response by triggering the expression of TNF-α as well as angiogenesis (Perez-Rodriguez et al. 2017).

OA is a disease that has recently become common among people and causes lesions in many different organs, including joints, with the symptoms it causes. Although there are different treatment modalities for the disease, a direct solution is still not available. The aim of this study was to investigate the damage caused by systemic inflammatory reactions in the kidney tissue due to OA and the protective effect of boric acid against this damage.

MATERIAL AND METHODS

Ethics committee approval of this study was obtained from Atatürk University Animal Experiments Local Ethics Committee (Ethical Report: 2023/04).

Wistar albino male rats, weighing 250-300 g, 12-16 weeks old, taken from Atatürk University Experimental Animals Laboratory, were fasted the night before for the experiment, but were given access to water. By applying ketamine (30mg/kg) and 2% xylazine (6mg/kg) intraperitoneally to the rats, 3mg monosodium iodoacetate (MIA) was dissolved in 0.9% NaCl under general anesthesia and injected. Of the rats intra articularly of 50µL at once and knee OA model was created with the application (Gundogdu et al. 2024). 28 rats were divided into 4 groups. The power analysis program was used to determine the number of animals in the group (Figure 1, G-Power 3.1.9.7. program). It was determined that at least 7 rats in each group and at least 28 rats in total were needed to obtain 95% working power (Type II error, β) with 0.05 error (Type I, α). Data from a previous study were used for this analysis (Bolat et al. 2023).

- *Group 1 (Control):* Rats were not treated.
- *Group 2 (OA):* Only knee OA model was created in rats.
- *Group 3 (OA + 4 mg Boric Acid):* Rats were administered oral 4 mg/kg boric acid 4 boric acid orally for 3 weeks after OA formation (Bolat et al. 2023).

- *Group 4 (OA + 10 mg Boric Acid):* Rats were administered oral 10 mg/kg boric acid 4 boric acid orally for 3 weeks after OA formation (Bolat et al. 2023).

After the administrations, the weights of all rats were measured and the rats were given a combination of 30mg/kg ketamine hydrochloride (Ketalar, Parke-Davis, İstanbul, Türkiye) and 6 mg/kg 2% xylazine hydrochlorideintraperitoneally it was sacrificed by decapitation under general anesthesia and was take kidney.

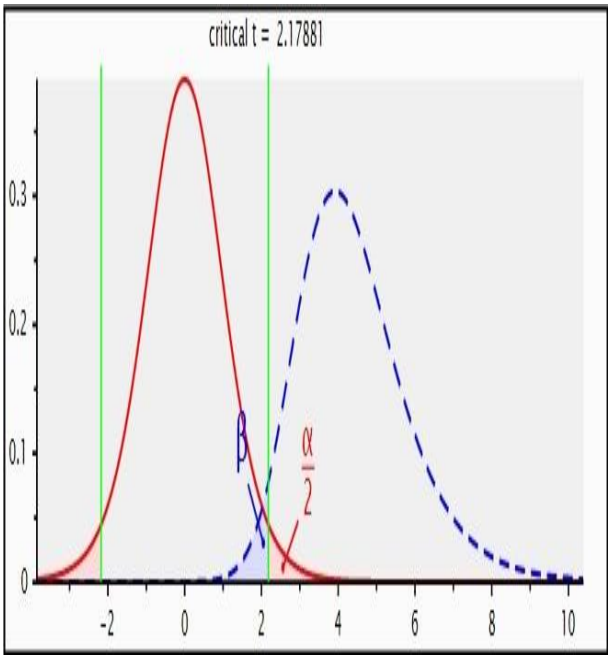

Figure 1: Power analysis graph obtained as a result of the power analysis applied to obtain a statistically significant difference of 14 scores between the groups in Knee Osteoarthritis scoring (Standard deviation±0.5).

Histopathological Examination

Tissues containing 10% buffered formaldehyde and kept for 48-72 hours. After routine tissue follow-up, the tissues were blocked with paraffin and 4 µm thick sections were taken from each block with a microtome device (Leica RM 2255). Afterwards, hematoxylin-eosin staining was performed and histopathological evaluations were made under the light microscope. Necessary areas were imaged with light microscope (OLYMPUS). Kidney tissues were examined histopathologically for mononuclear cell infiltration, hyperemia, and degeneration and necrosis in epithelial cells. Histopathological lesions were evaluated as no (-), mild (+), moderate (++) and severe (+++) (Terim et al. 2013).

Immunohistochemical Staining Method

Primary antibodies were used, (tumor necrosis factor α (TNF-α) Cat No: sc-52746 Dilution ratio: 1/100 US, interleukin 1β (IL1-β) Cat No:sc-52012 Dilution ratio: 1/100 US, Nnitric oxide synthase 2 (NOS2) Cat No: sc-7271 Dilution ratio: 1/100 US, matrix metalloproteinases 13 (MMP) Cat No: sc-101564 Dilution ratio: 1/100 US). Following the IHC kit protocol (ab64264), 3-3' Diaminobenzidine (DAB) chromegene was dropped onto the tissues. Necessary areas were imaged with light microscope (OLYMPUS). According to the immunopositivity status, it was scored as absent (-), mild (+), moderate (++), intense (+++) positivity (Kapakin et al. 2012).

Statistical Analysis

GraphPad Prism 8.0.2 program was used for statistical analysis in histopathological and immunohistochemical examinations and the data were evaluated with $p<0.05$ considered significant. Kolmogorov Smirnov test was applied to determine the normality of distributions in groups.

G*Power analysis was used to determine the number of animals in the group.The non-parametric Kruskal-Wallis test was used to detect group interaction, and the Mann Whitney U test was used to determine differences between groups (Bolat et al. 2023).

RESULTS

Histopathological Findings

*Group 1:*The kidneys were normal (Figure 2A).

Group 2: Severe degeneration and necrosis of renal tubular epithelial cells, severe mononuclear cell infiltration in the intertubular region and severe hyperemia in the veins were detected (Figure 2).

Group 3: Severe degeneration and mild necrosis in renal tubule epithelial cells, moderate mononuclear cell infiltration in the intertubular region and severe hyperemia in the veins were detected (Figure 2).

Group 4: Mild degeneration of renal tubular epithelial cells, mononuclear cell infiltration in the intertubular region and moderate hyperemia in the veins were detected (Figure2). A statistically significant difference ($p<0.05$) was found when compared with group 2. Histopathological findings were showed at Figure 3 and Table 1.

Table 1: Histopathological findings and scoring in kidney tissues (n=7).

	Group 1	Group 2	Group 3	Group 4
Degeneration of epithelium	-	+++	+++	+
Necrosis in epithelium	-	+++	+	-
Mononuclear cell infiltration	-	+++	++	-
Hyperemia in the veins	-	+++	+++	++

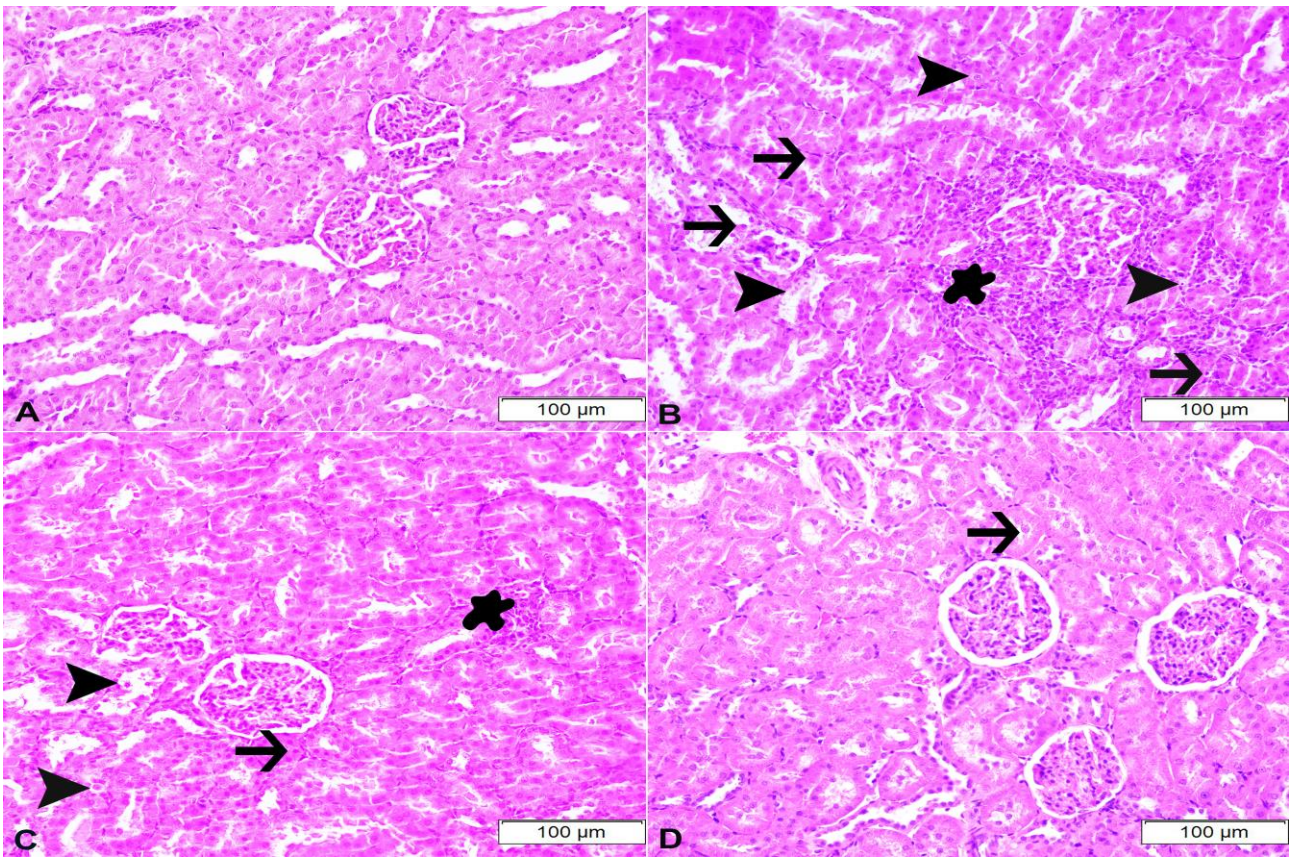

Figure 2: Kidney tissue, Group 1 (A), Group 2 (B), Group 3 (C), and Group 4 (D), renal tubule epithelial degeneration (arrows), necrosis (arrowheads) and inflammation (asteric), hematoxylin-eosin (H&E), Bar: 100µm.

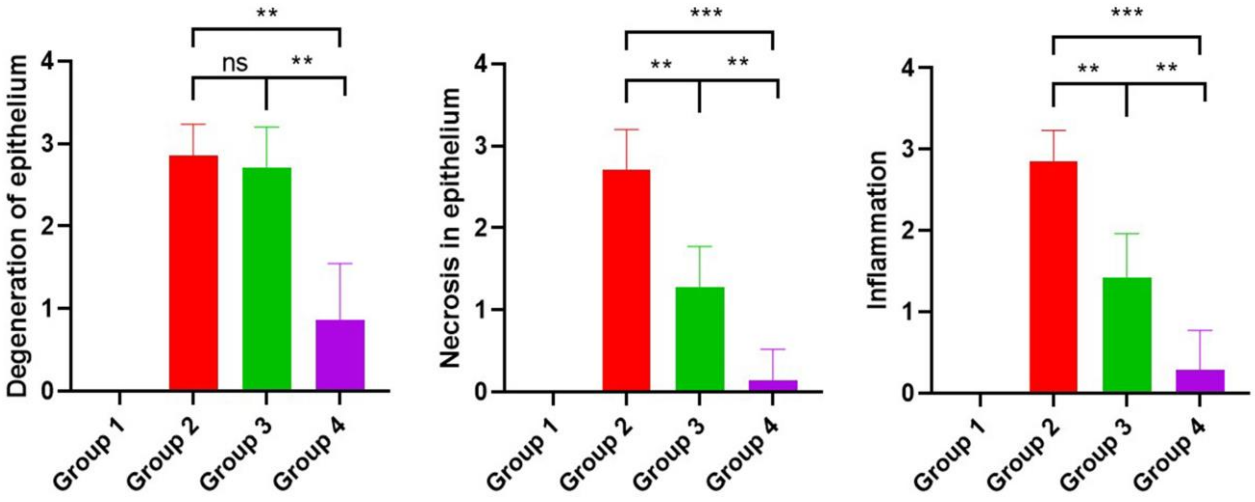

Figure 3: Scoring of histopathological findings in kidney tissue and statistical analysis findings. Degeneration in tubule epithelial cells (Group 2 vs 3 ns p=0.1865, Group 2 vs 4 ** p=0.0012, Group 3 vs 4 ** p=0.0017); Necrosis in tubule epithelial cells (Group 2 vs 3 ** p=0.0035, Group 2 vs 4 *** p=0.0006, Group 3 vs 4 ** p=0.0035); mononuclear cell infiltration (Group 2 vs 3 ** p=0.0023, Group 2 vs 4 *** p=0.0006, Group 3 vs 4 ** p=0.0087). (n=7). (ns: no significance).

Immunohistochemical Findings

Group 1: No TNF-α, IL1-β, MMP13 and NOS2 expression was observed in the kidneys (Figure 4).

Group 2: Severe TNF-α and IL1-β expressions were detected in glomeruli and inflammatory cells, and severe intracytoplasmic MMP13 and NOS2 expressions were seen in renal tubular epithelial cells (Figure 4).

Group 3: Mild TNF-α and moderate IL1-β expressions were detected in glomeruli and inflammatory cells, and moderate MMP13 and NOS2 expressions were identifed in renal tubular epithelial cells (Figure 4).

Group 4: Negative TNF-α and mild IL1-β expressions were detected in glomeruli and inflammatory cells, and mild MMP13 and NOS2 expressions were observed in renal tubular epithelial cells (Figure 4). A statistically significant difference ($p<0.05$) was found when immunohistochemical parameters were compared with Group 2. Immunohistochemical findings are presented in Figure 5 and Table 2.

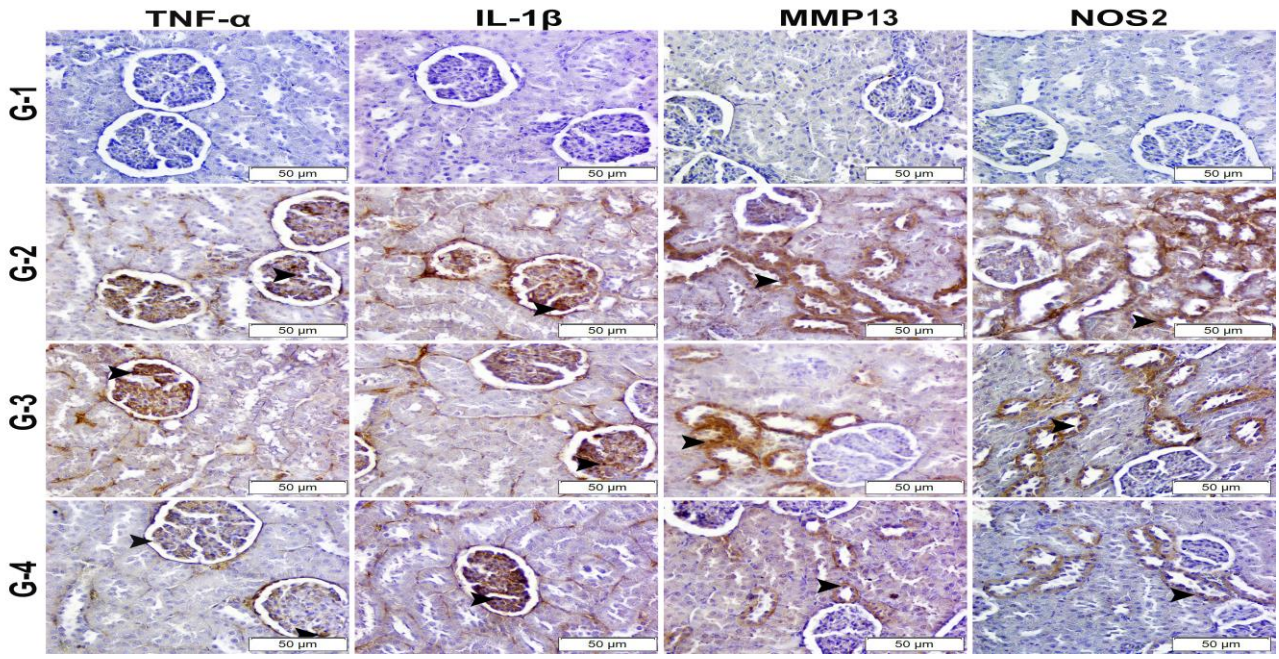

Figure 4: Kidney tissue, TNF-α and IL1-β expressions in glomeruli (arrowhead), MMP13 and NOS2 expressions in tubular epithelial cells (arrowhead), Immunoperoxidase-P (IHC-P), Bar: 50 µm.

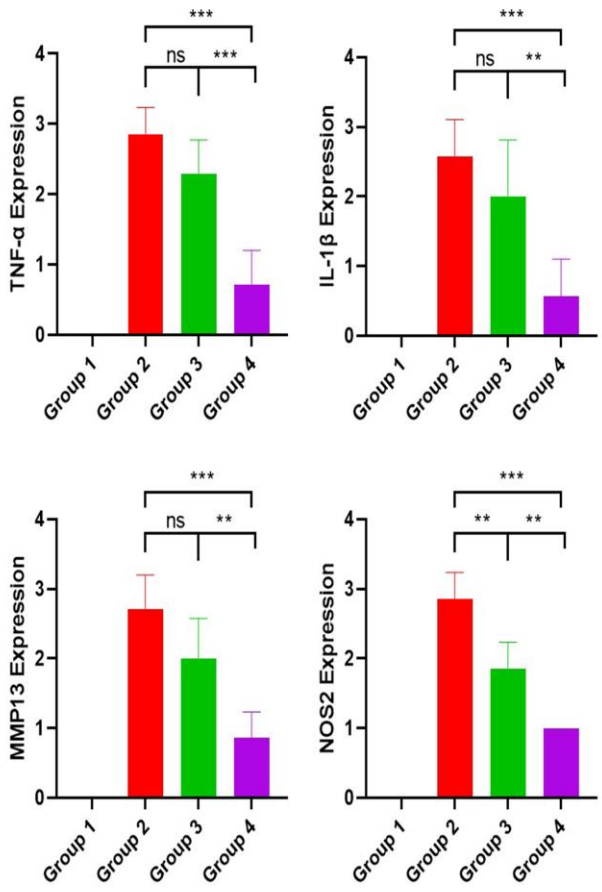

Figure 5: Immunohistochemical analysis results and statistical analysis data in kidney tissue.

TNF-α expression (Group 2 vs 3 ns p=0.1026, Group 2 vs 4 *** p=0.0006, Group 3 vs 4 *** p=0.0006); IL1-β expression (Group 2 vs 3 ns p=0.2739, Group 2 vs 4 *** p=0.0006, Group 3 vs 4 ** p=0.0087); MMP13 expression (Group 2 vs 3 ns p=0.0781, Group 2 vs 4 *** p=0.0006, Group 3 vs 4 ** p=0.0041); NOS2 expression (Group 2 vs 3 ** p=0.0041, Group 2 vs 4 *** p=0.0006, Group 3 vs 4 ** p=0.0047). (n=7). (ns: no significance).

Table 2: Scoring of immunohistochemical findings in kidney tissues (n=7).

	Group 1	Group 2	Group 3	Group 4
TNF-α	-	+++	++	+
IL-1β	-	+++	++	+
MMP13	-	+++	++	+
NOS2	-	+++	++	+

DISCUSSION AND CONCLUSION

Boron is a trace substance that needs oxygen and is widely found in nature. Boron is most abundant in nature in the form of boric acid and then borax. After BA is enter the body, it is absorbed very quickly in the gastrointestinal (GI) tract and enters the circulation. It takes an active role in different physiological and biochemical reactions by showing many protective activities in the body (Pawa and Ali 2006; Khaliq et al. 2018). Boric acid is used in the body, especially in steroid hormonal mechanisms, bone development and cellular mechanisms (King et al. 2015). Boric acid is widely found in vegetables and fruits, in nature especially in nuts, and people have easy access to it (Zhao and Wen et al. 2018).

There is not any study has been found examining the damage caused by the application of osteoarthritis in the kidney tissues. However, there are studies investigating the effects of boric acid against these damages in some studies that have created kidney damage with various substances (Malfait et al. 2016; Güney et al. 2022).

In a study where ethanol caused kidney damage, it was reported that boric acid prevented this damage in both biochemical and histopathological evaluations (Güney et al. 2022). Similarly, histopathologically and immunohistochemically, it has been determined that boric acid has a dose-dependent protective effect on kidney

tissue against the damage that occurs in kidney tissues with OA application.

MMPs induced by IL-1β, IL-6 and TNF-α secreted in the body as a result of OA; They increase cartilage destruction in tissues by activating inflammatory cytokines and chemokines (Malfait et al. 2016).Subsequently, nitric oxide (NO) and inducible nitric oxide synthase (iNOS), which are responsible for cartilage and bone degradation, induce the release of IL-1β in the body and systemic inflammatory reactions occur, causing damage to many tissues and organs (Mongkhon et al. 2014). Boric acid also plays a role in the body response by functioning in the hormonal and cellular mechanism in the body (Sogut et al. 2018). In addition, while BA plays a role in enysmatic activity in the body, it also increases the glutathione level in cells. In addition to its antiapoptotic activity at the cellular level, BA also reduces oxidative damage by suppressing ROS (Ince et al. 2018).

In recent studies; It has been reported that BA suppresses inflammatory reactions via the NF-kB pathway (Durick et al. 2005). Again, it was determined that BA application has an anti-inflammatory effect against inflammation caused by phytohemagglutinin (PHA-P) application (Armstrong et al. 2001). In the experimental study conducted in rats with OA; IL-1β, TNF-α, NOS2 and MMP13 expression levels in kidney tissues were increased by immunohistochemical methods as a result of systemic inflammatory reactions. However, it has been shown that BA significantly protects the kidney tissue against these inflammatory reactions in a dose-dependent manner, and new information has been added to the literature.

As a result, it was demonstrated for the first time that the systemic inflammatory response developed in rats with experimental osteoarthritis caused by MIA caused damage to the kidney tissues and that boric acid applied at different doses had a protective effect against such damage. For this reason, it was concluded that BA application may also be effective in other tissues and organs against the systemic inflammatory response that occurs with OA.

CONFLICTS OF INTEREST

The authors report no conflicts of interest.

AUTHOR CONTRIBUTIONS

Idea / Concept: İB, KATK
Supervision / Consultancy: İB, KATK, EMK
Data Collection and / or Processing: İB, KATK, EMK, ŞTT
Analysis and / or Interpretation: İB, GG, KG, SYT
Writing the Article: İB, KATK, EMK, GG
Critical Review: İB, KATK, FDM

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