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Investigation of the Effects of Boric Acid Used in the Treatment of Rat Models with Knee Osteoarthritis Induced by Monosodium Iodoacetate on Liver Tissue

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Abstract: Osteoarthritis (OA) is a significant joint disease that can affect the health of individuals in many sectors. Although OA is primarily known as a joint disease in the field of health, it can also cause damage to organs such as the liver due to the systemic inflammatory reactions (SIRS) it triggers. Boric acid (BA), which has been the subject of many studies and has been shown to have anti-inflammatory, anti-apoptotic, antimicrobial, and anticancer properties, is widely used in various areas of the healthcare sector. In this study conducted for this purpose, the protective effect of BA against liver damage caused by OA was investigated. A total of 28 rats were divided into four groups, each consisting of 7 rats. Except for the control group, all animals were induced with monosodium iodoacetate (MIA) to develop OA, and then rats were administered doses of BA ranging 4 and 10 mg/kg for 21 days. In histopathological examinations, severe hepatitis and degenerative changes were observed in the liver tissue of the OA-induced group, while it was found that BA application reduced these damages dose-dependently. Similarly, in immunohistochemical analyses, it was observed that OA induction significantly increased the expression levels of TNF-α, IL-1β, iNOS, and MMP-13 in the liver, but in the groups treated with BA, this expression level significantly decreased depending on the dose. This study observed that BA exhibited anti-inflammatory properties and had a protective effect on liver tissue against SIRS caused by OA.

Keywords: *Boric acid, Histopathology, Immunohistochemistry, Monosodium iodoacetate, Osteoarthritis.*

Monosodyum İyodoasetat ile Oluşturulan Diz Osteoartriti Olan Sıçan Modellerinin Tedavisinde Kullanılan Borik Asitin Karaciğer Dokusu Üzerine Etkilerinin Araştırılması

Özet: Osteoartrit (OA), bireylerin sağlığını birçok sektörde etkileyebilen önemli bir eklem hastalığıdır. OA sağlık alanında öncelikle bir eklem hastalığı olarak bilinse de tetiklediği sistemik inflamatuar reaksiyonlar (SIRS) nedeniyle karaciğer gibi organlarda da hasara yol açabilmektedir. Birçok çalışmaya konu olan ve anti-enflamatuar, anti-apoptotik, anti-mikrobiyal ve anti-kanser özelliklere sahip olduğu gösterilen borik asit (BA), sağlık sektörünün çeşitli alanlarında yaygın olarak kullanılmaktadır. Bu amaçla yapılan bu çalışmada, BA'nın OA'nın neden olduğu karaciğer hasarına karşı koruyucu etkisi araştırılmıştır. Toplam 28 rat, her biri 7 rattan oluşan dört gruba ayrılmıştır. Kontrol grubu hariç, tüm hayvanlar monosodyum iyodoasetat (MIA) ile indüklenerek OA geliştirilmiş ve ardından ratlara 21 gün boyunca 4 ve 10 mg/kg dozlarda BA uygulanmıştır. Histopatolojik incelemelerde, OA oluşturulan grubun karaciğer dokusunda şiddetli hepatit ve dejeneratif değişiklikler gözlenirken, BA uygulamasının bu hasarları doza bağlı olarak azalttığı tespit edilmiştir. Benzer şekilde, immünohistokimyasal analizlerde, OA indüksiyonunun karaciğerde TNF-α, IL-1β, iNOS ve MMP-13 ekspresyon seviyelerini önemli ölçüde artırdığı, ancak BA ile tedavi edilen gruplarda bu ekspresyon seviyesinin doza bağlı olarak önemli ölçüde azaldığı gözlenmiştir. Bu çalışma, BA'nın anti-inflamatuar özellikler sergilediğini ve OA'nın neden olduğu SIRS'e karşı karaciğer dokusu üzerinde koruyucu bir etki gösterdiğini gözlemledi.

Anahtar Kelimeler: *Borik asit, Histopatoloji, İmmünohistokimya, Monosodyum iyodoasetat,Osteoartrit.*

Introduction

Osteoarthritis (OA) is a common disease among humans caused by damage to articular cartilage and the disruption of the balance in the production and degradation of extracellular matrix components, in which inflammatory substances that occur in response to this damage play an active role. OA is a joint disorder known for being both progressive and causing severe degeneration (Steels et al., 2019). With the formation of OA, subchondral bone thickening, synovial inflammation, osteophyte formation, thickening of the joint capsule and degenerative changes in the ligaments are seen in the knee joint. Depending on these symptoms, pain, stiffness and even disability may occur in the joints (Chen et al., 2017). Osteoarthritis is one of the most common musculoskeletal disorders worldwide, primarily affecting the knee region. The research conducted in 2020 reported that knee OA affects approximately 16% of the global population, with a higher prevalence in women compared to men, and its prevalence continues to rise due to modern lifestyle conditions and a high obesity rate (Cui et al., 2020). While OA is mostly recognized as a joint disease characterized by cartilage damage and loss, it has a complex pathogenesis that can affect all tissues in the body (Primorac et al., 2020). With the formation of osteoarthritis, the amount of proinflammatory cytokines in the environment is increased by the release of certain enzymes and inflammatory substances from the body. This picture shows inflammation and subsequent morphological differentiation of the joint tissue. In addition, some chemokines exert chemotactic effects on cells that secrete proinflammatory substances, attracting inflammatory cells to these regions and creating disadvantages in the treatment of osteoarthritis (Molnar et al., 2021).

Monosodium iodoacetate (MIA) is well-known for reproducing symptoms similar to those observed in patients with osteoarthritis (OA) when used to induce OA in animal models (Fonsi et al., 2019). When injected into the joint cavity, MIA disrupts glycolytic energy metabolism in chondrocytes, causing cell death, inflammation and cartilage damage. MIA induction in the OA model increases inflammatory cytokines and gradually leads to pain that results in nerve cell damage and neuralgia (Kuyinu et al., 2016).

Boron is an element found in Group 3A of the periodic table, possessing both semiconductor and metal characteristics. Due to its reactivity with oxygen, boron is found in nature as oxides of various elements rather than in its free form (Kılıçarslan, 2020). Boron is known to have a significant and wide-ranging role in the field of health, from cancer treatment to obesity (Başkan et al., 2022). Several studies have demonstrated that boric acid positively affects hormone levels such as steroids, thyroid hormones, estrogen, and testosterone, regulates various minerals including calcium and vitamin D, and helps maintain bone structure (Şaylı et al., 2000). It is also known to have a protective effect against atherosclerosis. Studies on humans have shown that boron compounds exhibit cytotoxic effects against cancer cells and reduce the risk of lung cancer, prostate cancer, and abnormal cervical cytology (Söğüt et al., 2020). Additionally, boron compounds are used in the treatment of conditions such as joint inflammation, osteoporosis, and depression, as they increase antioxidant enzyme activity and affect collagen enzymes to prevent premature aging (Acaroz et al., 2019; TMMOB, 2016).

In conclusion, although OA is primarily recognized as a joint disease, it is known to cause damage in many tissues and organs, including liver tissue, due to the systemic inflammatory reactions it induces. Boric acid is known as an anti-inflammatory agent widely used in the healthcare sector. This study aims to elucidate the inflammatory reactions occurring due to OA and the protective efficacy of boric acid against them.

Material and Methods

Animals and experimental model: Male albino Wistar rats, aged 12-16 weeks and weighing 250-300 grams, were obtained from the Animal Laboratory of Atatürk University. The rats were intraperitoneally administered ketamine (30 mg/kg) and 2% xylazine (6 mg/kg) to induce general anesthesia. Under anesthesia, 3 mg of monosodium iodacetate (MIA) dissolved in 0.9% NaCl (0.1 mL) solution was intraarticularly administered to the right patellar ligament of the rats as a single dose of 50 µL to create a knee osteoarthritis (OA) model.

The rats were randomly divided into four equal groups. Ethics Committee Approval: Atatürk University Animal Experiments Local Ethics Committee 01.04.2023 (Ethical Report:2023/04). Group 1 (Control; n=7x1): No procedure was performed on the rats in this group. Group 2 (Knee OA + Saline; n=7x1): After creating the knee OA model in the rats, 0.1 mL of saline was orally administered on days 1, 7, 14, and 21. Group 3 (Knee OA + 4 mg Boric Acid Oral; n=7x1): After creating the knee OA model in the rats, 1 mL of 4 mg/kg boric acid was administered orally once a day for three weeks. Group 4 (Knee OA + 10 mg Boric Acid Oral; n=7x1): After creating the knee OA model in the rats, 1 mL of 10 mg/kg boric acid was administered orally once a day for three weeks. After 24 hours of all treatments, the weights of the rats in each group were measured. They were then sacrificed under general anesthesia by decapitation following the intraperitoneal administration of ketamine hydrochloride (30 mg/kg) and 2% xylazine hydrochloride (6 mg/kg). Liver tissue samples were collected from the sacrificed rats to assess tissue damage. Liver tissues were placed in 10% formaldehyde solution.

Histopathological and immunohistochemical examination: Liver tissue samples obtained from the rats were routine tissue processing, the tissues were blocked with paraffin. The sections were stained with hematoxylin and eosin and subjected to histopathological evaluation under a light microscope. For immunohistochemical examination (IHC Detection Kit cat no: ab236466, abcam, UK), primary antibodies (TNF-α Cat No: sc-52746, Diluent Ratio: 1/100, US IL-1β Cat No: sc-52012, Diluent Ratio: 1/100,

US iNOS Cat No: sc-7271, Diluent Ratio: 1/100, US MMP-13 Cat No: sc-101564, Diluent Ratio: 1/100) were used. DAB (3,3'-Diaminobenzidine) chromogen was used to visualize the binding of primary and secondary antibodies. After the staining process, the necessary areas were captured using the Olympus BX51 with DP72 camera system. Based on histopathological findings and immunopositivity, the staining was scored as absent (-), mild (+, 5-10% cell), moderate (++, 15-20% cell), or intense (+++, >20% cell) (Iskender et al., 2022).

Statistical analysis: Statistical analysis of the histopathological evaluations was performed using the SPSS 13.0 program, and data were evaluated considering $p<0.05$ as statistically significant. Duncan's test was used for intergroup comparisons. The non-parametric Kruskal-Wallis test was used to determine group interactions, and the Mann-Whitney U test was used to identify differences between groups.

Results

Histopathological and immunohistochemical findings

Group 1: When liver tissues were histopathologically examined, it was determined that the tissues had a normal histological appearance (Figure 1a). When liver tissues were examined immunohistochemically, TNF-α, IL-1β, iNOS, and MMP-13 expressions were evaluated as negative in the tissues (Figure 2a, 3a, 4a, and 5a). **Group 2:** In the liver, severe hepatitis, severe degeneration and necrosis in hepatocytes, and moderate dilation in the sinusoidal space were observed (Figure 1b). When liver tissues were examined immunohistochemically, TNF-α and IL-1β expressions were observed in inflammatory cells, and iNOS and MMP-13 expressions were detected severely in hepatocytes (Figure 2b, 3b, 4b, and 5b). **Group 3:** In the liver, moderate hepatitis, severe degeneration in hepatocytes, and moderate dilation in the sinusoidal space were detected (Figure 1c). When liver tissues were examined immunohistochemically were detected that moderately TNF-α and IL-1β expressions were in inflammatory cells, and cytoplasmic iNOS and MMP-13 expressions moderately in hepatocytes (Figure 2c, 3c, 4c, and 5c). **Group 4:** In the liver, mild hepatitis, mild degeneration in hepatocytes, and mild dilation in the sinusoidal space were observed (Figure 1d). A significant difference ($p<0.05$) was found when Group 4 was compared with Group 2. The histopathological findings are summarized in Table 1. When liver tissues were examined immunohistochemically, TNF-α and IL-1β expressions were observed in inflammatory cells, while cytoplasmic iNOS and MMP-13 expressions were detected mildly in hepatocytes (Figure 2d, 3d, 4d, and 5d). A significant difference ($p<0.05$) was found when Group 4 was compared with Group 2. The immunohistochemical findings are summarized in Table 1.

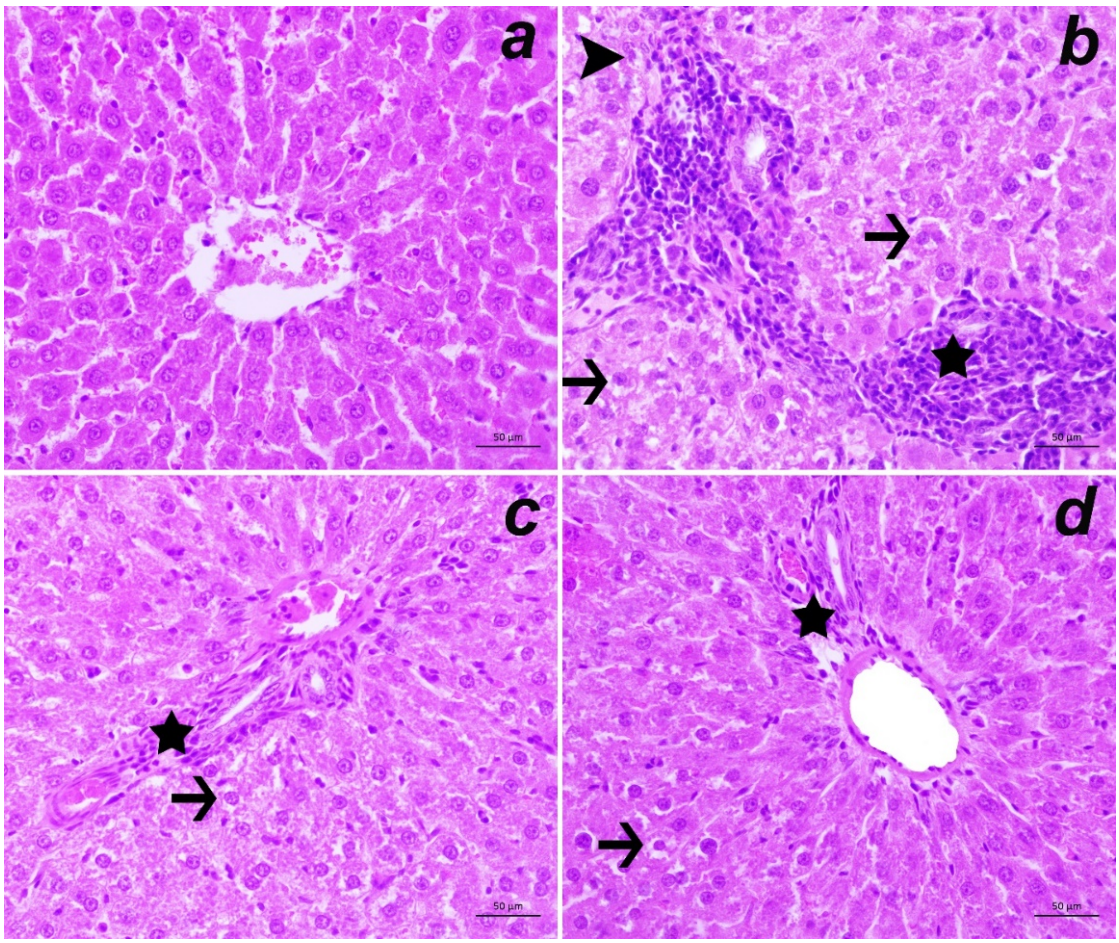

Figure 1. Liver tissue, Group 1(a), 2(b), 3(c), and 4(d), hepatitis (star), degeneration in hepatocytes (arrows), and necrosis (arrowhead), H&E, Scale bar: 50µm.

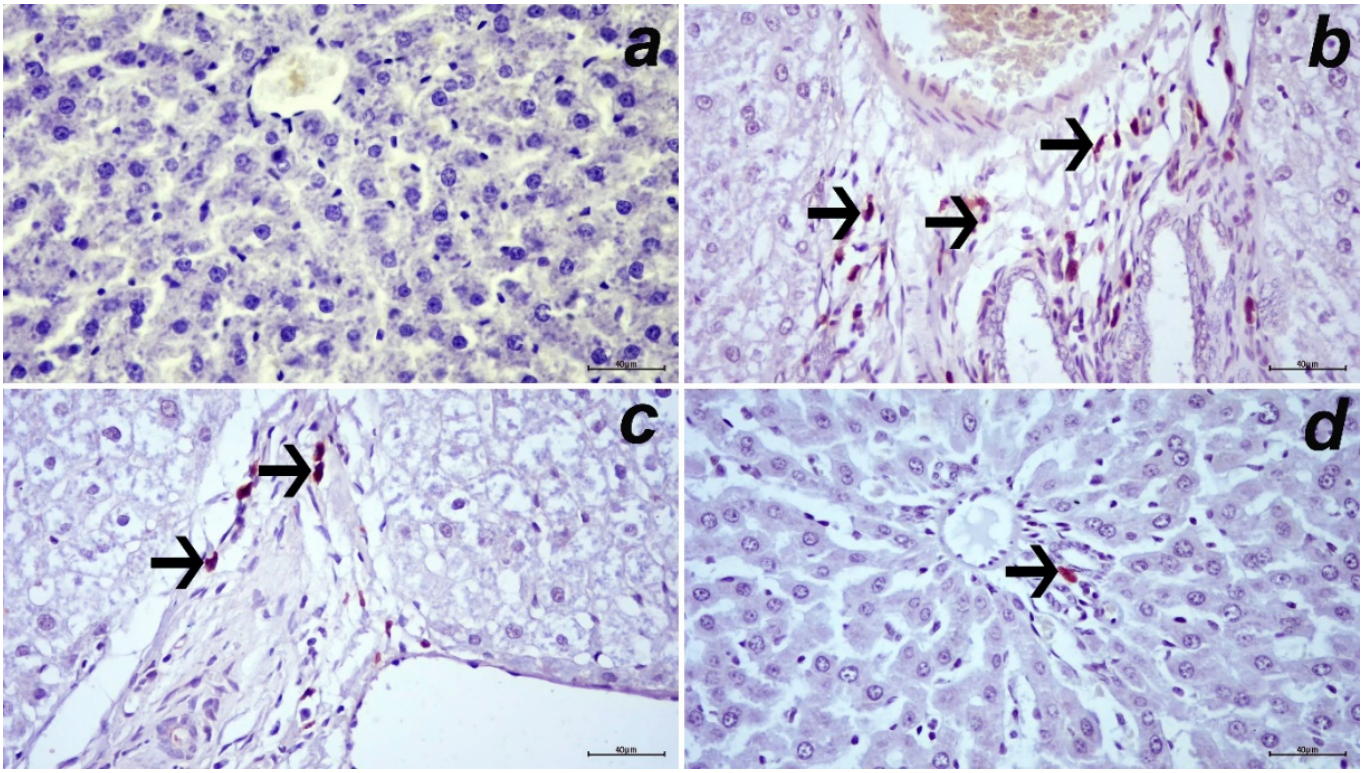

Figure 2. Liver tissue, Group 1(a), 2(b), 3(c), and 4(d), intracytoplasmic TNF-α expressions in inflammatory cells (arrows), IHC-P, Scale bar: 40µm.

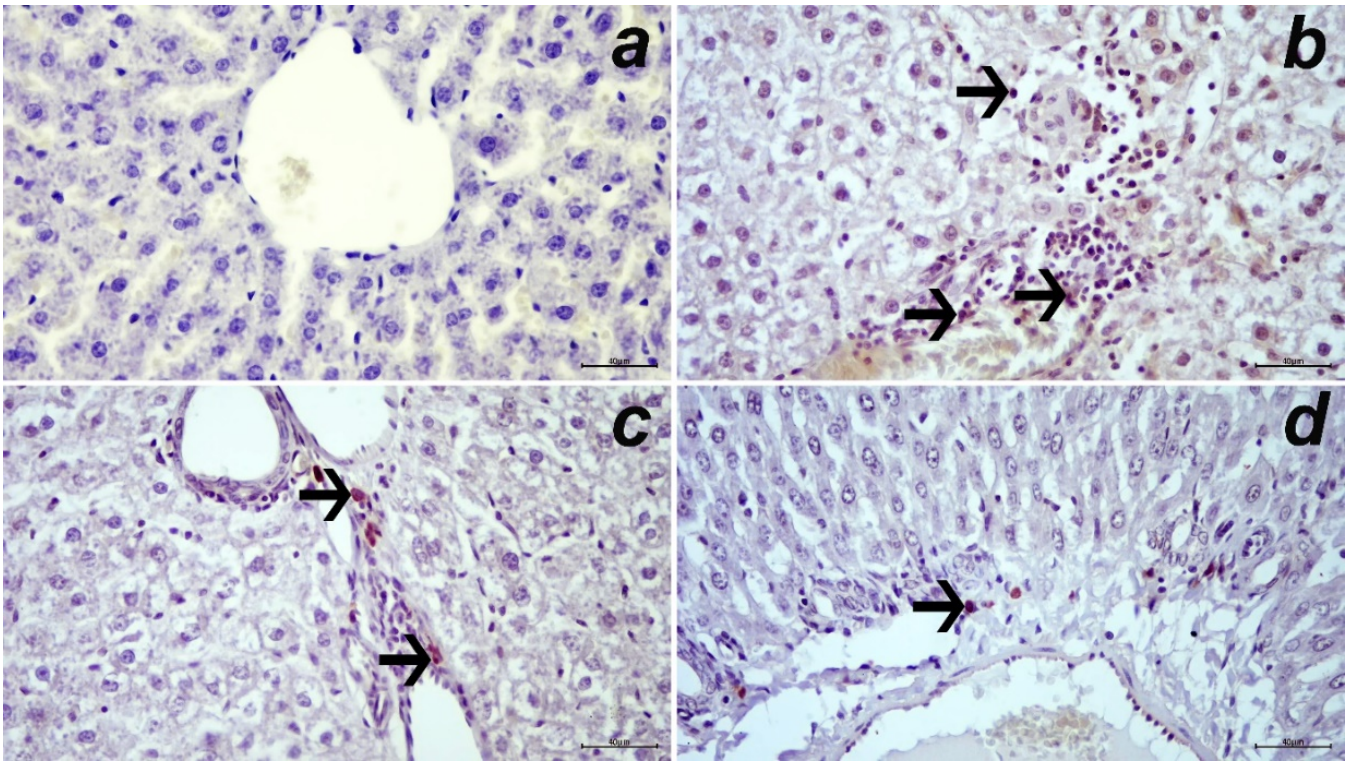

Figure 3. Liver tissue, Group 1(a), 2(b), 3(c), and 4(d), intracytoplasmic IL-1β expressions in inflammatory cells (arrows), IHC-P, Scale bar: 40µm.

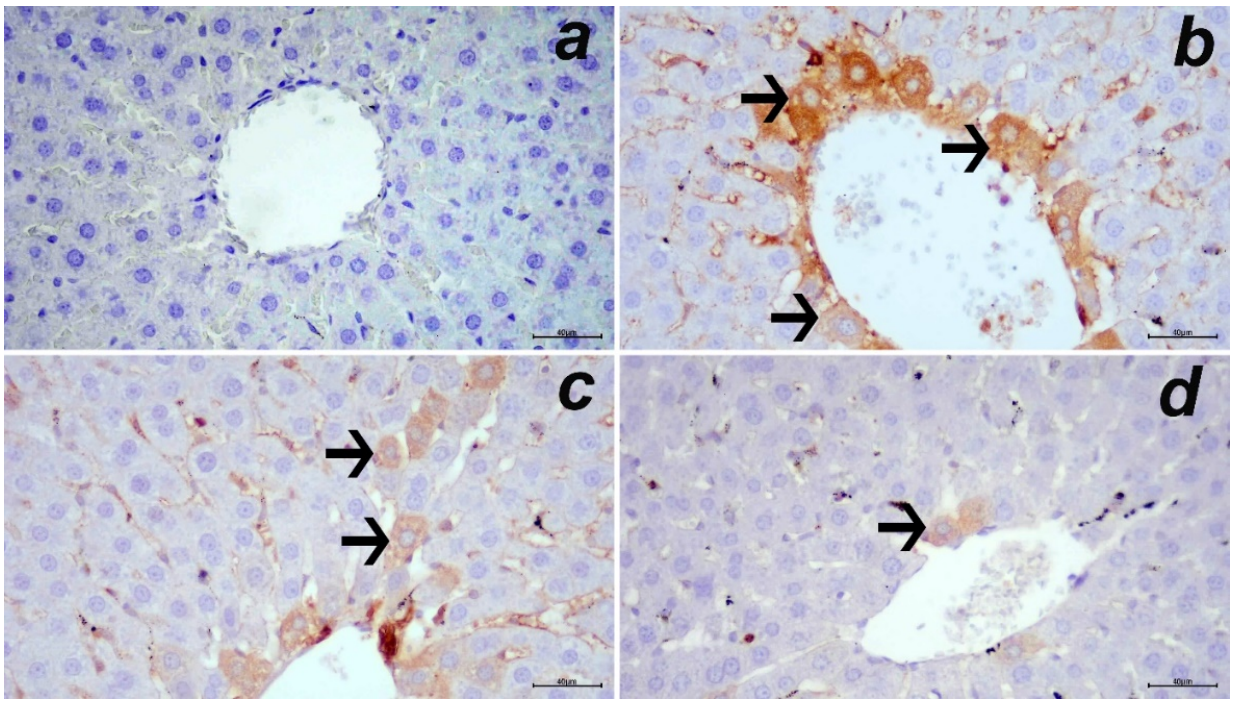

Figure 4. Liver tissue, Group 1(a), 2(b), 3(c), and 4(d), intracytoplasmic iNOS expressions in hepatocytes (arrows), IHC-P, Scale bar: 40µm.

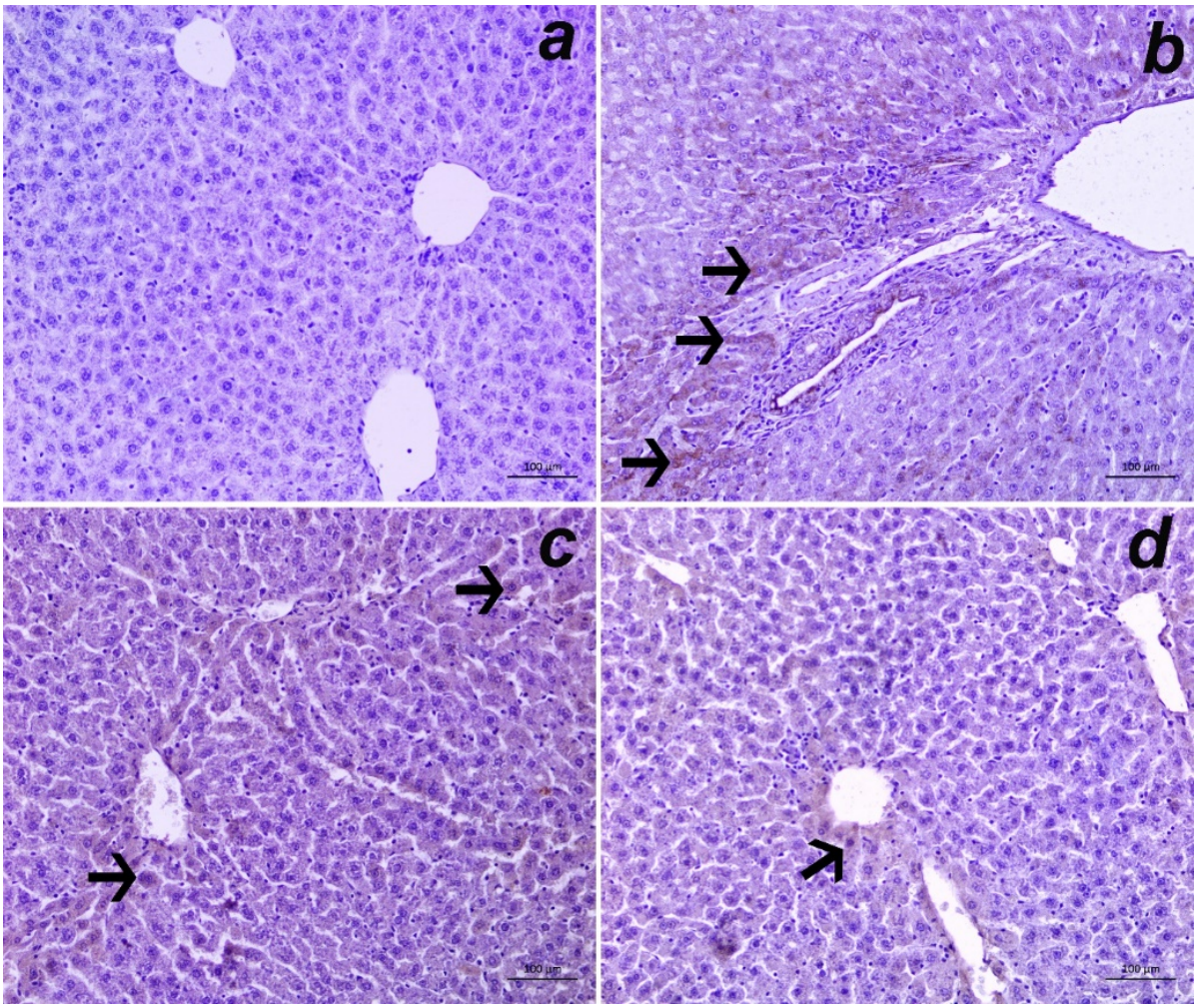

Figure 5. Liver tissue, Group 1(a), 2(b), 3(c), and 4(d), intracytoplasmic MMP-13 expressions in hepatocytes (arrows), IHC-P, Scale bar: 100µm.

Table 1. Histopathological and immunohistochemical findings in liver tissue and their scoring.

	Group 1	Group 2	Group 3	Group 4
Hepatitis	-	+++	++	+
Degeneration in hepatocytes	-	+++	+++	+
Necrosis in hepatocytes	-	+	-	-
Dilatation of sinusoids	-	++	++	+
TNF-α expressions	-	+++	++	+
IL-1β expressions	-	+++	++	+
iNOS expressions	-	+++	++	+
MMP-13 expressions	-	+++	++	+

Discussion and Conclusion

Boric acid is a mineral with antioxidant properties. It is widely used in various fields, including traditional healthcare, industry, and agriculture. Two hypotheses regarding the biochemical and physiological role of boric acid in living organisms have been proposed in the literature. Firstly, it acts as a response to hormone effects and cell membrane functions, affecting both transmembrane signaling and the movement of regulatory ions (Cengiz et al., 2018; Ince et al., 2012; Sogut et al., 2018). Secondly, boric acid can serve as a metabolic regulator in certain enzymatic systems. It increases the level of reduced glutathione, thereby reducing the effects of oxidative damage in the body and inhibiting the production of reactive oxygen species (ROS) and apoptosis (Ince et al., 2014). In a study conducted by Ince et al., it was found that boric acid exhibited strong hepatoprotective effects on liver damage. They demonstrated the protective efficacy of boric acid administration against histopathological changes induced by carbon tetrachloride in rat liver tissue. They suggested that this effect was due to both the activity of the antioxidant defense system and the inhibition of lipid peroxidation (Ince et al., 2012). Similarly, in a study by Cengiz et al., the protective effect of boric acid against damage induced by cyclophosphamide was emphasized (Cengiz et al., 2019). Based on these studies and literature findings, it is believed that boric acid has a protective effect against various types of liver damage. In accordance with the literature, the results of our study showed that boric acid showed antioxidant and antifilamatory activity in liver tissue against systemic inflammatory reactions in OA model.

Although there are few studies investigating the systemic inflammatory response in liver tissue associated with osteoarthritis (OA), no studies specifically examining boric acid in this context have been found. Some studies have indicated the occurrence of systemic inflammatory reactions in liver tissue associated with OA (Stammers et al., 1992). Additionally, studies on reperfusion-induced liver damage have shown that reperfusion, along with macrophage activation, leads to an increase in pro-inflammatory cytokine levels. The increased levels of pro-inflammatory cytokines such as IL-6 and TNF-α, which play a significant role in reperfusion injury, contribute to damage both in the reperfused tissue and in distant organs (Yildar et al., 2014). In an experimental study that induced hepatic ischemia-reperfusion injury, Crockett et al. observed histopathological findings such as sinusoidal congestion, cytoplasmic vacuolization, and neutrophil infiltration in the liver tissue (Crockett et al., 2006). It has been reported in the literature that inflammation occurs in liver tissues due to systemic inflammation. In this study, it was revealed that systemic inflammatory reactions occurring in the OA model also caused inflammatory reactions in the liver tissues of rats. Again, it was observed that proinflammatory cytokines IL-1β and TNF-α increased due to the increase in iNOS in liver tissues, and as a result, MMP-13 expression from hepatocytes also increased significantly. It is thought that this increase in MMP-13 develops due to systemic inflammatory response syndrome (SIRS) in the body.

No study investigating the effects of boric acid on liver damage in an OA model has been found. However, in a study conducted by Başbuğ et al., it was demonstrated that boric acid exhibited anti-inflammatory activity and protected liver tissue against inflammatory reactions induced by ischemia-reperfusion injury (Başbuğ et al., 2015). Similarly, in a study where ethanol-induced liver damage was examined, it was found that ethanol increased the sensitivity of Kupffer cells and the expression of inflammatory cytokines such as TNF-α (Dai et al., 2003). In a similar study on ethanol-induced liver injury, it was reported that boric acid application suppressed the increased expression of TNF-α (Sogut et al., 2018). These studies indicate that boric acid exhibits anti-inflammatory activity against inflammatory reactions occurring in the liver tissue associated with both ischemia-reperfusion injury and ethanol-induced liver damage. In this study, it was observed that TNF-α expressions due to inflammatory reactions in the liver tissue in the OA model were severely increased, and these expressions were significantly decreased due to boric acid administration. This is thought to be due to the anti-inflammatory activity of boric acid.

In conclusion, this study demonstrated that the systemic inflammatory response associated with the development of osteoarthritis induced by MIA leads to liver damage, and boric acid treatment exhibits a protective effect

against SIRS in the liver tissue. Furthermore, based on the obtained results, it is suggested that boric acid may have a protective effect against SIRS associated with OA in various tissues and organs.

Conflict of Interest

The authors stated that they did not have anyreal, potential or perceived conflict of interest.

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Author contribution

Idea, concept and design: I.B, K.A.T.K
Data collection and analysis: I.B, K.A.T.K, E.M.K
Drafting of the manuscript: I.B, G.G, K.G, S.Y.T
Critical review: I.B, K.A.T.K, F.D.M

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