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# The Effect of Boric Acid on Uterine Ischemia-Reperfusion Injury in the Rats: A Histopathological Assessment *

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The aim of this study was to investigate the protective effects of boric acid on uterine ischemia-reperfusion (IR) injury in rats. Twenty-four female rats were randomly divided into 3 groups. In the sham group, only laparotomy was performed. In the second group (IR), experimental ischemia (45 min) and reperfusion (1 hour) were induced. In the third group (BA+IR), boric acid was administered intraperitoneally at a dose of 200 mg/kg, 10 minutes before ischemia. Afterwards, uterine tissue was subjected to reperfusion for 1 hour. After the reperfusion period, the uterine tissues were surgically removed and fixed in formalin solution. Histopathological evaluation revealed significant differences in tissue damage among the groups ($p$<0.0001). The IR group exhibited marked edema, congestion, inflammation, necrosis and endometrial injury compared to the sham group ($p$<0.0001). In contrast, boric acid treatment significantly reduced these pathological changes. No significant histological difference was observed between sham and boric acid groups ($p$>0.05). In conclusion, boric acid treatment demonstrated a protective effect against uterine IR injury and contributed to the preservation of endometrial health. These findings suggest its potential as a fertility-preserving agent in uterine ischemia.

***Key Words:*** *Boric acid, ischemia, reperfusion, rat*

### Borik Asidin Rat Uterus Dokusunda İskemi-Reperfüzyon Hasarı Üzerine Etkisi: Histopatolojik Bir Değerlendirme

Bu çalışmanın amacı, borik asidin ratlarda uterus iskemi-reperfüzyon (IR) hasarı üzerindeki koruyucu etkilerini araştırmaktır. Yirmi dört dişi rat rastgele 3 gruba ayrıldı. Sham grubuna sadece laparotomi yapıldı. İkinci grupta (IR), deneysel iskemi (45 dakika) ve reperfüzyon (1 saat) oluşturuldu. Üçüncü grupta (BA+IR), iskemiden 10 dk önce 200 mg/kg dozda borik asit intraperitoneal olarak verildi. Sonrasında uterus dokusu 1 saat süreyle reperfüzyona maruz bırakıldı. Reperfüzyon sonrası, uterus dokusu cerrahi olarak alındı ve formalin çözeltisinde tespit edildi. Histopatolojik inceleme, gruplar arasında hasar düzeyi bakımından önemli farklılıklar olduğunu ortaya koydu ($p$<0.0001). IR grubu, sham grubuna kıyasla belirgin ödem, konjesyon, inflamasyon, nekroz ve endometriyal hasar sergilemiştir ($p$<0.0001). Buna karşılık, borik asit tedavisi, bu patolojik değişiklikleri önemli ölçüde azaltmıştır. Sham ve borik asit grupları arasında anlamlı bir histolojik fark gözlenmemiştir ($p$>0.05). Sonuç olarak, borik asit tedavisi uterus IR hasarına karşı koruyucu bir etki göstermiş ve endometriyal sağlığın korunmasına katkıda bulunmuştur. Bu bulgular, uterus iskemisinde fertiliteyi koruyucu bir ajan olarak potansiyelini göstermektedir.

***Anahtar Kelimeler:*** *Borik asit, iskemi, reperfüzyon, rat*

## Introduction

In veterinary medicine, uterine torsion is a major cause of dystocia, especially in farm animals. This condition is characterized by varying degrees of rotation along the long axis of the uterus. Depending on the stage of development, the disorder adversely affects vascular structure and tissue perfusion. Its incidence ranges between 1-7% among the causes of dystocia in cows (1-4). Torsion is more frequent in cows than in heifers and particularly common in Brown Swiss breed. Increased fetal activity may initiate the event. It may also occur due to the pregnant cow's movements while getting up from a lying position. Indoor housing and lack of exercise are another cause. In case of mild torsion, calving can be performed by manual intervention. However, in severe cases, laparotomy may be necessary to reposition the uterus. If the cervix is open, normal delivery can proceed; otherwise, cesarean section is required (5, 6). Delayed treatment can lead to organ rupture and hemorrhage. Subsequently, toxemia and peritonitis may develop, resulting in maternal death (4, 7).

Boron is an element ranked 5$^{th}$ in the periodic table. The atomic mass of this element, whose symbol is B, is 10.81 and is a semi-metal (8). It has various compounds, borax and boric acid, which are used in animal and human health. Boric acid is also called orthoboric acid and is a weak acid. Boron is an essential element for plants. In the human and animal body, it is a trace element with important physiological functions. This micromineral influences immunological reactions, energy metabolism,

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bone metabolism and the nervous system. It also contributes to steroid hormone production (9, 10). Boric acid deficiency causes vitamin D deficiency, resulting in decreased plasma triglyceride levels and elevated pyruvate levels (11).

Recent studies have revealed that boron compounds can exhibit antioxidant, anti-inflammatory, antimicrobial, anticarcinogenic and antimutagenic properties (12-14). Especially boric acid and borax derivatives have been reported to protect tissues against damage in experimental ischemia-reperfusion (IR) models in organs such as spinal cord, liver, kidney, spleen and pancreas (15-19). These studies suggest that boron can inhibit apoptosis, reduce inflammation and mitigate oxidative stress.

However, studies on the effects of boron on uterine tissue are quite rare (20). The uterus is sensitive to hormonal influences and contains complex cellular structures. It is also knowed known as a hormone producing site (21). These unique features may cause boron to show different effects in uterine tissue. Therefore, investigating the possible effects of boric acid application in uterine IR injury may fill the lack of knowledge in this field and contribute to the process of developing uterine protective pharmacological agents.

In the literature review, we did not find any study investigating the effects of boric acid on uterine IR injury. We hypothesized that boric acid may protect the uterus from IR injury. The aim of this study was to evaluate the histopathological effects of boric acid administration on uterine tissue using an experimental uterine IR model in rats. With the data to be obtained, this study aimed to reveal the reducing effects of boron on IR-induced damage in the uterus and to provide preclinical data that could form the basis for future clinical studies. Thus, the possibility of pharmacological strategies for organ preservation after gynecologic surgery can be increased and the therapeutic use of boron derivatives can be more clearly defined.

## Materials and Methods

**Research and Publication Ethics:** This study was conducted at the Experimental Animals Research and Application Center, Balikesir University, following ethical approval from the Balikesir University Experimental Animal Ethics Committee (date no. 26/12/2024, approval no. 2024/12-5).

**Materials:** Boric acid was obtained from Merck (Boric acid, Merck 1.00165 Cas No: 1 0043-35-3). Xylazine was procured from Alba Farma and ketamine was purchased from Doğa-Ilaç.

**Animals:** Twenty-four Wistar albino adult (8-12 weeks of age) female rats weighing 220-250 g were used as material. Animals were kept at a room temperature of 22±2ºC and 45-55% humidity, 12 hours light/dark period under standard laboratory conditions. No feed and water restriction was applied before the experiment.

**Experimental Protocol:** The sample size was determined based on the study design assumptions (α = 0.05, 1 - beta = 0.90), resulting in 8 animals per group. Although the Kruskal-Wallis test was ultimately used due to the non-normal distribution of the data, the sample size is expected to provide sufficient statistical power to detect meaningful differences among the groups. Before the experimental procedures, estrous cycles of all animals were monitored by vaginal smear method and animals showing regular estrus at least 3 times were identified and the experiment was performed during estrus phase (22, 23).

Group 1 (Sham, n=8): Saline was given intraperitoneally, and laparotomy was performed 10 minutes later.

Group 2 (IR, n=8): Saline was administered intraperitoneally, followed by 45 minutes of uterine ischemia and 1 hour of reperfusion.

Group 3 (BA+IR, n=8): Boric acid (200 mg/kg) dissolved in saline, was administered intraperitoneally 10 min before 45 minutes of uterine ischemia. Afterwards, reperfusion was performed for 1 hour.

The boric acid dose was based on previous studies (17, 18, 24, 25). In accordance with Kar et al. (26), it was freshly prepared by dissolving in saline, and the administration route and dosage reported by Güler et al. (25) were applied.

**Surgery and Pharmacological Applications:** All procedures were performed under general anesthesia and sterile conditions. Xylazine-ketamine protocol was used for anesthesia. Before the operation, 80 mg/kg ketamine hydrochloride (Keta-Control; Doğa İlaç) and 10 mg/kg xylazine hydrochloride (Beltazyn 2%; Alba Farma) were given intramuscularly. After anesthesia was achieved, the abdominal area was shaved and povidone-iodine was used for disinfection and laparotomy was performed with a 2-3 cm vertical incision in the midline of the lower abdomen. In the groups in which ischemia was to be induced, the distal abdominal aorta was clamped with an atraumatic microvascular clamp (Bulldog clamp; Aesculap (r), B. Braun Melsungen) approximately 0.5 cm from the bifurcation and left for 45 minutes. Bilateral ovaries were also clamped to prevent collateral circulation. Then, the incision was closed with a single suture and covered with sterile saline-soaked gauze to minimize intra-abdominal heat and fluid loss. At the end of 45 minutes, clamps were opened for reperfusion. The uteruses were reperfused for one hour. Following reperfusion, the uterine tissue was excised (27).

After reperfusion, rats were sacrificed by exsanguination method under anesthesia. The uterine tissues were then removed and placed in buffered formalin for histopathological examination. The evaluations were performed blindly by the same pathologist including edema, inflammatory cells, congestion, necrosis and endometrial cell loss.

**Histopathological Analysis:** The excised uterine tissue samples were first washed under running tap water overnight to remove residual formalin. Subsequently, the samples were subjected to routine tissue processing, including dehydration through a graded ethanol series, clearing in xylene, and embedding in paraffin blocks. Sections of 5 µm thickness were obtained using a microtome (Leica RM 2125 RT). For systematic evaluation, the first three sections and every tenth section thereafter were selected and mounted on glass slides. The slides were deparaffinized and rehydrated through xylene and graded ethanol series, then stained with hematoxylin and eosin (HE). All histopathological evaluations were conducted under a light microscope at 40-400 fold magnification (Olympus BX53-DIC with DP-73 camera, Tokyo, Japan). In each sample, 10 fields were randomly selected for scoring. Tissue sections were evaluated semi-quantitatively for edema, inflammation, congestion, necrosis, and endometrial injury. Each parameter was scored based on severity using a standardized scale: 0 = none, 1 = mild, 2 = moderate, 3 = severe. This scoring system was adapted from previously published protocols (28).

**Statistical Analysis:** GraphPad Prism 8 program (GraphPad Software, USA) was used for the statistical analyses. Since the histopathological parameters obtained from the groups in the study were semi-quantitative data, nonparametric tests were employed. Differences between the groups were first evaluated by Kruskal-Wallis test. Since significant results were obtained, post-hoc Dunn's multiple comparisons test was applied. $p<0.05$ was accepted as significant.

## Results

All groups were evaluated histopathologically for edema, inflammation, congestion, necrosis and endometrial damage. The histopathological scores of the groups are presented in Figure 1. Initially, the groups were compared using the Kruskal-Wallis test for each parameter (Table 1). This analysis revealed statistically significant differences among the groups in terms of all parameters ($p<0.0001$). Subsequently, a multiple comparison test was performed between paired groups for each parameter in order to find out which group was responsible for the difference. Regarding edema score (Figure 1a), the IR group exhibited a significant increase compared to the sham group ($p<0.0001$). In the BA+IR group, edema scores were significantly lower than in the IR group ($p<0.05$). No significant difference was observed between sham and BA+IR groups ($p>0.05$).

Severe congestion was observed in rats subjected to IR. In contrast, uterine degeneration was markedly reduced in boric acid-treated animals (Figure 1b). Compared to the BA group, the congestion score was significantly higher in the IR group ($p<0.05$). The IR group had very severe congestion values compared to the sham ($p<0.0001$). In the treatment group, the pathological score was significantly decreased ($p<0.05$).

In terms of necrosis, prominent degenerative changes were observed in the uterus of the IR group (Figure 1c, Figure 2b). This finding indicates severe cellular destruction and irreversible damage. In boric acid-treated animals, necrotic changes were reduced in the histopathological evaluation of the uterine tissue. The necrosis score was significantly lower in the treatment group compared to the IR group ($p<0.05$).

Histopathological examination also revealed significant differences in inflammation scores among the groups (Figure 1d). Post-hoc test results indicate a significantly higher level of inflammation in the IR rats compared to the BA+IRgroup ($p<0.05$). In the treatment group, the inflammation level decreased significantly and was similar to the sham group ($p>0.05$).

The endometrial tissue was normal in the sham group (Figure 2a). IR injury caused damage to the endometrial layer of the uterus (Figure 1e and 2b). In contrast, boric acid-treated rats exhibited mild degenerative changes in the uterus (Figure 2c). Compared to IR group, endometrial damage was significantly reduced in the treatment group ($p<0.05$).

**Table 1.** Distribution of histopathological parameters in the groups

| Variables[a] | Group 1 (Sham) (n=8) | Group 2 (IR) (n=8) | Group 3 (BA+IR) (n=8) | *p* |
|---|---|---|---|---|
| Edema | 0 [0–0] | 3 [2–3] | 1 [0–3] | 0.0001* |
| Congestion | 0 [0–0] | 3 [2–3] | 1 [0–2] | 0.0001* |
| Necrosis | 0 [0–0] | 3 [2–3] | 1 [0–2] | 0.0001* |
| Inflamation | 0 [0–0] | 2 [2–3] | 1 [0–2] | 0.0001* |
| Endometrial Injury | 0 [0–0] | 3 [3–3] | 1 [0–2] | 0.0001* |

*p* shows the differences between all groups.

[a] Median [minimum–maximum]: 0 (none), 1 (mild), 2 (moderate), 3 (severe).

*Significant at the 0.05 level (Kruskal–Wallis test).

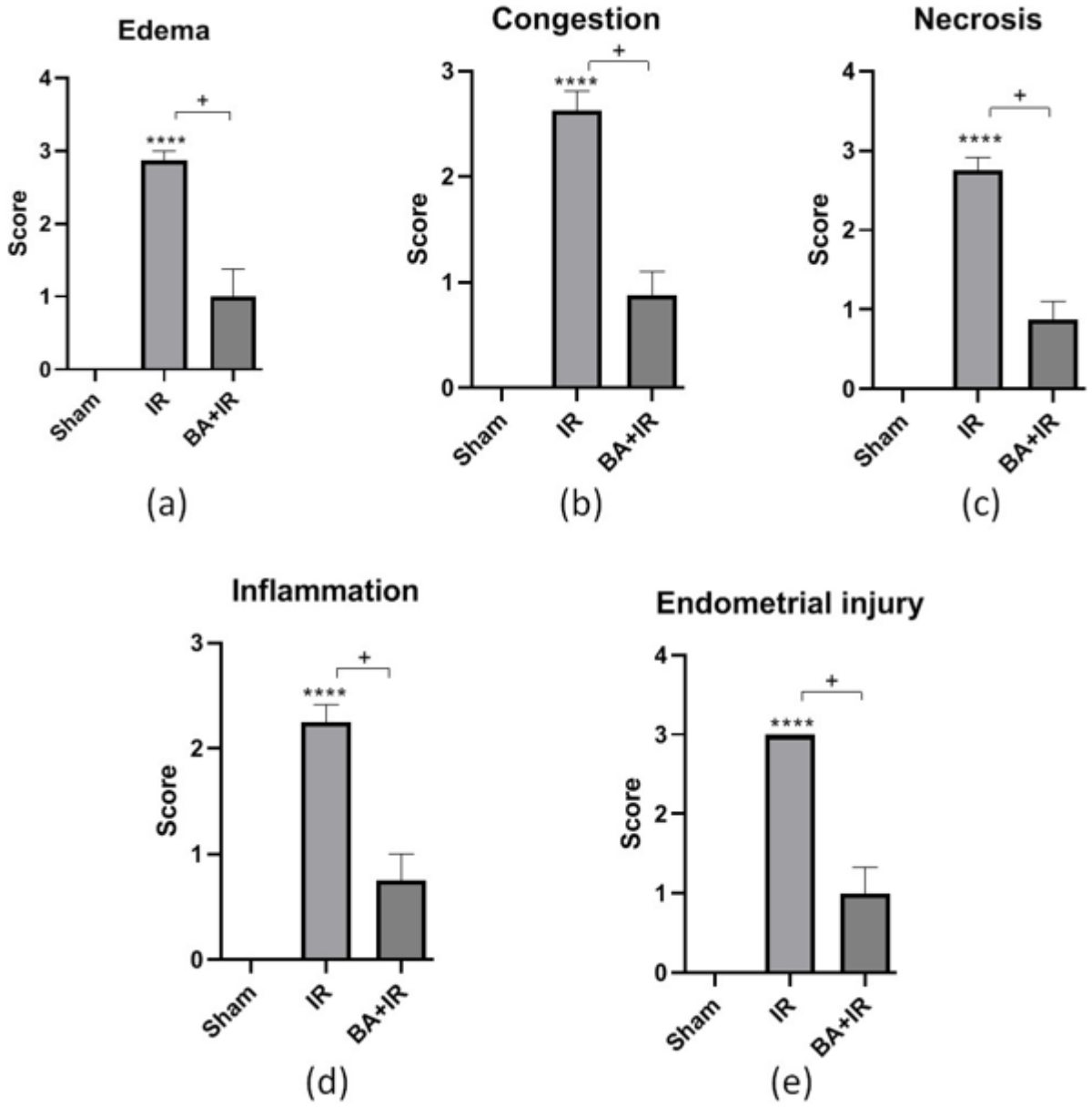


**Figure 1.** Histopathological evaluation of ischemia-reperfusion injury in the uterus. Comparison of scores in terms of edema, congestion, necrosis, inflammation and endometrial damage in the Sham (n=8), IR (n=8) and BA+IR group (n=8). Data are shown as mean ± standard error of the mean (SEM). Non-parametric Dunn's test was used for statistical analysis. p-values less than 0.05 were considered significant. ****: $p<0.0001$, different from Sham, +: $p<0.05$ different from BA+IR.

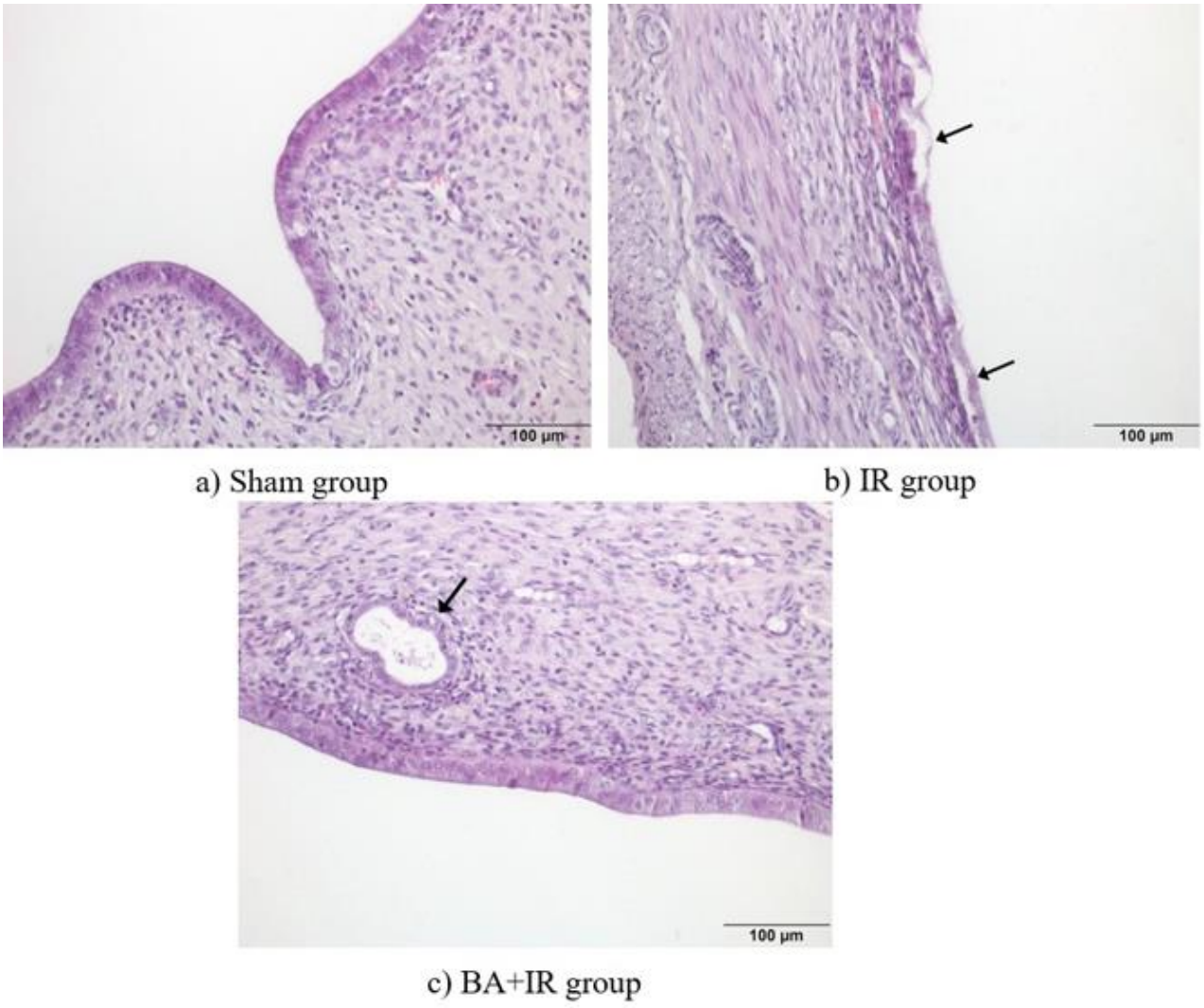


**Figure 2.** Representative histopathological images of the uterine tissues. A) A normal uterus from the sham group B) In the IR group, uterine tissue showing severe degeneration and necrosis (arrows) C) Very mild degeneration (arrow) in the uterus from the treatment group (HE x400).

## Discussion

Torsio uteri is a malposition of the uterus and is one of the causes of calving difficulty. As a result of torsion, IR injury may occur in the organ and this event may result in organ failure and even death. IR damage causes serious organ damage in experimental animal models (7). This injury triggers histopathological changes as well as biochemical, apoptotic and inflammatory responses. In some cases, remote organ injury and multiple organ failure may occur, ultimately leading to mortality (29). Previous studies have revealed that boric acid shows antioxidant, anti-inflammatory, antimicrobial, anticarcinogenic and antimutagenic properties (12-14). Studies have shown that boron derivatives protect organs such as spinal cord, liver, kidney, spleen and ovary against experimentally induced IR damage (15-19). In the literature survey, BA administration on uterine IR injury was investigated for the first time in this study.

Different durations have been used in experimental uterine IR models. The shortest time used for ischemia was 20 minutes and the longest was 2 hours. In the reperfusion period, the time interval is much more variable. This period varies from 30 minutes to 2 weeks (30-32). According to the literature, it can be seen that the most preferred ischemia time is 45 minutes (33-35). The most common reperfusion time is 1 hour (35-37). Accordingly, 45 minutes of ischemia followed by 1 hour of reperfusion was preferred in this experimental protocol.

In the current study, the effects of boric acid treatment were evaluated on histopathological changes in the uterus after IR injury. Our findings demonstrated that boric acid reduced histopathological organ damage and significantly preserved the uterine tissue. In particular, the alleviation of pathological parameters such as edema, congestion and necrosis indicates that the treatment is therapeutic. In addition, the reduction of

inflammation in the tissue and preservation of the endometrium suggest that boric acid is an agent that can be used to preserve fertility in such cases.

Previous studies have proven the protective effects of boric acid against IR damage in solid organs such as the ovaries, liver and kidneys (18, 19, 26). Karaman et al (19) reported that ovarian IR injury increased hemorrhage, edema and inflammation and decreased ovarian reserves. They determined that boron treatment alleviated ovarian damage and reversed these effects, resulting in follicle number similar to the control levels. These findings are in agreement with the histopathological improvement observed within the uterus in the present study. However, it should be taken into consideration that the uterus is a dynamic organ sensitive to hormones. Therefore, it seems that the boron related uterine IR pathophysiology should be investigated in depth.

Karaman and Yavuz (38) induced uterine damage in rats using cyclophosphamide in their study. The researchers reported that there were desquamations in the uterine epithelium and vacuolization occurred, endometrial glands were damaged, and lymphocyte infiltration formed. They also demonstrated that tissue damage was reduced in rats given boric acid in combination with cyclophosphamide, and that the uterus resembled that of the control group. Our study is consistent with that of Karaman and Yavuz (38). While IR injury caused severe pathological changes in the uterus, boric acid attenuated the damage, and the uterine tissue was similar to the control group.

In this study, we investigated the histopathology that developed in the uterus following IR injury. Çolak et al. (39) investigated the protective effects of boric acid on ovarian IR injury and reported that hemorrhage, degeneration, congestion, and necrosis developed in the ovaries following IR induction in a rat model. However, they found that these pathological changes were partially ameliorated with boric acid treatment. In parallel with the above study, our findings were similar. The extent of damage was reduced in the uteri of rats treated with boric acid. This situation indicates that boric acid has a protective effect in the uterus, as it does in the ovaries.

This study has some limitations. The first one is that the evaluations were based only on histopathological findings. No molecular techniques were performed to assess DNA damage, inflammation markers, apoptotic enzymes or antioxidant capacity. Second limitation is that a single boron dose and administration route were tested. Although the dose was based on the literature, the effects of different doses and application routes remain unknown. Third, only one IR duration was tested. These limitations make it necessary to reveal the effects of boric acid in the uterus with different studies.

In conclusion, our findings suggest that boric acid treatment exerts protective effects against uterine IR injury in rats. Especially, the preservation of endometrial tissue implies potential fertility protective properties. In addition, the reduction of inflammation score supports that this trace element may have anti-inflammatory effects. Although all these results offer the therapeutic potential of boric acid, obtained data provide promising results at the preclinical level. Future studies involving different doses, routes, and durations of boron administration are crucial to confirm its therapeutic potential, particularly in clinical and veterinary applications.

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