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RESEARCH

Ameliorative effect of boric acid on acute cadmium-induced oxidative stress, inflammation, histopathological injury and proapoptotic changes in rat liver

Sıçan karaciğerinde akut kadmiyum kaynaklı oksidatif stres, inflamasyon, histopatolojik hasar ve proapoptotik değişiklikler üzerine borik asidin iyileştirici etkisi

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Abstract

Purpose: This study aims to investigate the ameliorative effects of boric acid (BA) on cadmium (Cd)-induced hepatotoxicity were investigated in rats.

Materials and Methods: Twenty-eight male Wistar albino rats were randomly assigned to four groups (n=7 each): Control; Cd (single oral dose of Cd 15 mg/kg by gavage, no further treatment for 7 days); BA (boric acid 200 mg/kg/day intraperitoneally for 7 days); and Cd+BA (combined cadmium and boric acid). Serum malondialdehyde (MDA) and total oxidant status (TOS) were measured to assess oxidative stress.

Results: MDA and TOS levels were highest in the Cd group compared with the other groups, while these levels were reduced in the Cd+BA group. AST levels were significantly elevated in the Cd group (194.42 U/L) compared with the Cd+BA group (110.57 U/L). Similarly, LDH levels were significantly lower in the Cd+BA group. Histopathological evaluation of liver tissues showed markedly higher damage scores in the Cd group, whereas inflammatory and proapoptotic changes were less pronounced in the Cd+BA group.

Conclusion: These findings suggest that BA administration after acute Cd exposure may reduce hepatic injury by lowering oxidative stress, inflammation, and apoptosis.

Keywords: Cadmium, boric acid, inflammation, oxidative stress, hepatotoxicity

Öz

Amaç: Bu çalışmada, sıçanlarda borik asitin (BA), kadmiyum (Cd) kaynaklı hepatotoksisite üzerindeki iyileştirici etkileri araştırılmışitır.

Gereç ve Yöntem: Yirmi sekiz erkek Wistar albino sıçan rastgele dört gruba ayrıldı (her biri n=7): Kontrol; Cd (15 mg/kg Cd tek doz oral olarak gavaj ile verildi ve 7 gün boyunca ek tedavi uygulanmadı); BA (7 gün boyunca günde 200 mg/kg borik asit intraperitoneal olarak verildi); ve Cd+BA (kadmyum ve borik asidin birlikte uygulandığı grup). Oksidatif stres düzeyini değerlendirmek için serum malondialdehit (MDA) ve total oksidan durum (TOS) analiz edildi.

Bulgular: MDA ve TOS düzeyleri Cd grubunda diğer gruplara kıyasla daha yüksek bulunurken, bu düzeyler Cd+BA grubunda daha düşüktü. AST düzeyleri Cd grubunda (194.42 U/L), Cd+BA grubuna (110.57 U/L) göre anlamlı derecede daha yüksekti. Benzer şekilde, LDH düzeyleri de Cd+BA grubunda anlamlı olarak daha düşüktü. Karaciğer dokularının histopatolojik incelemesi, Cd grubunda hasar skorlarının belirgin şekilde daha yüksek olduğunu, Cd+BA grubunda ise inflamatuvar ve proapoptotik değişikliklerin daha hafif olduğunu göstermiştir.

Sonuç: Bu bulgular, akut Cd maruziyetinden sonra uygulanan BA'nın oksidatif stresi, inflamaayonu ve apoptozu azaltarak karaciğer hasarını hafifletebileceğini düşündürmektedir.

Anahtar kelimeler: Kadmiyum, borik asit, inflamasyon, oksidatif stres, hepatotoksisite

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INTRODUCTION

Cadmium (Cd) is a major environmental pollutant that poses serious health hazards to living organisms. With increasing industrialization worldwide, the concentration of this metal in nature has increased significantly. Mining-related industries, batteries, and pigment and ceramic manufacturing are major sources of cadmium release into the environment. Due to its non-biodegradable nature and increasing environmental emission, the risk of cadmium exposure to living organisms is gradually increasing[1,2]. Metals such as cadmium are important contributors to toxicity and oxidative stress[3,4]. Cd exerts its toxic effects by binding to oxygen-, nitrogen-, and sulfur-containing ligands in enzymes and proteins[5], interfering with essential bioelements[6], dysregulating apoptotic pathways[7], and inducing DNA damage as well as impairing DNA repair mechanisms[8].

Boric acid (H3BO3) is a naturally occurring boron compound found in the environment and in various foods. Boron is readily absorbed from the gastrointestinal tract[9], and in aqueous solution it predominantly exists as boric acid (BA)[10]. BA is generally considered to be non-toxic at low concentrations in humans[11]. It has long been recognized that boron-containing substances exhibit biochemical activity in both in vivo and in vitro settings[12]. Boron-containing compounds are increasingly investigated and used in anticancer and anti-inflammatory applications[13]. They are also known to have antioxidant[14], hepatoprotective, and antigenotoxic effects[15]. In this study, we investigated the mitigating effects of BA on Cd-associated hepatotoxic damage in rats.

Despite growing interest in the biological effects of boron-containing compounds, evidence regarding the potential therapeutic role of boric acid in cadmium-induced acute hepatotoxicity remains limited. In particular, the extent to which boric acid can modulate oxidative stress, inflammatory signaling, and apoptosis-related pathways following acute cadmium exposure has not been fully clarified. Therefore, the present study aimed to investigate the ameliorative effects of boric acid on cadmium-induced liver injury using a comprehensive experimental approach including biochemical, histopathological, and immunohistochemical analyses. We hypothesized that boric acid administration after cadmium exposure would attenuate oxidative stress–mediated hepatic injury and reduce inflammatory and proapoptotic responses in liver tissue.

MATERIALS AND METHODS

This experimental animal study was carried out at the Experimental Animal Research Center of Dicle University, Diyarbakır, Türkiye. All animal procedures complied with institutional and national guidelines and were approved by the Dicle University Animal Experiments Ethics Committee (DÜHADEK) on 24/02/2022 (Decision No.: 1, Protocol No.: 2021/38).

Sample

The sample size was determined based on previous experimental studies investigating cadmium-induced hepatotoxicity and the protective effects of antioxidant agents in rat models. Considering similar study designs and commonly used group sizes in the literature, seven animals were allocated to each experimental group to ensure adequate statistical power while adhering to the principles of reduction in animal research. Thus, a total of 28 rats were included in the study and randomly divided into four groups.

The rats were randomly selected and divided into four groups with an equal number of rats in each group. Rats in the Cd group received a single dose of Cd, while rats in the BA group received BA for seven days.

Group 1 (n=7): In the control group, no medication was administered. Group 2 (n=7): Cd was administered as a single dose of 15 mg/kg on the first day of the experiment and no other drug was administered during the experiment. The dose of Cd administered was based on literature data (18,19). Group 3 (n=7): The BA group received BA i.p. at a dose of 200 mg/kg daily for seven days. Group 4 (n=7): Cd+BA group, Cd 15 mg/kg/dose was given orally and BA was started immediately thereafter. BA treatment was initiated immediately after Cd administration and then administered intraperitoneally at a dose of 200 mg/kg/day for 7 consecutive days to evaluate its post-exposure ameliorative effect. Animals were sacrificed 24 hours after the 7th day of the experiment under general anaesthesia (Ketamine HCl 90 mg/kg (Ketalar, Pfizer Inc, USA) +Xylazine HCl 10 mg/kg (Rompun, Bayer

Health Care AG, Germany). The medical wastes were collected and disposed of according to the appropriate protocol.

Procedure

In this study, a total of 28 male Wistar albino rats weighing 320–400 g were used. The animals were housed in stainless steel cages under standard controlled conditions (temperature 22±2 °C, humidity 50%, 12-hour light-dark cycle), without movement restriction and with free access to feed and drinking water.

Each rat was weighed separately and their body weights were recorded and drug doses were calculated according to previously published studies. Animal welfare was maintained throughout the study and the researchers used protective equipment against the chemicals applied. All experimental procedures, including cadmium administration, boric acid treatment, and animal handling, were performed by researchers experienced in laboratory animal experiments. Biochemical analyses were conducted by trained laboratory technicians, while histopathological and immunohistochemical evaluations were carried out by an experienced histologist who was blinded to the study groups.

Determination of cadmium dose

Cadmium used in the study was obtained from Istanbul, Turkey, with 99.9% purity (CAS No: 7440-43-9). Cadmium was administered orally by gavage at a dose of 15 mg/kg body weight at the beginning of the experiment, and the animals were observed for seven days without any additional treatment to evaluate toxic effects[16].

Application of boric acid

BA used was obtained from Bursa, Turkey, with 99.5% purity (CAS No: 10043-35-3). It was administered intraperitoneally (i.p.) with a daily dose of 200 mg/kg for seven days. This dose of boric acid has been reported to be non-lethal and non-toxic[17].

Preparation of blood samples

Blood samples collected intracardially after anaesthesia were placed in a tube containing gel coagulation activator and separator and centrifuged at 3000 rpm for 10 minutes. The separated plasma was divided into two different Eppendorf tubes. Serum samples were separated and stored at -80 °C for biochemical analyses. Total antioxidant status (TAS), total oxidant status (TOS) and malondialdehyde (MDA) levels were analyzed in one Eppendorf tube and aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) levels were analyzed in the second Eppendorf tube. Liver tissues were placed in 10% formaldehyde solution and sent to histology laboratory for histopathological examinations.

Histochemical methods

Liver tissues fixed in 10% formaldehyde solution were subjected to routine histological follow-up as described by Bilge et al.[18]. The 5-µm-thick tissue sections taken on positively charged slides were deparaffinized in an oven at 58°C for 1 hour before staining and hematoxylin and eosin (H&E) staining protocol was applied for routine histological evaluation. Each section was examined under a light microscope (Zeiss Imager A2, Germany) and the method described by Camargo et al. was used for tissue damage scoring[19].

Immunohistochemical methods

After routine histological tissue follow-up, sections taken on positively charged slides were heated in ethylenediamine tetraacetic (EDTA) solution in a microwave oven for 15 minutes and then kept at room temperature. After washing in buffered phosphate saline (PBS) solution, tissue borders were determined with a hydrophobic pen. Tumor necrosis factor-alpha (TNF-α) (Santa Cruz), a pro-inflammatory cytokine, and apoptotic protease-activating factor-1 (APAF-1) (Santa Cruz) primary antibodies were incubated and kept overnight at +4 °C[20]. In immunohistochemical examinations, the damage in the liver tissues of rats was evaluated as follows: 0.1: <25%, 0.4: 26-50%, 0.6: 51-75%, 0.9: 76-100%, and the severity of immunoreactivity was scored as 0: none, +0.5: very little, +1: little, +2: moderate, +3: severe[21].

Total oxidant status (TOS)

TOS mmol/L (millimolar/litre) amounts in serum samples were determined by Rel Assay Diagnostic (Gaziantep, Turkey) kit. The method developed by Erel was used[22].

Total antioxidant status (TAS)

This method is used to measure the antioxidant margin of the organism against free radicals. The TAS µmol/L (micromolar/litre) amounts of serum samples were studied with Rel Assay Diagnostic

(Gaziantep, Turkey) kit. The method developed by Erel was used[23].

Measurement of oxidative stress index (OSI)

Evaluation of the oxidative stress index was calculated according to the formula[24] OSI=(TOS/TAS) x 100.

Measurement of blood serum MDA values

During MDA analysis; 0.5 ml serum sample was mixed using a vortex mixer and 2.5 ml of 20% tricholoroacetic acid (TCA) was added. Then, 1 ml of 0.6% thiobarbituric acid (TBA) was added and mixed using a vortex mixer for 10 minutes and kept in boiled water for half an hour. Data were calculated as (µmol/L) using dilution factors and extinction coefficient (1.56x105).

Statistical analysis

Statistical analyses were performed using SPSS version 20.0 (IBM Corp., Armonk, NY, USA). The normality of data distribution was assessed using the Shapiro–Wilk test. Variables showing normal distribution were expressed as mean ± standard deviation and analyzed using one-way analysis of variance (ANOVA) followed by appropriate post-hoc comparisons. Variables not showing normal distribution were expressed as median (minimum–maximum) values and analyzed using the Kruskal–Wallis test. When a significant difference was detected, pairwise comparisons between groups were performed using the Mann–Whitney U test with Bonferroni correction. A p value <0.05 was considered statistically significant.

RESULTS

Comparison of AST values showed a statistically significant difference between the Cd and Cd+BA groups (p=0.006) and the highest AST value was observed in the Cd group (194.42 U/L) (p<0.05, Table 1). Since the Kruskal-Wallis test was not significant for ALT values (p=0.258), no comparison was made between the groups (p>0.05, Table 1).

Table 1. Mean ± standard deviation values of liver enzymes (AST, ALT, LDH) levels.

Groups	AST (U/L)	ALT (U/L)	LDH (U/L)
Control	104.28±9.44[b]	45.57±7.48[AD]	393.57±131.58[b]
Cd	194.42±47.66[a,c,d]	58.28±21.63[AD]	1355.85±377.75[a,c,d]
BA	106.85±12.10[a,b]	39.85±5.45[AD]	429.71±229.60[b]
Cd+BA	110.57±34.36[b]	42.14±7.19[AD]	543.00±116.89[b]

Values are presented as mean± standard deviation. Superscript letters indicate statistically significant differences in pairwise comparisons (p<0.05): a vs Control, b vs Cd, c vs BA, d vs Cd+BA. AD indicates no statistically significant difference among groups for the relevant parameter (p>0.05); AST, aspartate aminotransferase; ALT, alanine aminotransferase; LDH, lactate dehydrogenase; BA, boric acid; Cd, cadmium.)

LDH levels were highest in the Cd group (1355.85 U/L) and significantly lower in the Cd+BA group (543.00 U/L) than in the Cd group. When the two groups were compared, this difference was statistically significant (p=0.003). (p<0.05, Table 1).

The normality examination of the statistically analyzed TAS values showed a normal distribution according to the Shapiro-Wilk test. As a result, ANOVA test was performed and the statistical analysis of the test result was not significant (p>0.05, Table 2). TOS values were highest in the Cd group (178.21 mmol/L) and the mean of the Cd+BA group (32.10 mmol/L) was significantly lower (p=0.002) than the Cd group (p<0.05, Table 2).

OSI values were calculated according to the method described by Tufek et al. As a result, when the Cd group was compared with the Cd+BA group, a significant difference was observed between the OSI values of the two groups (p=0.002). Although TAS values were not significant in the Cd+BA group, OSI values were significantly reduced (p>0.05, Table 2).

Statistical analysis of MDA values showed that the highest mean MDA level occurred in the Cd group (1.63 µmol/L), while the mean MDA value in the Cd+BA group (1.06 µmol/L) was significantly lower than the Cd group. In the comparison between the groups, the Cd group showed significantly higher values than the Cd+BA group. (p<0.05, Table 2).

The mean difference between the control group and BA group (p=0.442) was not significant, and Cd+BA group was positively affected in terms of MDA values (p>0.05, Table 2).

Table 2. Mean ± standard deviation values of TAS, TOS, OSI, MDA levels.

Groups	TAS (µmol/L)	TOS (mmol/L)	OSI (AU)	MDA (µmol/L)
Control	1.24±0.27^{AD}	48.63±26.34^{b}	3929.66±2058.89^{b}	1.19±0.08^{b}
Cd	1.10±0.12^{AD}	178.21±46.45^{a,c,d}	16436.02±5306.42^{a,c,d}	1.63±0.24^{a,c,d}
BA	1.28±0.25^{AD}	54.45±27.09^{b}	4224.44±2112.54^{b}	1.15±0.15^{b}
Cd+BA	1.21±0.21^{AD}	32.10±11.91^{b}	2654.42±905.59^{b}	1.06±0.22^{b}

Values are presented as mean± standard deviation. Superscript letters indicate statistically significant differences in pairwise comparisons (p<0.05): a vs Control, b vs Cd, c vs BA, d vs Cd+BA. AD indicates no statistically significant difference among groups for the relevant parameter (p>0.05).; TAS, total antioxidant status; TOS, total oxidant status; OSI, oxidative stress index; MDA, malondialdehyde; BA, boric acid; Cd, cadmium.)

All liver tissues were examined individually under a light microscope. The histological damage score of the Cd group was significantly higher than that of the Cd+BA group (p=0.002). The damage scoring was 3.14 in the Cd group and 2.14 in the Cd+BA group (p<0.05, Table 3).

Table 2. Mean ± standard deviation values of TAS, TOS, OSI, MDA levels.

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Control	1.24±0.27^{AD}	48.63±26.34^{b}	3929.66±2058.89^{b}	1.19±0.08^{b}
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BA	1.28±0.25^{AD}	54.45±27.09^{b}	4224.44±2112.54^{b}	1.15±0.15^{b}
Cd+BA	1.21±0.21^{AD}	32.10±11.91^{b}	2654.42±905.59^{b}	1.06±0.22^{b}

Values are presented as mean± standard deviation. Superscript letters indicate statistically significant differences in pairwise comparisons (p<0.05): a vs Control, b vs Cd, c vs BA, d vs Cd+BA. AD indicates no statistically significant difference among groups for the relevant parameter (p>0.05); TAS, total antioxidant status; TOS, total oxidant status; OSI, oxidative stress index; MDA, malondialdehyde; BA, boric acid; Cd, cadmium.)

Examination of H&E-stained liver sections showed that the control group sections (Figure 1) and BA group had normal histological appearance (Figure 2), but histological damage occurred in each section of the Cd group. Histopathological changes such as haemorrhage, congestion, mononuclear cell infiltration, degeneration of the central vein, pycnosis in hepatocyte nuclei, dilatation in the sinusoidal space were observed in the tissues of the Cd group (Figure 3). However, in the Cd+BA group, the analysis revealed that the pathological changes were improved and only mild congestion persisted (Figure 4).

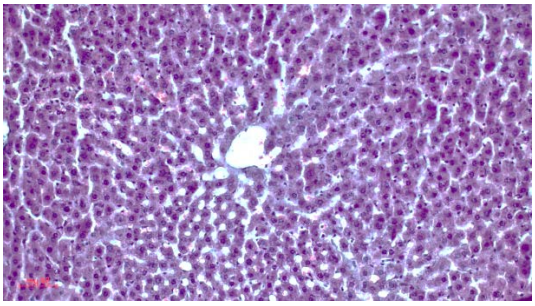

Figure 1. Representative liver histology of the control group. Bar: 50 µm

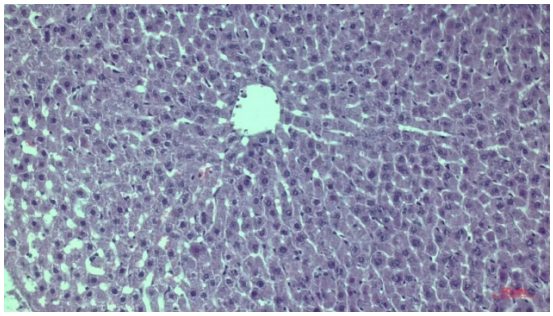

Figure 2. Liver histology of the boric acid group. (H&E, Bar: 50 µm)

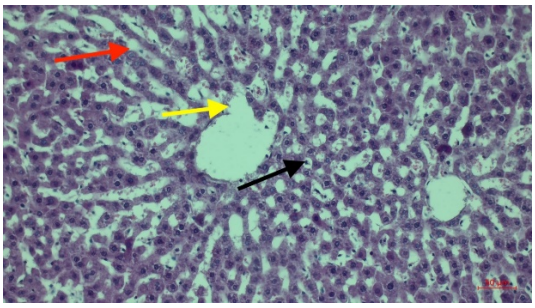

Figure 3. Micrograph of liver tissue of cadmium group, sinusoidal dilatation (red arrow), degeneration of the central vein (yellow arrow), pyknosis in hepatocyte nuclei (black arrow). (H&E, Bar: 50 µm).

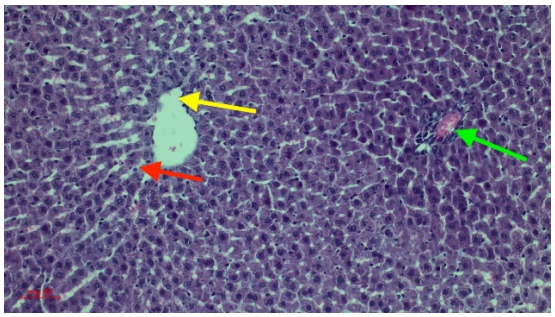

Figure 4. Cadmium+Boric acid group, persistent congestion was observed (green arrow), degeneration of the central vein appeared to improve (yellow arrow), the dilatation in the sinusoidal arrangement improved and took a near normal appearance (red arrow). (H&E, Bar: 50 µm).

Histoscopic evaluation of immunological changes in the liver tissue (summarized in Table 3) showed that the most severe damage occurred in the Cd group (3.14±0.3), while the damage in the Cd+ BA group (2.14±0.3) was significantly lower ($p=0.002$) ($p<0.05$, Table 3).

Table 3. Mean ± standard deviation values determined by statistical analysis of liver injury scoring, TNF-α and APAF-1 histoscoring tissues.

Groups	Liver Damage Scoring	TNF-α	APAF-1
		Histoscoring	Histoscoring
Control	$0.00\pm0.00^{b,d}$	$0.01\pm0.02^{b,d}$	$0.01\pm0.00^{b,d}$
Cd	$3.14\pm0.37^{a,c,d}$	$2.10\pm0.60^{a,c,d}$	$0.32\pm1.45^{a,c,d}$
BA	$0.14\pm0.37^{b,d}$	$0.02\pm0.02^{b,d}$	$0.02\pm0.01^{b,d}$
Cd+BA	$2.14\pm0.37^{a,b,c}$	$0.71\pm0.25^{a,b,c}$	$0.13\pm0.62^{a,b,c}$

Values are presented as mean± standard deviation. Superscript letters indicate statistically significant differences in pairwise comparisons ($p<0.05$): **a** vs Control, **b** vs Cd, **c** vs BA, **d** vs Cd+BA; Cd, cadmium; BA, boric acid; TNF-α, tumor necrosis factor-alpha; APAF-1, apoptotic protease-activating factor-1.)

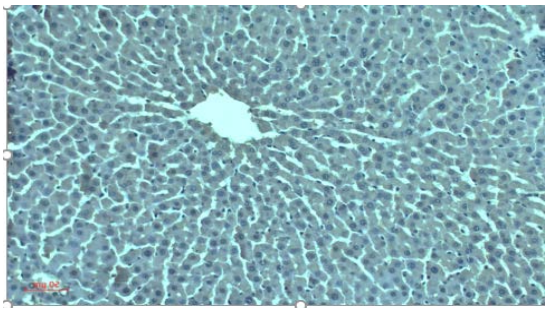

Figure 5. Light microscopic image of liver tissue (Staining: TNF-α, Counterstaining: Hematoxylin, Bar: 50 µm). Control group.

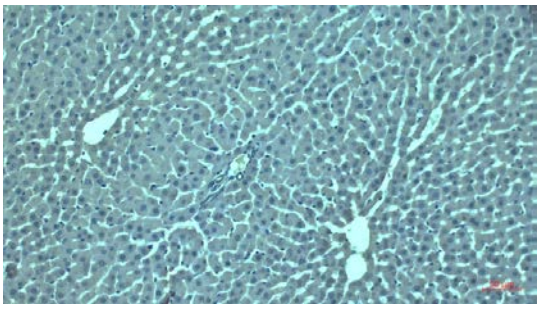

Figure 6. Light microscopic image of liver tissue (Staining: TNF-α, Counterstaining: Hematoxylin, Bar: 50 µm). Boric acid group, the prevalence of TNF-α positive expressions was very mild and the severity was very low.

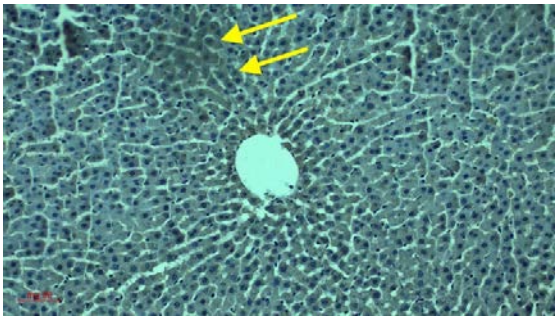

Figure 7. Light microscopic image of liver tissue (Staining: TNF-α, Counterstaining: Hematoxylin, Bar: 50 µm). Cadmium group, the severity and prevalence of TNF-α positive expressions were observed at a diffuse level (yellow arrow).

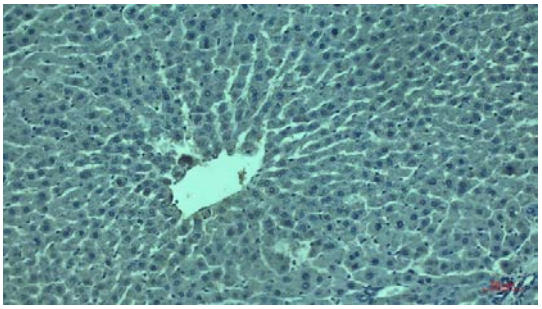

Figure 8. Light microscopic image of liver tissue (Staining: TNF-α, Counterstaining: Hematoxylin, Bar: 50 µm). Cadmium+Boric acid group, the severity and prevalence of TNF-α positive expressions were observed at a low level.

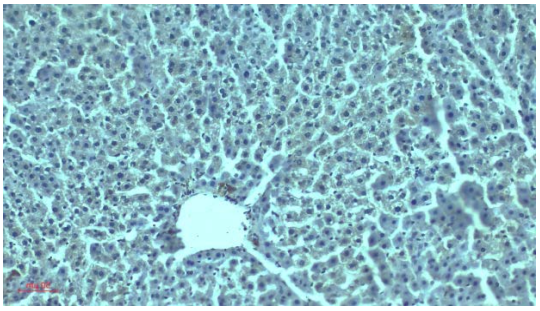

Figure 9. Light microscopic image of liver tissue (Staining: APAF-1, Counterstaining: Hematoxylin, Bar: 50 µm). Control group APAF-1 positive expressions were very mild and very low in intensity.

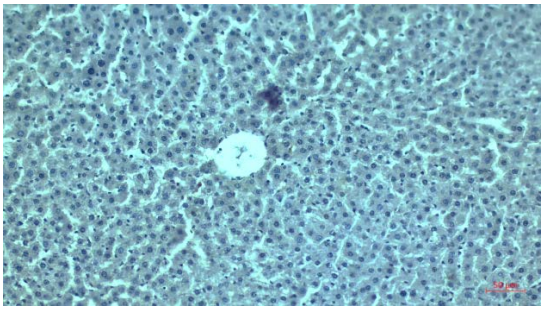

Figure 10. Light microscopic image of liver tissue (Staining: APAF-1, Counterstaining: Hematoxylin, Bar: 50 µm). Boric acid group APAF-1 positive expressions were very mild and very low in intensity.

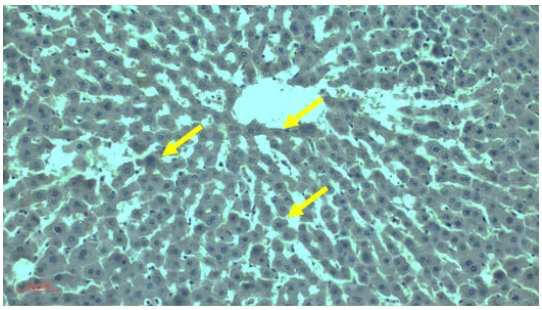

Figure 11. Light microscopic image of liver tissue (Staining: APAF-1, Counterstaining: Hematoxylin, Bar: 50 µm). Cadmium group APAF-1 positive expressions were moderate in intensity and prevalence (yellow arrow).

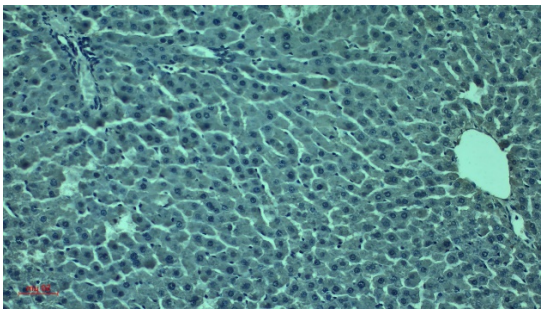

Figure 12. Light microscopic image of liver tissue (Staining: APAF-1, Counterstaining: Hematoxylin, Bar: 50 µm). Cadmium+Boric acid group APAF-1 positive expressions were observed at low level (yellow arrow).

Light microscopic examination of the tissues showed very weak TNF-α and APAF-1 immunoreactivity in the control and BA groups. In the Cd group, strong TNF-α immunoreactivity (Figure 5,6,7,8) and APAF-1 (Figure 9,10,11,12) expressions were observed, whereas in the Cd+BA group, the expression levels

were reduced compared to the Cd group, but not as much as in the control or BA group.

DISCUSSION

Cadmium (Cd) is a toxic environmental metal whose environmental levels have increased markedly since the 20th century due to industrialization and anthropogenic activities. Cd exerts hepatotoxic effects through several interconnected mechanisms, including binding to sulfhydryl groups in proteins and enzymes, disruption of essential metal homeostasis, and induction of oxidative stress[25,26]. Excessive production of reactive oxygen species can lead to lipid peroxidation, DNA damage, mitochondrial dysfunction, and activation of apoptotic pathways[27]. Because of its persistence in the environment and its tendency to accumulate in biological tissues such as the liver and kidneys, cadmium exposure represents an important public health concern[27,28].

At the cellular level, Cd-related hepatotoxicity is frequently accompanied by mitochondrial dysfunction, cytochrome c release, and activation of the intrinsic (mitochondria-mediated) apoptotic cascade. In this pathway, APAF-1 serves as a central scaffold for apoptosome formation and subsequent caspase-9 activation[29]. Consistent with this mechanism, cadmium-induced oxidative stress has been linked to apoptosis and DNA damage in hepatic cells[30], and inflammatory responses mediated by TNF-α–related signaling pathways have also been implicated in cadmium toxicity[31].

The biochemical activities of boron-containing compounds have long been recognized in both in vivo and in vitro studies[32]. BA exhibits antioxidant, hepatoprotective, antigenotoxic, anti-inflammatory, and antineoplastic effects[14-16,33,34]. Kar et al. demonstrated the anti-inflammatory effect of BA in their study[35]. Türkez et al. investigated the efficacy of BA on hepatotoxicity induced by aluminium and found that it was effective[36]. In the present study, it was determined that BA demonstrated protective effects against cadmium-induced hepatotoxicity with TOS and MDA parameters, which are oxidative stress markers. TOS values, which reflect oxidative stress as described by Erel, were significantly higher in the Cd group. The Cd group showed significantly higher values than the Cd+BA group[22,23].

MDA is a highly reactive product formed during lipid peroxidation. MDA exerts several harmful effects such as disruption of permeability by affecting ion exchange in cell membranes and gaining a mutagenic character by reacting with DNA bases[37]. MDA levels were highest in the Cd group and there was a significant difference between the other study groups.

Zhang et al. observed that the oedema in hepatocytes gradually increased after Cd exposure and the degree of cell necrosis increased in parallel when Cd dose was increased. They also reported that chronic inflammatory changes occurred when Cd exposure was increased to 20 mg/kg[38]. In this study, Cd was administered at a dose of 15 mg/kg, it was found that histopathological alterations such as haemorrhage and congestion, mononuclear cell infiltration occurred in liver tissues and TNF-α expression, which is one of the inflammatory cytokines, increased.

These light microscopic findings are consistent with the spectrum of Cd-associated hepatic injury reported in experimental models, including sinusoidal dilatation/congestion, hepatocellular swelling (ballooning/vacuolar degeneration), focal necrosis, and inflammatory cell infiltration. Importantly, such lesions can co-exist with apoptotic bodies that are more specifically reflected by proapoptotic immunomarkers, including APAF-1. These histological alterations may be interpreted as morphological correlates of oxidative injury and mitochondrial dysfunction, complementing the biochemical evidence of increased lipid peroxidation (MDA) and global oxidant burden (TOS/OSI)[39].

Söğüt et al. also investigated the potential hepatoprotective effects of boric acid in a rat model of hepatotoxicity induced by chronic alcohol exposure. In their study, MDA, total sialic acid (TSA), and the antioxidant enzymes superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) were evaluated as markers of alcohol-related hepatic injury. In addition, caspase-3 and TNF-α levels were assessed as apoptosis- and inflammation-related biomarkers. Compared with the control group, the ethanol group exhibited increased MDA, TSA, and TNF-α levels, whereas SOD and CAT activities were decreased. In the ethanol + boric acid group, MDA, TSA, caspase-3, and TNF-α levels were reduced, while SOD and CAT activities were increased compared with the ethanol group. Furthermore, histopathological evaluation of light microscopy images demonstrated reduced immunohistochemical caspase-3 and TNF-α activity

in the ethanol + boric acid group relative to the ethanol group[34].

In the present study investigating the effects of BA on Cd-associated hepatotoxicity, we evaluated our samples using a comprehensive approach including biochemical parameters (AST, ALT, LDH), histological staining (H&E), and immunohistochemical markers (APAF-1, TNF-α). To further characterize oxidative stress, we measured TAS and TOS and calculated the OSI. Additionally, we assessed MDA levels to strengthen the evaluation of lipid peroxidation. Based on this multi-dimensional analysis, BA administration initiated after Cd exposure was found to significantly ameliorate oxidative stress, inflammatory signaling, and proapoptotic changes, supporting its therapeutic potential in acute Cd-mediated liver injury.

Güler et al. reported that BA prevented biomolecular changes caused by liver ischaemia[40]. Ince et al. showed that BA had protective activity against liver damage caused by carbon tetrachloride (CCl_4)[41]. Söğüt et al. reported that BA had antioxidant and antiapoptotic effects when they investigated hepatoxicity of rats fed with chronic alcohol[42]. In the present study, apoptotic and inflammatory damage in the liver tissues were analyzed. The Cd group showed the most severe damage, whereas BA administration significantly reduced the extent and severity of the damage. BA significantly improved histopathological and immunohistochemical changes in liver tissue.

Taken together, the concurrent improvement in oxidative stress indices (TOS/OSI, MDA), inflammatory readouts (TNF-α), and apoptosis-related staining (APAF-1) supports a model in which BA mitigates Cd-related injury by dampening redox-driven inflammatory signaling and limiting intrinsic apoptotic execution in hepatocytes[30].

This study has several limitations that should be acknowledged. First, the relatively small sample size may limit the generalizability of the findings. Second, the experimental design evaluated only the short-term effects of boric acid following acute cadmium exposure, and therefore the long-term protective or therapeutic effects could not be assessed. Third, although biochemical, histopathological, and immunohistochemical analyses were performed, additional molecular markers related to oxidative stress and apoptosis could provide a more comprehensive understanding of the underlying mechanisms. Finally, as this study was conducted in an experimental animal model, the findings may not be directly extrapolated to human clinical conditions.

Overall, the findings of the present study demonstrate that acute cadmium exposure leads to marked oxidative stress, inflammatory activation, and hepatocellular injury in the liver tissue. Administration of boric acid immediately after cadmium exposure significantly attenuated these pathological changes, as evidenced by improvements in oxidative stress markers, inflammatory mediators, and apoptosis-related parameters. These results suggest that boric acid may exert a protective or therapeutic effect by modulating redox balance and limiting inflammation- and apoptosis-related pathways involved in cadmium-induced hepatotoxicity. Nevertheless, further experimental and clinical studies are required to clarify the precise molecular mechanisms and to evaluate the potential translational relevance of these findings in human cadmium exposure.

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Ethical Approval: All animal procedures complied with institutional and national guidelines and were approved by the Dicle University Animal Experiments Ethics Committee (DÜHADEK) on 24/02/2022, Decision No.: 1, Protocol No.: 2021/38. Efforts were made to minimize animal suffering and reduce animal numbers. All procedures involving animals were carried out in strict accordance with relevant ethical guidelines and regulations.
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