RESEARCH ARTICLE / *Araştırma Makalesi*

Boric Acid Ameliorates Liver Injury in Rat Induced by Cyclophosphamide

Borik Asit Sıçanlarda Siklofosfamidin Neden Olduğu Karaciğer Hasarını İyileştirir

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Abstract

Introduction — The present study aimed to look into any potential ameliorative benefits of boric acid on liver damage in rats caused by cyclophosphamide (CTX).

Materials and Methods — Four groups, control, boric acid, CTX, and boric acid+CTX, were created. Female Wistar albino rats were given daily injections of CTX (75 mg/kg) to create the liver damage model. Cyclophosphamide (75 mg/kg) was administered intraperitoneally, and boron (1.3 g/rat/day) was administered by gavage every day for two weeks in the boric acid+CTX group. The histopathological changes were evaluated in liver tissue staining with hematoxylin and eosin, Masson trichrome, and periodic acid Schiff. In addition, we assessed liver tissue enzyme activity as malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px).

Results — Images in the boric acid+CTX group had lower histological evaluations than those in the CTX group under the light microscope. According to the findings, boric acid reduced MDA levels in the liver tissues. Additionally, boric acid improved the actions of oxidative stress indicators to reduce oxidative stress brought on by CTX and upregulated antioxidant parameters.

Conclusion — In conclusion, our study has demonstrated that CTX- induced liver injury can be alleviated by reducing the tissue MDA levels, and increasing the liver's SOD, GSH-Px, and CAT activities. In order to reduce the liver damage caused by CTX, boric acid may be administered as a dietary supplement or functional food.

Keywords — Boric acid; Cyclophosphamide; Liver injury

Öz

Amaç — Mevcut çalışmanın amacı, borik asidin sıçanlarda siklofosfamidin (CTX) neden olduğu karaciğer hasarı üzerindeki olası iyileştirici etkilerini araştırmaktır.

Yöntem ve Gereçler — Çalışmada dört grup oluşturuldu; kontrol, borik asit, CTX ve borik asit+CTX. Karaciğer hasarı modelini oluşturmak için Wistar albino cinsi dişi ratlara günlük 75 mg/kg CTX intraperitoneal (i.p.) olarak verildi. Borik asit+CTX grubuna 75 mg/kg CTX (i.p.) ve 1,3 g/rat/gün boron gavaj ile iki hafta boyunca her gün verildi. Karaciğer dokusundaki histopatolojik değişiklikler hematoksilen ve eozin, masson trikrom ve periyodik asit-Schiff boyamaları ile değerlendirildi. Karaciğer dokusunda malondialdehit (MDA), süperoksit dismutaz (SOD), katalaz (CAT) ve glutatyon peroksidaz (GSH-Px) enzim aktiviteleri belirlendi.

Bulgular — Işık mikroskobunda borik asit+CTX grubundaki histolojik değişikliklerin CTX grubuna göre daha düşük olduğu belirlendi. Bulgulara göre borik asit, karaciğer dokularındaki MDA seviyesini azalttığı gözlendi. Buna ek olarak, borik asit, CTX'in neden olduğu oksidatif stresi azaltmak için oksidatif stres belirteçlerinin etkilerini iyileştirdiği ve antioksidan parametrelerini artırdığı belirlendi.

Sonuç — Sonuç olarak, çalışmamız, CTX'in neden olduğu karaciğer hasarının, doku MDA seviyelerinin düşürülmesi, karaciğerin SOD, GSH-Px ve CAT aktivitelerinin arttırılması ile hafifletilebileceğini göstermiştir. CTX'in neden olduğu karaciğer hasarını azaltmak için borik asit, diyet takviyesi veya fonksiyonel gıda olarak verilebilir.

Anahtar Kelimeler — Borik asit, Karaciğer hasarı, Siklofosfamid.

INTRODUCTION

One of the most prevalent diseases in society, liver disease is a frequent health issue. Chemical toxins can readily harm the liver, which results in metabolic and coagulatory diseases.[1] Cyclophosphamide (CTX), an anticancer medication, is the standard of care for a variety of human malignant tumors.[2] However high the therapeutic index of CTX and variety of CTX's medical applications, its clinical use is limited because of the drug's side effects, which include immunotoxicity, bone marrow suppression, cardiotoxicity, nephrotoxicity, and neurotoxicity.[3,4] Since CTX and its metabolites are mostly metabolized and excreted through the liver and kidney, hepatotoxicity and nephrotoxicity are two of the most common adverse effects.[5] High doses of CTX exposure can cause immediate hepatotoxic consequences, characterized by liver inflammation and oxidative stress caused by the production of inflammatory cytokines and free radicals.[6] According to reports, phosphoramide mustard has antineoplastic properties, and acrolein adds to the hepatotoxicity brought on by CTX.[7] The production of reactive oxygen species (ROS) by acrolein and the inhibition of antioxidant defense systems are closely connected processes known as lipid peroxidation.[8] The disruption of the glutathione defense system, oxidative stress, and apoptosis can also result from ROS production.[9] Therefore, finding natural substances to reduce CTX-induced hepatotoxicity would be very important.

A non-metallic element, borax (sodium tetraborate; Bx) and boric acid (BA) are two natural forms. Foods are rich in boron, which has been found to be present in human plasma at levels between 10 and 20 M.[10] The physiological effects of boron were the subject of several investigations.[11] Additionally, boron has been found to have anticancer, antigenotoxic, anti-oxidant, and hepatoprotective properties by various studies.[12,13] Therefore, we wanted to study the role of oxidant/antioxidant pathways and the potential curative benefit of BA in the treatment of CTX toxicity.

MATERIALS and METHODS

Chemicals and reagents

In this work, we used Wistar albino female rats (16–24 weeks old, average body weight 250 g) from the Erciyes University's animal house. The Erciyes University's Animal Research Ethics Committee approved this work (No:23/013). Rat feed and filtered tap water were readily available, and the rats were kept under temperature control (24 °C), relative humidity control (50% relative humidity), and lighting control (12 hours of light and 12 hours of darkness). Application of BA: MIAFERT provided the boric acid solution for human food directly. In the study, the treatment groups received daily oral gavage administration of 1.3 g/rat/day of 200 µL of liquid BA solution under the MIAFERT brand. In the CTX group, sterile physiological saline was used to dissolve CTX and administered at a dose of 75 mg/kg/day for two weeks by intraperitoneal injection (Sigma-Aldrich, St. Louis, MO, USA).

Animal experimental design

The rats were assigned to four groups randomly (n = 10) (temperature: 23 25 °C, 12-hour light/dark cycle):

- Group 1 (the control group, n = 10); normal saline was administered orally.
- Group 2 (the CTX group, n = 10); 75 mg/kg, intraperitoneally, CTX was given once a week on the 1st and 8th days of the experiment.[14,15]
- Group 3 (the Boric acid group, n = 10), received 1.3 g/rat/day and 200 µL boric acid orally every day by gavage for 15 days.[16,17]
- Group 4 (the CTX+ Boric acid group, n = 10), received 75 mg/kg intraperitoneally once a week for two weeks on the 1st and 8th days, and 1.3 g/rat/day of 200 µL boric acid was administered orally by gavage every day for 15 days.

The study rats were sacrificed by cervical dislocation, and liver tissue samples were obtained for tissue assays and histopathological analysis.

Histological analysis of liver

Saline was used to gently wash the liver tissue samples. They were immediately fixed in a 10% formaldehyde solution, then histologically produced utilizing conventional methods. Tissue sections that were five micrometers thick were obtained and stained with hematoxylin and eosin (H&E), Masson's trichrome (MT) and periodic acid-Schiff (PAS). As previously reported, a histologist assessed the extent of liver damage for inflammatory cell infiltration, hepatocyte vacuolization, sinusoidal dilatation and blood vessel congestion. The histopathological findings were evaluated as no (0), mild (1), moderate (2), and severe (3). Malondialdehyde and antioxidant enzyme activity measurements

After the sacrifice, liver tissue was removed and twice-washed in phosphate buffer solution (PBS) to remove any blood or blood clots. After the liver tissue was homogenized in ice-cold PBS, then centrifuged (2000 g, 4°C, 15 min). Next, the supernatant was collected to measure malonyldialdehyde to evaluate hepatic lipid peroxidation (MDA). Additionally, commercial kits were used to measure the levels of antioxidant enzymes such as glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT) (Bio-diagnostics Co., Cairo, Egypt and BioVision, Inc., California, USA). As stated by the manufacturer's recommendations, each assay was carried out three times.

Statistical analysis

The expression of all data was the mean and standard deviation. Differences between the groups were analyzed by one-way analysis of variance with the Tukey multiple comparison tests using GraphPad Prism 8.0. P values less than 0.05 could be considered statistically significant changes.

RESULT

Effect of boric acid on liver histopathology induced by CTX

When histopathology was evaluated using H&E staining, it was apparent that the liver tissue from the CTX-induced and control group had distinct structural differences. According to HE staining, the liver tissue from the control group had a normal structure, complete hepatic lobules, and obvious borders. Figure 1 demonstrates how significant pathological changes were seen after CTX stimulation compared to the control group.

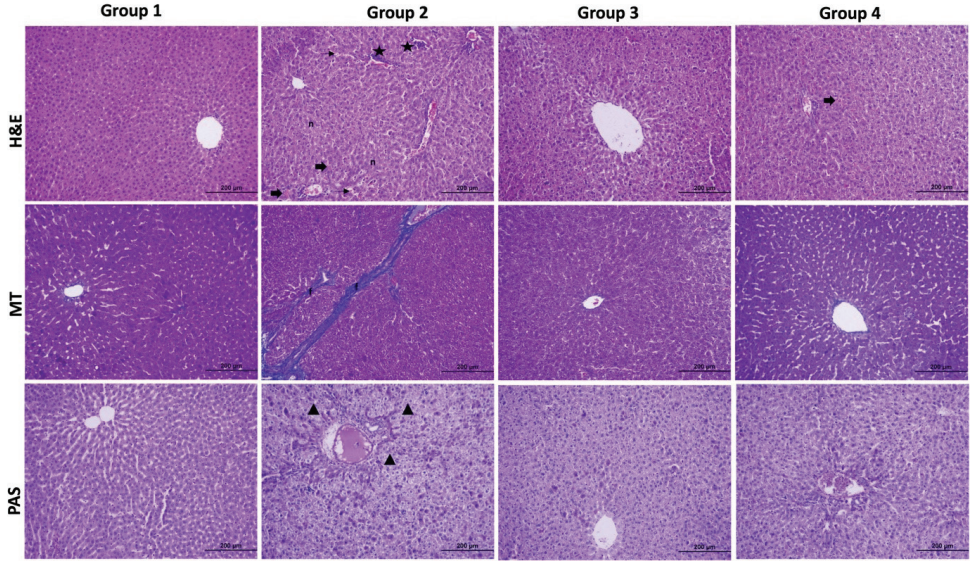

Figure 1. Liver tissue of al experimental groups stained with H&E, MT and PAS. X200. n, necrosis; arrow, sinusoid dilation; thick arrow, congestion; star, mononuclear cell infiltration; f, fibrosis, triangle, reduced glycogen content in hepatocytes.

Hepatocyte degeneration and lymphocyte infiltration in liver slices caused by CTX alone were used as evidence. BA considerably reduced the degree of hepatocyte degeneration and inhibited lymphocyte infiltration, demonstrating that the treatment could slow the histological progression of CTX-induced hepatotoxicity. Histopathological scorings in all groups are reported in Table 1.

Table 1. Statical analysis of histopathological scoring.

Group 1	Group 2	Group 3	Group 4	p
0.0 (0.0-1.0)[a]	2.0 (1.0-3.0)[bc]	0.5 (0.0-1.0)[a]	1.0 (0.8-2.0)[ac]	.0093

Data are expressed as median (quarter1-quarter3). The same letters on the same line indicate similarity between groups and different letters indicate difference between groups.

According to the MT data, blue pigments stained collagen fibers in liver tissue in all groups, although the CTX group's staining was more intense (Figure 1). The wall of the vena centralis and surrounding veins in the control and BA groups' liver sections showed normal connective tissue supporting the vein and portal area. Little or no fibrosis was seen in the BA-treated group.

PAS stained sections showed that the control group's hepatocytes had intense glycogen staining. Compared to the control group, the cells along the central vein had less glycogen in the CTX group. Treatment with BA increased the glycogen content compared to the CTX group (Figure 1).

Oxidative stress findings

The levels of MDA, GSH-Px, SOD and CAT were assessed in liver tissue samples. The model group had significantly higher MDA levels than the control group, as shown in Table 2 ($p<0.001$). Additionally, compared to the control group, treatment with CTX injection dramatically reduced the GSH-Px, SOD, and CAT activities ($p<0.001$). However, treatment with boric acid markedly decreased the MDA levels and markedly improved SOD, GSH-Px, and CAT activity ($p<0.001$). These findings showed that boric acid decreased MDA levels, increased antioxidant enzyme activity, and reduced oxidative stress caused by CTX.

DISCUSSION

The liver tissue damage that was experimentally produced in the current study was carried on by CTX metabolites. The increased tissue oxidative stress parameters are consistent with these pathogenic changes in the findings. The liver tissue has likely significantly improved as seen by a decrease in abnormal pathological signs, such as tissue damage at the BA treatment.

The liver is susceptible to toxicity and damage due to its vital function in metabolizing drugs and toxins.18 The number of chemotherapeutic drugs that can treat cancer is constrained because of severe side effects.[19] A drug known as CTX that nitrogen mustard alkylates has a well-known anti-cancer effect and a variety of anti-tumor activities.[20] Studies show that prolonged or severe CTX use can harm the liver and kidneys.[21] Because CTX will unavoidably be used in therapeutic treatments, it is essential to research different compounds that can reduce its side effects.
Antioxidants include enzymes such as CAT and GSH-Px,

Table 2. Descriptive statistics of tissue MDA, SOD, CAT, and GSH-Px levels.

	Group 1	Group 2	Group 3	Group 4	p
MDA	(1.65±0.09)[a]	(1.67±0.22)[a]	(2.54±0.23)[b]	(1.86±0.28)[a]	.001
SOD	(17.28±6.20)[a]	(17.23±1.06)[a]	(4.80±3.00)[b]	(12.40±2.96)[a]	.001
CAT	(32.66±10.55)[a]	(30.44±1.26)[a]	(15.63±0.91)[b]	(25.42±4.77)[ab]	.001
GSH-Px	(41.87±4.09)[a]	(38.67±3.61)[ac]	(21.68±3.73[bc]	(30.62±8.34)[c]	.001

Data are expressed as mean (X) ± standard deviation (SD). The same letters on the same line indicate similarity between groups and different letters indicate difference between groups.

as well as minerals such as zinc, selenium, vitamins, and boron. It has been shown to protect cells from DNA damage and lipid peroxidation, which are the early phases of many disease processes.[22] Numerous writers have noted that the increased free radical generation causes the antioxidant levels in the experimental study to decrease.[23-25] The naturally occurring mineral boron, together with BA, is extensively employed in agricultural, industrial, and cosmetic applications, besides its traditional medical application. In many studies, BA has been shown to have antioxidant,[26] hepatoprotective,[27] and anti-genotoxic effects.[22] Additionally, it has been hypothesized that BA increases the body's glutathione stores and prevents oxidative damage by blocking other reactive oxygen species. Additionally, it has been proposed that BA reduces oxidative damage by increasing glutathione levels in the body and preventing the production of additional reactive oxygen species.[26] In light of all of this, the current investigation sought to ascertain the preventive effects of BA on liver damage brought on by CTX in rats.

However, BA can act as a metabolic manager in some enzymatic systems. In addition to decreasing oxidative damage's effects and inhibiting ROS generation and apoptosis, BA also increases the body's level of reduced glutathione.[22] One study found that just 1.5 g/kg of ethanol increased the levels of AST, ALT, MDA, and caspase-3 while decreasing the SOD and CAT levels. However, in the 100 mg/kg BA + ethanol group, AST, ALT, MDA, and caspase-3 levels decreased while levels of SOD and CAT increased.[27] In mice, Ince et al. found that 200 mg/kg boric acid significantly reduced the liver damage caused by carbon tetra chloride. This may be because it activates the body's antioxidant defense mechanism and reduces lipid peroxidation.[28] These researchers' conclusions and those of the current investigation are similar. BA may also limit inflammatory processes, reduce ROS levels, and increase antioxidant levels (likely by limiting GSH depletion). Drug exposure can lead to impaired liver function, which is typically carried on by oxidative stress. ROS damages the cells by targeting the polyunsaturated fatty acids in the phospholipids of the biofilms and triggering lipid peroxidation. MDA is an indirect marker of lipid peroxidation and serves as an indirect indicator of oxidative stress-related damage.[29] Generally, hepatic GSH-Px, SOD, and CAT are significant antioxidant system enzymes that scavenge ROS and keep the antioxidant system functioning properly, preventing oxidative stress from damaging the liver.[30] As a result, the liver's level of antioxidant enzymes can indirectly determine its ability to fight free radicals. In our investigation, treatment with BA markedly reduced the MDA level and enhanced GSH-Px, SOD, and CAT activities in the groups treated with BA. These findings suggest that BA's anti-oxidant abilities may be used to reduce the liver damage caused by CTX.

CONCLUSIONS

In conclusion, the current study further showed that CTX could cause liver damage while inducing oxidative stress in rats. The BA treatment considerably decreased the MDA level, and the GSH-Px, SOD, and CAT activities were significantly increased in the BA-treated groups. These findings suggest that BA's anti-oxidant properties may be used to decrease the liver damage caused by CTX. Therefore, BA could prevent all of these negative consequences of CTX, and its protective mechanism against oxidative stress brought on by CTX might include BA's effects on reducing oxidative stress. The findings of this study could lead to the development of a BA treatment that is used in combination with CTX to reduce the side effects of cancer treatment. The observed benefits herein may therefore be attributed to the anti-inflammatory and tissue-regenerating characteristics of BA, which are carried on by the compound's numerous active components, in addition to its antioxidant activities.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Authorship contribution statement

Arzu Yay, Gozde Ozge Onder, Ozge Goktepe, Esra Balcioglu: Conceptualization, Methodology, Writing – review & editing. Arzu Yay, Gozde Ozge Onder, Ozge Goktepe, Esra Balcioglu: Methodology. Ozge Cengiz Mat, Demet Bolat, Eda Okur, Esra Balcioglu: Methodology, Data analyses. Arzu Yay, Esra Balcioglu: Data analyses. Arzu Yay: Supervision.

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