Research Article
Pamukkale Medical Journal
doi:https://dx.doi.org/10.31362/patd.1438593

Effects of boric acid on oxidant-antioxidant, proinflammatory cytokine levels, and biochemical parameters in aged rats

Yaşlı sıçanlarda borik asidin oksidan-antioksidan, proinflamatuar sitokin seviyeleri ve biyokimyasal parametreler üzerine etkisi

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Posted date:17.02.2024 Acceptance date:19.03.2024

Abstract

Purpose: As a result of the literature studies, it was seen that boric acid was the subject of many studies, and its effects on living things were investigated and examined. This study aimed to investigate the effects of oral boric acid supplementation at pharmacologic doses on physiological and biochemical systems in aged rats.

Material and methods: A total of 32 Wistar Albino male-aged rats were randomly and equally divided into the following four groups: 1st; Control=1 ml saline; 2nd; Low-dose boric acid (L-BA)=10 mg/kg; 3rd; Medium-dose boric acid (M-BA)=20 mg/kg; 4th; High-dose boric acid (H-BA)=40 mg/kg. Boric acid was given orally to aged rats for 28 days. Blood, liver, and kidney samples of rats were collected on day 29 to be analyzed for oxidants, antioxidants, proinflammatory cytokines, and biochemical changes.

Result: Boric acid significantly increased albumin, total protein, calcium levels equally in all boric acid groups compared to the control group (p<0.05), increased cholesterol parameter only in H-BA group (p<0.05), increased phosphor level in M-BA and H-BA groups compared to control and L-BA groups (p<0.05), total bilirubin level was increased only in L-BA group (p<0.05), blood urea nitrogen level was increased in L-BA and M-BA groups (p<0.05), alanine aminotransferase level was increased only in M-BA group (p<0.05), creatine kinase and glucose levels were increased boric acid in all groups compared to control group (p<0.05). However, boric acid did not affect globulin, creatine, alkaline phosphatase, and amylase levels in a dose-dependent manner (p>0.05). Boric acid significantly decreased MDA levels (p<0.05) and increased GSH, SOD, and CAT enzyme activities (p<0.05) in liver and kidney tissues in a dose-dependent manner. In addition, boric acid decreased plasma IL-6 and TNF-α proinflammatory cytokine levels (p<0.05).

Conclusion: This study demonstrated that boric acid supplementation has ameliorative effects in a dose-dependent manner on lipid peroxidation, immunomodulation, and regulation of many blood biochemical parameters in aged rats.

Keywords: Age, antioxidant, boric acid, oxidant, proinflammatory cytokine.

Basegmez M, Dogan MF. Effects of boric acid on oxidant-antioxidant, proinflammatory cytokine levels, and biochemical parameters in aged rats. Pam Med J 2024;17:369-379.

Öz

Amaç: Yapılan literatür çalışmaları neticesinde borik asit ’in birçok çalışmaya konu olduğu ve canlıya olan etkilerinin araştırılarak incelendiği görülmüştür. Bu çalışmada yaşlı sıçanlarda farmakolojik dozlarda uygulanan oral borik asit takviyesinin fizyolojik ve biyokimyasal sistemlerdeki etkilerinin araştırılması amaçlanmıştır.

Gereç ve yöntem: Toplam 32 adet Wistar Albino erkek yaşlı sıçan rastgele ve eşit olarak aşağıdaki dört gruba ayrılmıştır: 1.; Kontrol=1ml salin, 2.; Düşük-doz borik asit (L-BA)=10 mg/kg, 3.; Orta-doz borik asit (M-BA)=20 mg/kg, 4.; Yüksek-doz borik asit (H-BA)=40 mg/kg. Yaşlı sıçanlara 28 gün boyunca borik asit oral olarak verilmiştir. Sıçanların kanı, karaciğer ve böbrek örnekleri oksidan, antioksidan, proinflamatuar sitokinler ve biyokimyasal değişiklikler açısından analiz edilmek üzere 29. günde toplanmıştır.

Bulgular: Borik asit, albümin, total protein ve kalsiyum düzeylerini kontrol grubuna kıyasla tüm borik asit gruplarında eşit oranda artırırken (p<0,05), kolesterol parametresini sadece H-BA grubunda artırmıştır (p<0,05). Borik asit fosfor düzeyini kontrol ve L-BA gruplarına kıyasla M-BA ve H-BA gruplarında artırırken (p<0,05), total bilirubin düzeyini sadece L-BA grubunda (p<0,05), kan üre nitrojen düzeyini L-BA ve M-BA gruplarında artırmıştır (p<0,05). Ayrıca alanin aminotransferaz düzeyini sadece M-BA grubunda artırırken (p<0,05), kreatin kinaz ve glukoz düzeylerini kontrol grubuna kıyasla tüm gruplarda artırdığı tespit edilmiştir (p<0,05). Ancak borik asidin globulin, kreatin, alkalin fosfataz, amilaz düzeyleri üzerinde doza bağlı bir etkisi olmamıştır (p>0,05). Borik asit karaciğer ve böbrek dokularında MDA düzeylerini önemli ölçüde azaltmış (p<0,05) ve GSH, SOD ve CAT enzim aktivitelerini artırmıştır (p<0,05). Ayrıca, borik asit plazma IL-6 ve TNF-α proinflamatuar sitokin düzeylerini azaltmıştır (p<0,05).

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Sonuç: Bu çalışma borik asit takviyesinin yaşlı sıçanlarda lipid peroksidasyonu, immünomodülasyon ve birçok kan biyokimyasal parametresinin düzenlenmesi üzerinde doza bağlı olarak iyileştirici etkileri olduğunu göstermiştir.

Anahtar kelimeler: Yaş, antioksidan, borik asit, oksidan, proinflamatuar sitokin.

Başeğmez M, Doğan MF. Yaşlı sıçanlarda borik asidin oksidan-antioksidan, proinflamatuar sitokin seviyeleri ve biyokimyasal parametreler üzerine etkisi. Pam Tıp Derg 2024;17:369-379.

Introduction

The aging process is one of the most significant examples of homeostasis disruption, which affects physiological systems, especially the immune, nervous, and endocrine systems, including oxidative stress and inflammation [1]. Thus, aging can be characterized as a progressive and widespread decline in the organism's functioning that results in a reduced capacity to adapt to changes and maintain homeostasis [2].

Boric acid, which is widely used in both industrial and consumer products, exists in nature as a mineral substance [3]. It has been proven in various experimental studies that boron and its compounds are beneficial for humans and animals [4]. Boric acid has been shown to play a significant function in preserving health through nutritional and pharmacological intakes [5]. The effects of boric acid on enzyme, mineral, and hormone metabolism have been previously demonstrated [5]. Indeed, boron supplements in diets have been demonstrated to play key roles in bone development by reducing calcium excretion and increasing plasma-ionized calcium and phosphorus levels [6]. It is known that boron plays an important role in the inflammatory response by suppressing the activities of some enzymes, such as 6-phosphogluconate [4]. There are also a few studies showing the importance of boron on antioxidant activity by destroying reactive oxygen species [7]. It has been reported that oral boric acid administration increases GSH and CAT antioxidant enzyme levels in the body and prevents oxidative damage by inhibiting reactive oxygen species [8]. In a previous study, boric acid and borax supplementation were shown to reduce lipid peroxidation and increase antioxidant activity by decreasing blood MDA levels and increasing blood GSH levels [9]. Boric acid has been suggested to be better than borax in combating heavy metal toxicity [10]. Furthermore, boron compounds, particularly boric acid and borax, have been demonstrated to decrease oxidative stress by boosting the antioxidant system in several disease and toxicity models [3].

The most important sources of boron and its derivatives are plants and drinking water. A report by the World Health Organization (WHO) reports that nearly 1.2 mg of boron is consumed daily through nutrients, with 0.2-0.6 mg ingested with drinking water. It has been reported that the safe dosage range for individuals is 1-13 mg/day and that a daily intake of less than 1 mg is inadequate [11]. Oral boric acid supplements have been observed to be easily absorbed from the gastrointestinal system in humans and animals and quickly dispersed throughout the body. Regardless of the mode of administration, boric acid has been observed to be quickly excreted unchanged in the urine in less than 24 hours in both people and animals [12].

Considering previous studies, the importance of boric acid, a boron compound, for homeostasis is emphasized. In this study, different doses of oral boric acid supplementation were administrated to aged rats. It investigated how oral boric acid supplementation influenced biochemical parameters in serum, as well as oxidant and antioxidant markers in kidney and liver tissue.

Materials and methods

Chemical

Boric acid ($H3BO3$) (Code number: V55901), purchased from Chemistry Lab Istanbul, Türkiye, was used as a test compound.

Animals and experimental model

This study began following the approval of the Pamukkale University Animal Experiments Ethics Committee. In this study, thirty-two male-aged rats (aged between 24-28 months) were purchased from Pamukkale University Experimental Surgery Application and Research

Center, Denizli, Türkiye. The rats were checked daily at regular intervals under the supervision of a veterinarian. Rats were housed in cages in the experimental animal unit at a temperature of 22±1°C, 50% humidity, 12 hours of light/darkness, and regular ventilation. To feed the rats, standard rat food and fresh drinking water were given ad libitum every day. The control group of rats received 1 ml of physiological saline orally by gastric gavage daily for 28 days. Boric acid was applied by oral gastric gavage for 28 days at the dose ranges specified in Table 1.

Table 1. The procedure process and experimental groups

Groups	28. days	29. days
Control group	1mL oral saline was given for twenty-eight days	Rats in all groups will be sacrificed on day 29 of the experiment
Boric acid 10 mg/kg group (L-BA)	10 mg/kg of boric acid was given for twenty-eight days	
Boric acid 20 mg/kg group (M-BA)	20 mg/kg of boric acid was given for twenty-eight days	
Boric acid 40 mg/kg group (H-BA)	40 mg/kg of boric acid was given for twenty-eight days	

After the application period, which lasted a total of 28 days, the animals were sacrificed by anesthetizing with a combination of xylazine HCI and ketamine HCI after a 24-hour fast following the last application. Samples of blood were extracted from the abdominal aorta of each rat into lithium heparin and serum separator tubes. After centrifugation of blood samples at 3000 rpm for 10 minutes at 4°C, plasma and serum samples were obtained.

Determination of TNF-α and IL-6

Plasma TNF-α (Cat. No. E0764Ra) and IL-6 (Cat. No. E0135Ra) levels were determined using a commercial rat ELISA kit from Bioassay Technology Laboratory, according to the manufacturer's instructions.

Biochemical evaluation

Albumin (ALB), total protein (TP), globulin (GLO), calcium (CA), creatine (CRE), phosphorus (P), total bilirubin (TBIL), blood urea nitrogen (BUN), alanine aminotransferase (ALT), alkaline phosphatase (ALP), creatine kinase (CK), amylase (AMY), glucose (GLU), and cholesterol (CHOL) were measured from serum samples using the MNCHIP Veterinary Chemistry Analyzer Pointcare v3 device. To determine oxidant and antioxidant markers in the liver and kidney tissues of rats, the tissues were first washed with 0.9% NaCl. Subsequently, tissues were homogenized at a 1:40 w/v ratio in 0.1 M phosphate buffer (PH=7.4). The tissue homogenates were then centrifuged at 4°C and 3500 rpm for 10 minutes to obtain the supernatant [13]. The supernatants that were collected were used to test the activities of glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA). The MDA concentration in tissue supernatants, an indication of lipid peroxidation, was measured using Ohkawa et al. [14] methods. The GSH concentration was determined in tissue supernatants using the method presented by Beutler et al. [15]. For SOD enzyme activity, Sun et al. [16] and for CAT enzyme activity, using the method described by Aebi [17] were used.

Statistical analysis

The results obtained from the study were analyzed in SPSS 28.0 (IBM SPSS Statistics 28 software (Armonk, NY: IBM Corp.)). GraphPad Prism 9.5 (GraphPad Software, San Diego, CA, USA) was used for graphical presentations. All results are expressed in mean±standard error of the mean (SEM). The normal distribution of the data was performed by the Shapiro-Wilk test, and data normality was found to be greater than 0.05. In addition, the assumption of homogeneity of the variance of the data was examined by Levene's statistical test, and a value greater than 0.05 was obtained. When the parametric test assumptions were met, one-way analysis of variance was used to compare independent group means, and the level of statistical significance between the means was determined by the Duncan test. In all analyses, $p<0.05$ was considered statistically significant.

Results

In the study, the effects of oral boric acid supplements administered at pharmacological doses [3] on aged rats on serum biochemical parameters are given in Table 2. While oral boric acid supplementation did not affect GLO, AMY, ALP, and CRE levels (p>0.05), it was observed to significantly increase ALB, TP, and CA levels without any dose-related statistical effect (p<0.05). The GLU and CK levels significantly increased in the boric acid group compared with the control group (p<0.05). Also, the M-BA and H-BA groups demonstrated a similar statistical rate of increase in GLU and CK levels (p>0.05).

The P level was not different between the control and L-BA groups (p>0.05), but considerably increased in the M-BA and H-BA groups compared to the control and L-BA groups (p<0.05). The H-BA group demonstrated a significant reduction in CHOL levels compared to the control and boric acid groups (p<0.05). BUN levels were statistically similar in control and H-BA doses, whereas L-BA and M-BA doses significantly increased BUN levels (p<0.05). Significant increases in ALT and TBIL levels were observed in the M-BA and L-BA groups, respectively (p<0.05).

Table 2. Effects of boric acid supplementation on serum biochemical parameters (n=8)

Parameters	Control	L-BA	M-BA	H-BA
ALB (g/dL)	2.56±0.14^{b}	3.20±0.12^{a}	3.30±0.08^{a}	3.31±0.06^{a}
TP (g/dL)	6.03±0.13^{b}	6.74±0.09^{a}	6.81±0.16^{a}	6.82±0.08^{a}
GLO (g/dL)	3.61±0.09	3.61±0.11	3.43±0.10	3.51±0.12
CA (mg/dL)	8.08+0.10^{b}	9.48±0.13^{a}	9.60±0.11^{a}	9.64±0.06^{a}
CRE (mg/dL)	0.48±0.06	0.52±0.02	0.54±0.05	0.49±0.03
P (mg/dl)	6.08±0.20^{b}	6.56±0.26^{b}	7.50±0.15^{a}	7.66±0.19^{a}
TBIL (mg/dL)	0.18±0.02^{b}	0.24±0.02^{a}	0.22±0.01^{ab}	0.19±0.02^{ab}
BUN (mg/dL)	15.54±0.46^{c}	16.95±0.41^{b}	18.08±0.25^{a}	16.08±0.26^{bc}
ALT (U/L)	48.00±1.83^{b}	46.25±1.92^{b}	55.25±2.09^{a}	48.37±1.44^{b}
ALP (U/L)	152.75±9.28	128.00±10.59	130.75±6.30	135.50±7.76
CK (U/L)	587.25±15.99^{c}	710.37±22.80^{b}	771.56±25.98^{a}	885.12±25.87^{a}
AMY (U/L)	522.25±29.40	527.25±14.13	559.88±21.64	526.63±15.86
GLU (mg/dL)	175.12±4.82^{c}	201.50±6.05^{b}	229.37±7.31^{a}	240.75±6.03^{a}
CHOL(mg/dL)	115.38±6.04^{a}	119.50±7.56^{a}	118.00±4.85^{a}	91.50±5.55^{b}

The values were expressed as means ± SEM
^{a,b,c} In the same line values with different letters show statistically significant differences in serum (p<0.05)
ALB: Albumin, TP: Total Protein, GLO: Globulin, CA: Calcium, CRE: Creatine, P: Phosphor TBIL: Total bilirubin, BUN: Blood Urea Nitrogen
ALT: Alanine Aminotransferase, ALP: Alkaline Phosphatase, CK: Creatinine Kinase, AMY: Amylase, GLU: Glucose
CHOL: Cholesterol.L-BA: Boric Acid 10mg/kg, M-BA: Boric Acid 20mg/kg, H-BA: Boric Acid 40mg/kg

In the study, the effects of oral boric acid supplements administered at pharmacological doses to aged rats on liver MDA, GSH, SOD, and CAT activities are given in Table 3. Boric acid did not statistically affect MDA levels in the L-BA group (p>0.05) and significantly reduced MDA levels in the M-BA and H-BA groups (p<0.05). Boric acid significantly increased dose-dependent GSH levels compared to the control group (p<0.05). Boric acid did not statistically affect SOD levels in the L-BA group (p>0.05) and increased SOD levels in the M-BA and H-BA groups (p<0.05). Boric acid did not statistically affect CAT levels in the L-BA group and dose-dependently increased CAT levels in the M-BA and H-BA groups.

Table 3. Effects of boric acid supplementation on MDA, GSH, SOD, and CAT activities in the liver tissue of rats (n=8)

Groups	Liver MDA (nmol/g tissue)	Liver GSH (nmol/g tissue)	Liver SOD (nmol/g tissue)	Liver CAT (nmol/g tissue)
Control	4.51±0.52^{a}	19.52±0.64^{c}	3.24±0.16^{c}	1.48±0.11^{c}
L-BA	4.35±0.32^{a}	23.82±0.74^{b}	3.69±0.13^{c}	1.78±0.09^{c}
M-BA	3.13±0.38^{b}	25.06±1.29^{b}	4.30±0.72^{b}	2.18±0.10^{b}
H-BA	1.57±0.12^{c}	31.44±1.25^{a}	5.19±0.23^{a}	2.51±0.12^{a}

The values were expressed as means ± SEM, ^{a,b,c} Different letters in the same column represent statistically significant differences (p<0.05)
MDA: Malondialdehyde, GSH: Glutathione, SOD: Superoxide Dismutase, CAT: Catalase, L-BA: Boric Acid 10mg/kg
M-BA: Boric Acid 20mg/kg, H-BA: Boric Acid 40mg/kg

In the study, the effects of oral boric acid supplements administered at pharmacological doses to aged rats on kidney MDA, GSH, SOD, and CAT activities are given in Table 4. Boric acid did not statistically affect MDA levels in the L-BA group (p>0.05) and significantly reduced MDA levels in the M-BA and H-BA groups (p<0.05). Boric acid significantly increased dose-dependent GSH levels compared to the control group (p<0.05). Boric acid did not statistically affect SOD levels in the L-BA group (p>0.05) and increased SOD levels in the M-BA and H-BA groups (p<0.05). Boric acid did not statistically affect CAT levels in the L-BA group and increased CAT levels in the M-BA and H-BA groups (p<0.05).

Table 4. Effects of boric acid supplementation on MDA, GSH, SOD, and CAT activities in the kidney tissue of rats (n=8)

Groups	Kidney MDA (nmol/g tissue)	Kidney GSH (nmol/g tissue)	Kidney SOD (nmol/g tissue)	Kidney CAT (nmol/g tissue)
Control	7.15±0.63^{a}	19.86±0.55^{c}	3.98±0.21^{c}	0.44±0.02^{b}
L-BA	6.69±0.43^{ab}	25.36±0.50^{b}	4.31±0.27^{bc}	0.54±0.03^{b}
M-BA	5.21±0.61^{bc}	30.40±0.77^{a}	4.83±0.23^{ab}	0.73±0.04^{a}
H-BA	4.83±0.34^{c}	32.28±1.30^{a}	5.06±0.19^{a}	0.89±0.04^{a}

The values were expressed as means ± SEM, ^{a,b,c} Different letters in the same column represent statistically significant differences (p<0.05)
MDA: Malondialdehyde, GSH: Glutathione, SOD: Superoxide dismutase, CAT: catalase, L-BA: Boric Acid 10mg/kg
M-BA: Boric Acid 20mg/kg, H-BA: Boric Acid 40mg/kg

In the study, the effects of oral boric acid supplements administered at pharmacological doses to aged rats on plasma TNF-α levels are given in Figure 1. As shown in Figure 1, boric acid caused a significant dose-dependent decrease in TNF-a levels in the other study groups compared to the control group (p<0.05).

In the study, the effects of oral boric acid supplements administered at pharmacological doses to aged rats on plasma IL-6 levels are given in Figure 2. As demonstrated in Figure 2, L-BA decreased IL-6 levels in comparison to the control group, but the difference was not statistically significant. Boric acid significantly reduced IL-6 levels in the M-BA and H-BA groups in a dose-dependent manner compared to the control group (p<0.05).

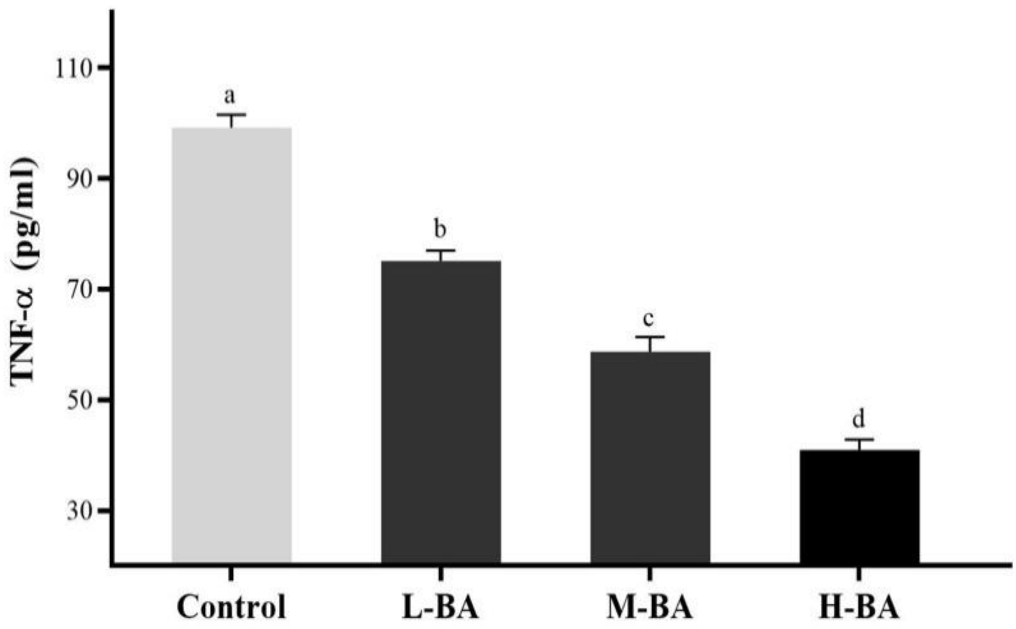

Figure 1. Plasma TNF-α levels in boric acid-treated rats with pharmacological dose

Each value represents the mean±SEM (n=8). The letters (a, b, c, d) indicate statistically significant differences between the groups, p<0.05
L-BA: Boric Acid 10mg/kg, M-BA: Boric Acid 20mg/kg, H-BA: Boric Acid 40mg/kg

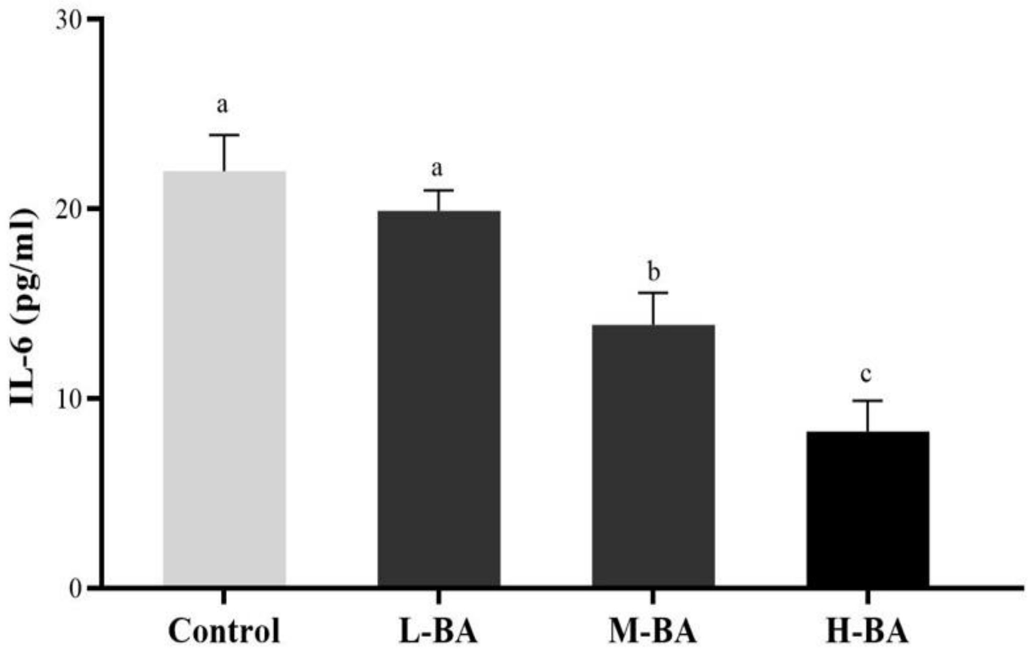

Figure 2. Plasma IL-6 levels in boric acid-treated rats with pharmacological dose

Each value represents the mean±SEM (n=8). The letters (a, b, c) indicate statistically significant differences between the groups, p<0.05
L-BA: Boric Acid 10mg/kg, M-BA: Boric Acid 20mg/kg, H-BA: Boric Acid 40mg/kg

Discussion

The study investigated how the pharmacological dose of boric acid supplementation affects serum biochemical parameters, liver and kidney tissue oxidant and antioxidant enzyme levels, and IL-6 and TNF-α proinflammatory cytokine levels, which are important in immune system regulation.

The liver is the primary source of serum proteins and produces albumin, globulin, fibrinogen, and the majority of other clotting factors [18]. In the study, oral boric acid supplementation increased serum TP and ALB levels equally in all doses compared to the control group and did not affect GLO levels. Similarly, previous studies have shown no effect on GLO levels when boric acid is added to broiler diets [19] and when rabbits were administered boric acid dose-dependently [20]. In addition, Kan and Kucukkurt [21] showed that 10 and 20 mg/kg of boric acid supplementation did not change the TP level in rats compared to the control group. In contrast, TP and ALB levels in aged rats increased in a dose-independent manner in the boric acid groups compared to the control group. This finding suggests that different animal species do not respond in the same way to boric acid supplementation. This could be due to the study used aged rats and administered boric acid orally.

Considering the reports that dietary or supplemental boric acid affects many biochemical parameters [22, 23], it is important to reveal the effects of dose-dependent boric acid supplementation on biochemical parameters in aged rats. In the study, serum calcium concentrations increased in rats supplemented with oral boric acid for 28 days. This study is consistent with reports that the boron diet prevents prenatal metabolic disorders by increasing serum calcium levels and dietary boron intake increases calcium content in the femur bones of rats [24] and chickens [25]. In addition, the increase in fracture strength in the lumbar vertebrae of rats [26], femur bones [6] and layers [27] of rabbits with the addition of boron to diets supports this study. Also, it has been reported that boron species, which have an essential function in bone development and mineralization, ensure the incorporation of calcium into the bone, joints, and cartilage, leading to a significant improvement in bone development observed in 95% of the patients [28]. The findings of the study suggest that oral boric acid intake may be a useful tool for increasing serum calcium levels and that dose-dependent supplementation may not provide additional benefits.

In this study, creatine did not change depending on the dose of oral boric acid. This finding is similar to reports showing that serum creatinine levels were unchanged when boron and boric acid were added to the diets of rabbits [20] and rats [21]. In addition, the dose-dependent effect of boron was reported to cause an increase in creatinine levels in rats exposed to acrylamide [29]. When the impact of boric acid on creatinine levels is examined, different results emerge. In most of the studies, creatinine levels were not affected by boric acid supplementation in a dose-dependent manner, as in this study. We also analyzed that creatine kinase enzyme activity was significantly higher in all boric acid groups compared to the control group. This finding was consistent with previous research [19] showing boron supplementation in broiler diets at levels of 750 and 1000 mg/kg increased creatine kinase enzyme activity compared to the control group.

During the aging period, several cellular and physiological changes occur in the liver tissue, which has a remarkable ability to renew and maintain its function throughout life [30]. It has been observed that serum AST, ALT, ALP, and bilirubin activities are commonly used to assess liver structural integrity [31]. Kan and Kucukkurt [21] reported that the different doses of boron supplementation had no statistically significant effect on serum ALP activity levels in rats compared to the control group. In the present study, serum ALP levels did not change in the L-BA and H-BA groups but increased only in the M-BA group. This is in line with the previous report that there was no effect on ALT levels in rats given boric acid at a dose of 10 mg/kg [21]. In addition, when oral borax decahydrate was given to rabbits at a dose of 10 mg/kg, it did not affect ALT levels [32]. In this study, TBIL levels increased only in the L-BA group. Indeed, these results show that BA produces different effects on the liver depending on the dose.

Previous reports have indicated that boron supplements can alter the amounts of nitrogen metabolites in blood and urine by affecting the consumption of certain amino acids or proteins [22]. When compared with the control group, the BUN level was statistically higher in the group given L-BA and M-BA boric acid. Similarly, it was reported that boron given with acrylamide caused an increase in urea nitrogen level [29]. In addition, similar to our study, boron has been shown to increase serum urea levels in laying hens [33]. However, it was reported that increased serum BUN levels in gentamicin-treated rats decreased in dose-dependent boron groups (5, 10, and 20 mg/kg) [34]. The main reason for the different results of the studies on the effect of boric acid on serum BUN activity may be due to differences between doses and administration methods. Therefore, further studies with different doses and administration methods should be performed to clarify the issue in aged rats.

The increased blood GLU levels of boric acid administered orally in the study support the previous report [35] on increased blood GLU levels when rats were given feed enriched with boric acid at doses of 250, 500, and 1000 ppm. Some studies have shown that boron does not affect increasing or decreasing blood glucose levels [21]. This may be due to differences in the dose, route of administration, absorption, distribution, and catabolism of boric acid with age, sex, and race differences between living organisms.

In a recent study, it was concluded that boron supplements affect lipid metabolism as a result of the relationship between total cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triglyceride levels in rats fed a high-fat diet [36]. In our study, except for an important decrease in the H-BA group, the other study groups did not affect serum CHOL levels in aged rats. This finding is in agreement with the report [37] that dietary boron supplementation in Japanese quail at a dose of 1000 mg/kg compared to 500 mg/kg and 750 mg/kg boric acid supplementation decreased total cholesterol levels. In this case, boron-containing drugs could have a reducing effect on serum cholesterol levels.

Free oxygen radicals are highly reactive and can cause damage to cell membranes and organelles through lipid peroxidation [9]. In our study, MDA levels, which indicate lipid peroxidation, decreased significantly in the liver and kidney tissues of aged rats given oral boric acid supplementation at M-BA and H-BA dosages. We also found that oral boric acid supplementation dose-dependently increased GSH levels, SOD, and CAT antioxidant enzyme activities in aged rats. Consistent with our results, oral boric acid supplementation acted as a scavenger of superoxide, hydroxyl radical, and singlet molecular oxygen [38]. Similarly, Kar et al. [39] found a substantial decrease in MDA levels in the group given boric acid after ischemic reperfusion injury of kidney tissue, while an increase in SOD, CAT, and GSH levels was observed. However, low doses of boric acid supplementation (15-50 mg/L) did not alter MDA and CAT concentrations in human peripheral blood cultures [40]. It was also reported that boric acid supplementation in rats does not change the MDA level in liver and kidney tissues, while SOD and CAT antioxidant enzyme parameter levels decrease [9]. The reason for the difference may be the difference in the dose of boric acid used in the study, the method of administration, the duration, and the age of the rats used. Indeed, this suggests that oral boric acid supplementation may play a significant role in maintaining homeostasis by increasing the activity of these enzymes in a dose-dependent manner.

It is known that boric acid affects the function of the immune system [41]. The need for boric acid can vary greatly due to infection, increased inflammation, and metabolic disorders, and this can be particularly pronounced for malnourished individuals. In particular, diet boron is known to help control the normal inflammatory process by serving as a signaling suppressor that down-regulates certain enzymatic activities typically elevated at the site of inflammation [42]. In the present study, a decrease in IL-6 and TNF-α cytokine levels was observed in aged rats due to the pharmacologic dose of boric acid. It has been similarly shown that boron supplementation decreased proinflammatory cytokine levels such as IL-6 and TNF-α [43]. This study was also consistent with the report [44] that boric acid supplementation significantly decreased TNF-α proinflammatory cytokine levels, which play an important role in the pathophysiology of the knee osteoarthritis model in rats. In the previous study, oral boric acid supplementation similarly reversed BPA-induced TNF-α, IL-6, and IL-1β expression levels [45] as well as acrylamide-induced TNF-α expression levels [29]. These results show that giving aged rats a boric acid supplement in pharmacologic doses lowers their elevated levels of IL-6 and TNF-α.

In summary, this study demonstrated that dose-dependent supplementation of boric acid has ameliorative effects on lipid peroxidation, immunomodulation, and regulation of many blood biochemical parameters in aged rats. The study found that boric acid supplementation reduced systemic inflammation markers such as IL-6 and TNF-α. This suggests that boric acid may help reduce the risk of inflammatory diseases in the elderly. Boric acid contributes to the maintenance of antioxidant capacity, which is an important approach in the treatment of aging-related disorders. Furthermore, oral boric acid supplementation for humans and animals would be useful to determine its nutritional value. In the future, our study will provide perspectives that will contribute to new studies to compare the effects of different doses and routes of administration of boric acid in different age and gender groups.

Conflict of interest: No conflict of interest was declared by the authors.

References

1. De La Fuente M. Role of neuroimmunomodulation in aging. Neuroimmunomodulation 2008;15:213-223. https://doi.org/10.1159/000156465
2. De La Fuente M, Miquel J. An update of the oxidation-inflammation theory of aging: the involvement of the immune system in oxi-inflamm-aging. Curr Pharm Des 2009;15:3003-3026. https://doi.org/10.2174/138161209789058110
3. Ince S, Keles H, Erdogan M, Hazman O, Kucukkurt I. Protective effect of boric acid against carbon tetrachloride-induced hepatotoxicity in mice. Drug Chem Toxicol 2012;35:285-292. https://doi.org/10.3109/01480545.2011.607825
4. Uluisik I, Karakaya HC, Koc A. The importance of boron in biological systems. J Trace Elem Med Biol 2018;45:156-162. https://doi.org/10.1016/j.jtemb.2017.10.008
5. Singh AK, Kewalramani N, Mani V, Sharma A, Kumari P, Pal RP. Effects of boric acid supplementation on bone health in crossbred calves under tropical condition. J Trace Elem Med Biol 2021;63:126647. https://doi.org/10.1016/j.jtemb.2020.126647
6. Hakki SS, Dundar N, Kayis SA, et al. Boron enhances strength and alters mineral composition of bone in rabbits fed a high energy diet. J Trace Elem Med Biol 2013;27:148-153. https://doi.org/10.1016/j.jtemb.2012.07.001
7. Karabağ F, İnce S, Demirel HH. Boric acid is associated with the suppression of apoptosis and endoplasmic reticulum stress in rat model of paracetamol -induced hepatotoxicity. J Taibah Univ Sci 2023;17:1-9. https://doi.org/10.1080/16583655.2023.2250565
8. Cengiz M. Boric acid protects against cyclophosphamide-induced oxidative stress and renal damage in rats. Cell Mol Biol (Noisy-le-grand) 2018;64:11-14.
9. Ince S, Kucukkurt I, Cigerci IH, Fidan AF, Eryavuz A. The effects of dietary boric acid and borax supplementation on lipid peroxidation, antioxidant activity, and DNA damage in rats. J Trace Elem Med Biol 2010;24:161-164. https://doi.org/10.1016/j.jtemb.2010.01.003
10. Turkez H, Geyikoglu F, Tatar A, Keles MS, Kaplan I. The effects of some boron compounds against heavy metal toxicity in human blood. Exp Toxicol Pathol 2012;64:93-101. https://doi.org/10.1016/j.etp.2010.06.011
11. WHO (2003) Boron in Drinking-water Background document for development of WHO Guidelines for Drinking-water Quality. Available at: https://cdn.who.int/media/docs/default-source/wash-documents/wash-chemicals/boron-2003-background.pdf?sfvrsn=8b397e68_4. Accessed 15 February, 2024
12. Murray FJ. A comparative review of the pharrnacokinetics of boric acid in rodents and humans. Biol Trace Elem Res 1998;66:331-341. https://doi.org/10.1007/BF02783146
13. Ince S, Kucukkurt I, Demirel HH, Arslan Acaroz D, Akbel E, Ciğerci IH. Protective effects of boron on cyclophosphamide induced lipid peroxidation and genotoxicity in rats. Chemosphere 2014;108:197-204. https://doi.org/10.1016/j.chemosphere.2014.01.038
14. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351-358. https://doi.org/10.1016/0003-2697(79)90738-3
15. Beutler E, Duron O, Kelly BM. Improved method for the determination of blood glutathione. J Lab Clin Med 1963;61:882-888.
16. Sun Y, Oberley LW, Li Y. A simple method for clinical assay of superoxide dismutase. Clin Chem 1988;34:497-500. https://doi.org/10.1093/clinchem/34.3.497
17. Aebi H. Catalase. Methods of Enzymatic Analysis (Second Edition) 1974;2:673-684. https://doi.org/10.1016/B978-0-12-091302-2.50032-3
18. Thapa BR, Walia A. Liver function tests and their interpretation. Indian J Pediatr 2007;74:663-671. https://doi.org/10.1007/s12098-007-0118-7

19. Eren M, Uyanik F, Kocaoglu Guclu B, Atasever A. The influence of dietary boron supplementation on performance, some biochemical parameters and organs in broilers. Asian J Animal Veterinary Advances 2012;7:1079-1089. https://doi.org/10.3923/ajava.2012.1079.1089

20. Yiğit P, Eren M, Soyer Sarica Z, Şentürk M. Tavşanlarda borik asidin kan kimyasina etkisi. Erciyes Üniversitesi Veteriner Fakültesi Dergisi 2013;10:77-85.

21. Kan F, Kucukkurt I. Investigation of the effect of boron on thyroid functions and biochemical parameters in hypothyroid induced-rats. J Biochem Mol Toxicol 2022;36:e23186. https://doi.org/10.1002/jbt.23186

22. Nielsen FH. Boron in human and animal nutrition. Plant and Soil 1997;193:199-208.

23. Hazman Ö, Bozkurt MF, Fidan AF, Erkan Uysal F, Çelik S. The effect of boric acid and borax on oxidative stress, inflammation, er stress and apoptosis in cisplatin toxication and nephrotoxicity developing as a result of toxication. Inflammation 2018;41:1032-1048. https://doi.org/10.1007/s10753-018-0756-0

24. Nielsen FH. Dietary fat composition modifies the effect of boron on bone characteristics and plasma lipids in rats. Biofactors 2004;20:161-171. https://doi.org/10.1002/biof.5520200305

25. Mızrak C, Yenice E, Can M, Yıldırım U, Atik Z. Effects of dietary boron on performance, egg production, egg quality and some bone parameters in layer hens. South African J Animal Sci 2010;40:257-264. https://doi.org/10.4314/sajas.v40i3.10

26. Naghii MR, Ebrahimpour Y, Darvishi P, et al. Effect of consumption of fatty acids, calcium, vitamin D and boron with regular physical activity on bone mechanical properties and corresponding metabolic hormones in rats. Indian J Exp Biol 2012;50:223-231.

27. Cufadar Y, Olgun O, Bahtiyarca Y. Effects of different sources of boron supplementation to diet on egg shell quality and bone characteristics in laying hens. Archiva Zootechnica 2011;14:37-42.

28. Devirian TA, Volpe SL. The physiological effects of dietary boron. Crit Rev Food Sci Nutr 2003;43:219-231. https://doi.org/10.1080/10408690390826491

29. Acaroz U, Ince S, Arslan Acaroz D, et al. The ameliorative effects of boron against acrylamide-induced oxidative stress, inflammatory response, and metabolic changes in rats. Food and Chemical Toxicology 2018;118:745-752. https://doi.org/10.1016/j.fct.2018.06.029

30. Frith J, Jones D, Newton JL. Chronic liver disease in an ageing population. Age Ageing 2009;38:11-18. https://doi.org/10.1093/ageing/afn242

31. Adewale OB, Adekeye AO, Akintayo CO, Onikanni A, Saheed S. Carbon tetrachloride (CCl4)-induced hepatic damage in experimental Sprague Dawley rats: Antioxidant potential of Xylopia aethiopica. J Phytopharmacol 2014;3:118-123. https://doi.org/10.31254/phyto.2014.3207

32. Basoglu A, Baspinar N, Sagkan Ozturk A, Peker Akalin P. Effects of boron administration on hepatic steatosis, hematological and biochemical profiles in obese rabbits. Trace Elements and Electrolytes 2010;27:225-231. https://doi.org/10.5414/tep27225

33. Eren M, Uyanik F. Influence of dietary boron supplementation on some serum metabolites and egg-yolk cholesterol in laying hens. Acta Vet Hung 2007;55:29-39. https://doi.org/10.1556/AVet.55.2007.1.4

34. Ince S, Kucukkurt I, Demirel HH, Arslan Acaroz D, Varol N. Boron, a trace mineral, alleviates gentamicin-induced nephrotoxicity in rats. Biol Trace Elem Res 2020;195:515-524. https://doi.org/10.1007/s12011-019-01875-4

35. Akman Alacabey N, Ozdemir H, Oto G. Hyperglycemic effect of dietary boron in rats with experimental diabetes mellitus induced by streptozotocin. Van Vet J 2023;34:244-250. https://doi.org/10.36483/vanvetj.1298344

36. Kucukkurt I, Ince S, Eryavuz A, et al. The effects of boron-supplemented diets on adipogenesis-related gene expressions, anti-inflammatory, and antioxidative response in high-fat fed rats. J Biochem Mol Toxicol 2023;37:e23257. https://doi.org/10.1002/jbt.23257

37. Eren M, Guclu B, Uyanik F, Karabulut N. The effects of dietary boron supplementation on performance, carcass composition and serum lipids in Japanese quails. J Anim Vet Adv 2006;5:1105-1108.

38. Ince S, Kucukkurt I, Demirel HH, Turkmen R, Sever E. Thymoquinone attenuates cypermethrin induced oxidative stress in Swiss albino mice.Pestic BiochemPhysiol 2012;104:229-235. https://doi.org/10.1016/j.pestbp.2012.09.003

39. Kar F, Hacioglu C, Senturk H, Burukoglu Donmez D, Kanbak G. The role of oxidative stress, renal inflammation, and apoptosis in post ischemic reperfusion injury of kidney tissue: the protective effect of dose-dependent boric acid administration. 2020;195:150-158. https://doi.org/10.1007/s12011-019-01824-1

40. Türkez H, Geyikoglu F, Tatar A, Keleş S, Ozkan A. Effects of some boron compounds on peripheral human blood. Z Naturforsch C J Biosci 2007;62:889-896. https://doi.org/10.1515/znc-2007-11-1218

41. İlhan AO, Can B, Kar F, Çakır Gündoğdu A, Söğüt İ, Kanbak G. An investigation into the protective effects of various doses of boric acid on liver, kidney, and brain tissue damage caused by high levels of acute alcohol consumption. Biol Trace Elem Res 2023;201:5346-5357. https://doi.org/10.1007/s12011-023-03699-9

42. Hunt CD, Idso JP. Dietary boron as a physiological regulator of the normal inflammatory response: a review and current research progress. 1999;12:221-233.

43. Naghii MR, Mofid M, Asgari AR, Hedayati M, Daneshpour MS. Comparative effects of daily and weekly boron supplementation on plasma steroid hormones and proinflammatory cytokines. Journal of Trace Elements in Medicine and Biology 2011;25:54-58. https://doi.org/10.1016/j.jtemb.2010.10.001
44. Gundogdu K, Gundogdu G, Demirkaya Miloglu F, Demirci T, Yılmaz Tascı S, El Aty AMA. Anti-inflammatory effects of boric acid in treating knee osteoarthritis: biochemical and histopathological evaluation in rat model. Biol Trace Elem Res 2023:e1-11 https://doi.org/10.1007/s12011-023-03872-0
45. Acaroz U, Ince S, Arslan Acaroz D, et al. Bisphenol-A induced oxidative stress, inflammatory gene expression, and metabolic and histopathological changes in male Wistar albino rats: Protective role of boron. Toxicol Res (Camb) 2019;8:262-269. https://doi.org/10.1039/c8tx00312b

Acknowledgment: This study was supported by the Pamukkale University Scientific Research Coordination Unit (project code: 2022BSP010).

Ethics committee approval: Permission was obtained from the Pamukkale University Animal Experiments Ethics Committee for the study (permission date (meeting dated 01.09.2022 and numbered 2022/07), permission number PAUHDEK-2022/26).

Author contributions

M.B. and M.F.D. designed the study and provided the relevant materials and analytical tools for experiments. M.B. and M.F.D. performed the experiments. M.B. and M.F.D. analyzed the data. M.B. wrote the manuscript.