

Boric acid supplementation affects antioxidant activity in ovariectomized female rats

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¹Program of Pharmacy Services, Gölhisar Vocational School of Health Services,
Burdur Mehmet Akif Ersoy University, Burdur, Turkey

²Department of Biochemistry, Faculty of Veterinary Medicine, Aydın Adnan Menderes University, Aydın, Turkey

Received 25.01.2022

Accepted 09.05.2022

Tekeli H., Ekren Asıcı G. S., Bildik A.

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Summary

This study aimed to investigate the protective effect of boric acid (BA) supplementation against oxidative stress (OS) in ovariectomized rats. A total of 48 nonpregnant female Wistar albino rats (180-250 g) were used in the experiment. The rats were divided into six equal groups (n = 8): Control, OVX, OVX + 5 mg/kg BA (OVX + BA₅), OVX + 10 mg/kg BA (OVX + BA₁₀), 5 mg/kg BA (BA₅), and 10 mg/kg BA (BA₁₀). The rats were intraperitoneally anesthetized with a combination of ketamine hydrochloride (85 mg/kg) and xylazine (10 mg/kg) and underwent a bilateral ovariectomy. Supplementation with 5 mg/kg and 10 mg/kg BA via oral gavage was started 2 weeks after ovariectomy and continued for 20 days. Catalase (CAT), glutathione S-transferase (GST), glutathione peroxidase (GPx), glutathione (GSH), and malondialdehyde (MDA) levels in the liver, kidney, and brain tissues of rats were analyzed by the spectrophotometric method. MDA concentrations in the liver tissue was increased in the OVX rats compared to the control group (p < 0.05). Liver GST activity was significantly decreased in the OVX rats, but it was increased with 5 mg/kg and 10 mg/kg BA supplementation (p < 0.05). Mean kidney GSH, GPx, and CAT activities in the OVX rats were increased with 5 mg/kg and 10 mg/kg BA supplementation (p < 0.05). In the brain tissue, GST activity was increased in the OVX rats treated with 10 mg/kg BA (p < 0.05). These results indicate that BA supplementation can enhance antioxidant defense mechanisms against OS after ovariectomy in female rats.

Keywords: antioxidant activity, boric acid, ovariectomy, oxidative stress

Menopause is associated with various short-term consequences, such as urogenital difficulties or joint pains, and long-term consequences, including mood changes, vasomotor symptoms, osteoporosis, neurological disorders, cardiovascular diseases, obesity, depression, sexual difficulties, insulin resistance, and metabolic dysfunction (12). Menopause in female rats shows in a significantly increased weight gain and bone loss (8). A decline in estrogen concentration is caused by increasing free radical production (42). The effect of metabolic disorders due to estrogen deficiency on the liver is clinically interesting because an excess of reactive oxygen species (ROS) can play a potential role in aggravation of liver, brain and kidney diseases (8).

Oxygen free radicals are extremely reactive and can damage cells and tissues by interacting with cell membranes and organelles. Malondialdehyde (MDA) is one of the most important products of lipid peroxidation (LPO) (45). A stressful condition leads to an excessive production of free radicals, which results

in oxidative stress, an imbalance in the oxidant/antioxidant systems (28). Abundant evidence from animal studies indicates that ovariectomy can induce a redox imbalance characterized by increased levels of lipoperoxidation markers, such as MDA and ROS, and decreased concentrations of antioxidant enzymes, such as catalase (CAT), glutathione-S transferase (GST), and glutathione peroxidase (GPx) (18, 34).

Boron is a bioactive food ingredient beneficial for bone growth and maintenance (36), brain functions, and hormone regulation (5, 39). It has been added to many new synthetic molecules tested as potential drugs in recent years (22). BA (H₃BO₃) is the most popular boron compound used in cosmetics and other industries (2). BA in human and animal nutrition has a considerable effect on various physiological and metabolic systems of the organism (19). Two hypotheses have been developed about the biochemical and physiological functions of BA. According to the first of them, BA activates cell membrane functions that influence the

response to hormone action, transmembrane signaling, and transmembrane movement of regulatory ions (5). According to the second hypothesis, BA works as a metabolic regulator in various enzymatic systems (30). It is thought that boron increases antioxidant capacity by increasing the storage of glutathione and thus reduces intracellular ROS. BA also acts as a Lewis acid, accepting a hydroxyl ion from water and releasing a proton (16, 17, 19).

Some pharmacological agents, such as vitamins, trace elements, and medicinal plants, have been successfully used to ameliorate unfavorable metabolic states due to oxidative stress (OS) after menopause (11, 13). The aim of the present study was to evaluate the effect of BA supplementation on antioxidants and OS in ovariectomized (OVX) female rats, which are a well-established animal model for postmenopausal changes.

Material and methods

Animals and experimental design. Experimental procedures were reviewed and approved by the Animal Ethics Committee of Aydın Adnan Menderes University, Aydın, Turkey (ADU-HADYEK, Decision number 64583101, 2013/029). Experiments on the effects of BA were performed on 48 nonpregnant female Wistar Albino rats (180-250 g) obtained from the Experimental Application and Research Center, Aydın Adnan Menderes University (Turkey). Before the experiment, the rats were kept 10 days for adaptation. During the study, all rats were provided with a relative humidity of 40-60%, optimal room temperature (22°C), and 12 h of light and 12 h of darkness.

The rats were randomized according to their weight into six groups of eight animals each: Control (n = 8), Ovariectomy (OVX) (n = 8), Ovariectomy + 5 mg/kg BA (OVX + BA₅) (n = 8), Ovariectomy + 10 mg/kg BA (OVX + BA₁₀) (n = 8), 5 mg/kg BA (BA₅) (n = 8), and 10 mg/kg BA (BA₁₀) (n = 8). They were anesthetized intraperitoneally with a combination of ketamine hydrochloride (85 mg/kg), and xylazine (10 mg/kg) and underwent a bilateral ovariectomy. The ovaries were excised and removed by opening the oviducts with minimal disruption to the soft tissues; then the incisions were closed with an ellipse (44). The abdominal and skin incisions were closed with 4-0 and 2-0 vicryl suture, respectively. Ovariectomy was performed on rats in the OVX, OVX + BA₅, and OVX + BA₁₀ groups, and sham ovariectomy was performed simultaneously on rats in the control group. BA (Sigma-B0252, USA) supplementation by oral gavage was started 2 weeks after ovariectomy and continued for 20 days. BA was supplemented at 5 mg/kg and 10 mg/kg, taking into account the 1998 data of WHO and other studies (43). At the end of the 21st day, the rats were sacrificed, and liver, kidney, and brain tissues were collected. The tissues were homogenized in phosphate buffer (pH 7.4) in 0.1 M KCl, and the homogenates were centrifuged at 10,000 g for 15 min. All samples were stored at -20°C until analysis.

Biochemical analysis. MDA, glutathione (GSH), GPx, GST, and CAT levels were analyzed in liver, kidney

and brain tissues. MDA concentration was measured by a method described by Yoshioka et al. (45). GSH levels were measured with 5,5-dithiobis(2-nitrobenzoate) at 412 nm according to a method described by Beutler et al. (4). Tissue GPx activity was determined by a coupled assay of Paglia and Valentine, using t-butylhydroperoxide as substrate (31). GST and CAT activities were measured with a spectrophotometer (Shimadzu, Japan) by methods of Habig et al. (14) and Aebi et al. (1), respectively. Total amounts of protein in tissues were measured according to Lowry's method using bovine serum albumin as standard (23).

Statistical analysis. The findings of the study were analyzed by one-way analysis of variance (ANOVA) and with Tukey's test as a post-hoc test, using SPSS v. 21 (IBM NY, USA). Data were presented as mean ± standard error (mean ± SEM), and statistical significance was set at p < 0.05.

Results and discussion

Liver, kidney, and brain MDA concentrations and antioxidant activities are given in Tables 1, 2, and 3, respectively. MDA activities in the liver tissue of OVX rats were significantly higher than those of the control group (p < 0.05). The mean MDA concentration in the liver and kidney tissues of rats supplemented with 5 mg/kg and 10 mg/kg BA were higher than for the control and OVX groups (p < 0.05).

The liver GSH content increased significantly in the OVX group compared to the control group (p < 0.05). With 5 mg/kg and 10 mg/kg BA supplementation, it decreased in liver tissue, but increased in kidney tissue (p < 0.05).

The mean GPx activity in the liver and kidney tissue of OVX rats was higher than it was in the control, BA₅, and BA₁₀ groups (p < 0.05). Supplementation with 5 mg/kg and 10 mg/kg BA increased GPx activity in liver tissue (p < 0.05). Liver GST activity in the OVX group was lower than it was in the control, BA₅, and BA₁₀ groups (p < 0.05). With the administration of 5 mg/kg and 10 mg/kg BA, GST activity increased in liver tissue (p < 0.05). GST activity in brain tissue was higher in the OVX rat groups administered 5 mg/kg BA than it was in the BA₅, BA₁₀, and OVX + BA₁₀ groups (p < 0.05). With 5 mg/kg and 10 mg/kg BA supplementation, CAT activity increased in kidney tissue, but decreased in liver tissue (p < 0.05).

The ovariectomy-induced menopause model in female rats is widely used to study biological processes and test new interventions that can minimize harmful effects associated with menopause (3, 24, 32). The experimental model most commonly used to observe the menopause period is a bilateral ovarian surgery (34, 35). OS is a condition characterized by an imbalance between pro-oxidant molecules and antioxidant defenses, including reactive oxygen and nitrogen species. MDA is the final product of LPO, and therefore it is commonly used as an indicator of OS (40). In the present study, liver MDA levels increased with ovariectomy, but there was no statistically significant change

Tab. 1. Effect of BA supplementation on MDA, GSH, and enzymatic antioxidant levels in liver tissues of OVX female rats (mean $\pm$ SEM)

Parameters	Groups (Mean $\pm$ SEM)					
	C	OVX	OVX + BA ₅	OVX + BA ₁₀	BA ₅	BA ₁₀
MDA (μ mol/g)	13.22 $\pm$ 5.57 ^d	34.62 $\pm$ 8.66 ^c	81.48 $\pm$ 10.83 ^a	71.83 $\pm$ 6.16 ^b	51.93 $\pm$ 4.42 ^b	46.14 $\pm$ 2.56 ^b
GSH (nmol/g)	3.36 $\pm$ 0.38 ^c	7.56 $\pm$ 1.17 ^a	6.24 $\pm$ 0.75 ^{ab}	4.57 $\pm$ 0.47 ^{bc}	5.22 $\pm$ 0.38 ^b	3.83 $\pm$ 0.24 ^c
GPx (U/g)	53.55 $\pm$ 3.25 ^c	156.75 $\pm$ 19.49 ^a	103.97 $\pm$ 8.57 ^b	63.46 $\pm$ 5.37 ^c	67.74 $\pm$ 3.20 ^c	49.22 $\pm$ 6.76 ^c
GST (nmol/g)	4125.92 $\pm$ 152.83 ^{ab}	2481.61 $\pm$ 408.06 ^d	3129.90 $\pm$ 229.67 ^c	3728.71 $\pm$ 200.20 ^b	3846.32 $\pm$ 200.46 ^b	4677.66 $\pm$ 220.91 ^a
CAT (kat/mg)	72.24 $\pm$ 33.01 ^a	42.97 $\pm$ 13.67 ^b	37.81 $\pm$ 8.63 ^b	6.61 $\pm$ 2.03 ^c	25.05 $\pm$ 25.36 ^b	37.70 $\pm$ 15.36 ^b

Explanation: ^{a, b, c, d} – superscripts in rows indicate statistically significant differences, $p < 0.05$

Tab. 2. Effect of BA supplementation on MDA, GSH, and enzymatic antioxidant levels in kidney tissues of OVX female rats (mean $\pm$ SEM)

Parameters	Groups (Mean $\pm$ SEM)					
	C	OVX	OVX + BA ₅	OVX + BA ₁₀	BA ₅	BA ₁₀
MDA (μ mol/g)	16.53 $\pm$ 2.73 ^c	15.13 $\pm$ 2.84 ^c	94.57 $\pm$ 10.11 ^a	40.46 $\pm$ 3.65 ^b	39.45 $\pm$ 5.61 ^b	38.12 $\pm$ 5.00 ^b
GSH (nmol/g)	14.60 $\pm$ 0.86 ^b	14.88 $\pm$ 1.80 ^b	26.11 $\pm$ 3.19 ^a	26.01 $\pm$ 2.15 ^a	12.53 $\pm$ 0.90 ^b	10.48 $\pm$ 0.60 ^b
GPx (U/g)	111.87 $\pm$ 9.74 ^c	188.22 $\pm$ 11.81 ^b	258.30 $\pm$ 25.96 ^a	246.98 $\pm$ 15.16 ^a	108.07 $\pm$ 11.00 ^c	95.77 $\pm$ 5.79 ^c
GST (nmol/g)	288.44 $\pm$ 38.77 ^b	319.20 $\pm$ 23.12 ^b	167.53 $\pm$ 25.91 ^c	152.85 $\pm$ 20.99 ^c	515.78 $\pm$ 53.49 ^a	491.28 $\pm$ 34.98 ^a
CAT (kat/mg)	6.90 $\pm$ 2.60 ^c	13.02 $\pm$ 4.82 ^c	34.75 $\pm$ 7.40 ^{ab}	52.22 $\pm$ 14.09 ^a	12.38 $\pm$ 2.74 ^c	20.52 $\pm$ 5.63 ^{bc}

Explanation: ^{a, b, c} – superscripts in rows indicate statistically significant differences, $p < 0.05$

Tab. 3. Effect of BA supplementation on MDA, GSH, and enzymatic antioxidant levels in brain tissues of OVX female rats (mean $\pm$ SEM)

Parameters	Groups (Mean $\pm$ SEM)					
	C	OVX	OVX + BA ₅	OVX + BA ₁₀	BA ₅	BA ₁₀
MDA (μ mol/g)	15.89 $\pm$ 3.45 ^b	14.56 $\pm$ 2.00 ^b	17.67 $\pm$ 2.53 ^b	15.75 $\pm$ 2.57 ^b	30.45 $\pm$ 6.32 ^a	30.82 $\pm$ 2.87 ^a
GSH (nmol/g)	31.98 $\pm$ 2.74	26.09 $\pm$ 1.49	25.50 $\pm$ 0.83	34.27 $\pm$ 4.35	32.62 $\pm$ 6.99	30.38 $\pm$ 3.00
GPx (U/g)	54.48 $\pm$ 5.41	65.64 $\pm$ 6.64	56.62 $\pm$ 5.16	45.56 $\pm$ 7.01	64.19 $\pm$ 11.78	62.32 $\pm$ 4.27
GST (nmol/g)	96.35 $\pm$ 8.81 ^a	98.54 $\pm$ 11.19 ^a	100.16 $\pm$ 5.30 ^a	76.78 $\pm$ 9.66 ^{bc}	66.78 $\pm$ 7.66 ^{bc}	56.44 $\pm$ 3.54 ^c
CAT (kat/mg)	51.77 $\pm$ 6.14	33.62 $\pm$ 8.69	35.50 $\pm$ 2.89	48.88 $\pm$ 7.25	37.22 $\pm$ 6.55	46.53 $\pm$ 5.95

Explanation: ^{a, b, c} – superscripts in rows indicate statistically significant differences, $p < 0.05$

in MDA levels in the kidney and brain tissues of OVX rats. Ovariectomy related with estrogen deficiency may affect not only reproductive organs but also metabolic activities in many tissues including brain, liver and kidney tissues (7). Increased MDA levels in the liver may be a result of estrogen deficiency after ovarian surgery and increased free radical formation in the liver (27). Conflicting results are reported in previous studies. Contini et al. (11) and Osmanova et al. (29) reported that MDA concentration did not increase in the kidney and brain tissues of OVX rats. According to another study, however, there was an increase in MDA concentration in the brain and kidney tissues of OVX rats (28).

The development of new therapeutic agents with fewer side effects to be used after menopause has been an interesting subject. Boron plays important roles in healthy bone development and steroid hormone metabolism, as well as prevents tissue damage (33). Its deficiency causes abnormal bone development, de-

terioration of growth, and a decrease in blood steroid hormone levels (5). BA may play a role as an antioxidant by neutralizing free radicals that are formed during LPO. It has been reported that MDA levels decreased along with BA in the brain and kidney tissues of arsenic-induced damage in female rats (21). Boron deficiency in animals decreases the electrical activity of the brain and short term-memory, whereas boron supplementation improves brain function (33). In the present study, 5 mg/kg and 10 mg/kg BA administered to OVX rats increased MDA levels in liver and kidney tissues. This increase may be related to high-dose boron accumulation or long-term exposure. But the effect of BA on the generation of free radicals may be revealed by the activation of antioxidant enzyme systems, which prevent the development of free radical reactions and the accumulation of superoxidation-peroxide.

The liver is a critical organ in mammals. It is responsible for a number of functions that, among others, support metabolism, immunity, digestion,

detoxification, and vitamin storage (24, 25). It has been reported that physiological functions deteriorate and oxidative damage increases in the liver of aged rats after ovariectomy and that pro-inflammatory and antioxidant activity also changes significantly (20). Chong et al. (9) found that GSH contents increased in the liver tissue of OVX rats although GPx activity did not change. In the present study, liver GSH and GPx activities increased significantly in the OVX rats, but GST and CAT activities decreased. This may be due to estrogen deficiency after ovariectomy. Antioxidant balance may change due to ROS production in kidney and brain tissues (15, 37). Nazıroğlu et al. (28) report that kidney and brain GSH and GPx activities did not differ between control and OVX rats (28). In another study, it is noted that there was not change in brain CAT activity after ovariectomy (29). In our study, kidney GPx activity increased in the OVX rats compared to the control rats, but brain and kidney GSH, GST, and CAT activities did not change. This might be due to unchanged MDA activity in kidney and brain tissues.

Boron repairs different body organs in mice with a dissimilar advanced antioxidant mechanism of defense. It also acts as a metabolic regulator in several enzymatic systems and is well-known to be involved in cell membrane functions and enzymatic antioxidant activity. Previous studies have shown that BA increases antioxidant levels in rats (10, 38). Ince et al. (18) noted that high doses of BA reduced GSH contents in the rat's liver, while GPx activity did not change. In the present study, supplementation with 5 mg/kg BA decreased GPx activity in the liver tissue of OVX rats, while GST activity increased. Similarly, 10 mg/kg BA decreased GSH, GPx, and CAT activities, while GST activity increased. In general, a high energy expenditure associated with GSH depletion occurs in the liver, which has the largest reservoir of GSH. Increasing liver LPO might be a result of depleted liver GSH stores (25, 26). The GST enzyme protects membrane components from LPO and works in interaction with GSH (13). In our study, the increase in liver GST activity may have been associated with the change in GSH and GPx levels. In particular, 10 mg/kg BA administered to the OVX rats increased their liver GST levels. At the same time, the highest GST activity was observed in the liver tissue of the BA₁₀ group. BA may reduce liver damage due to oxidative stress caused by OVX. There are no reports in the literature with which we could compare our findings on the effects of BA supplementation on antioxidant activities in the liver, brain, and kidney tissues of rats after ovariectomy.

Kidney GSH, GPx, and CAT activities were found to be higher in rats treated with 5 mg/kg and 10 mg/kg BA compared to the control and OVX rats. This may be due to an increase in renal intracellular GSH biosynthesis. BA may regulate NADPH production, thereby increasing GSH concentration in the body. The increase in GSH and GPx activities in kidney tis-

sue decreased GST levels. GPx activity may decrease OS by increasing glutathione reserves that neutralize oxidants with BA administration. Ulaş and Çay (41) investigated the relationship between GSH and GPx activities in the kidney tissue of diabetic OVX rats and reported that GPx is a key component of the antioxidant defense mechanism and is necessary for the normal functions of GSH. Also the CAT activity increasing with BA supplementation may be explained by the fact that BA (a Lewis acid) is capable of accepting a hydroxyl ion. An increase in CAT activity may reduce damage in the kidney due to LPO after ovariectomy.

Boric acid has an antioxidant effect that prevented damage caused by alcohol to membranes of the cerebral cortex of rat pups (37). In our study, GST activities in the brain tissue of OVX rats increased with 5 mg/kg BA supplementation, but GSH, GPx, and CAT activities did not change. Furthermore, the activities of these enzymes have been shown to be lower in the brain than in other tissues, for example, in the liver and kidney (6). It was also observed that the unchanged MDA concentrations in the brain tissues of the control, OVX and BA-treated rats were in line with GSH, GPx, and CAT levels.

Our results indicate that the supplementation of BA at 5 mg/kg and 10 mg/kg can enhance antioxidant defense mechanisms against OS after ovariectomy in female rats. BA itself is not an antioxidant, but it might strengthen the antioxidant defense system of the tissues. However, the usability and potential side effects of BA in animals should be well investigated. In addition, new evidence on the antioxidant and metabolic activity of BA should be supported by *in vivo* and *in vitro* studies at different doses.

References

1. Aebi H.: Catalase in vitro. *Methods Enzymol.* 1984, 105, 121-126.
2. Akkoyun H. T.: Effect of boric acid on some elemental levels on rat's liver and kidney tissues during mercury chloride exposure. *Cell. Mol. Biol.* 2008, 64, 84-88.
3. Akyüz E., Makav M., Mushap K., Bayram P.: The effect of Tarantula cubensis extract on gentamicin-induced acute kidney injury in ovariectomized rats. *Kafkas Univ. Vet. Fak. Derg.* 2021, 27, 559-566.
4. Beutler E., Dubon O., Kelly B. M.: Improved method for the determination of blood glutathione. *J. Lab. Clin. Med.* 1963, 61, 882-888.
5. Bolt Hermann M., Başaran N., Duydu Y.: Effects of boron compounds on human reproduction. *Arch. Toxicol.* 2020, 94, 717-724.
6. Brocardo P. S., Gil-Mohapel J., Christie B. R.: The role of oxidative stress in fetal alcohol spectrum disorders. *Brain Res. Rev.* 2011, 67, 209-225.
7. Chantal N. M., Désiré D. D. P., Caude B. D., Sandrine M. N. Y., Lohik M. N., Francine M. M., Pierre K.: Neuroprotective effects of the Anthocleista schweinfurthii Gilg. (loganiaceae) stem bark extract in postmenopause-like model of ovariectomized wistar rats. *J. Complement Integr. Med.* 2019, 16, 1.
8. Choi J. S., Song J.: Effect of genistein on insulin resistance, renal lipid metabolism, and antioxidative activities in ovariectomized rats. *J. Nutr.* 2009, 25, 676-685.
9. Chong C. L. G., Farida H., Faizah O.: Hepatoprotective effects of morinda Citrifolia leaf extract on ovariectomized rats fed with thermoxidized palm oil diet: evidence at histological and ultrastructural level. *Oxidative medicine and cellular longevity* 2019.
10. Cikler-Dulger E., Sogut I.: Investigation of the protective effects of boric acid on ethanol induced kidney injury. *Biotech. Histochem.* 2020, 95, 186-193.
11. Contini M. C., Millen N., Gonzalez M., Mahieu S.: Melatonin prevents oxidative stress in ovariectomized rats treated with aluminium. *Biol. Trace Elem. Res.* 2011, 144, 924-943.

12. *El-Fattah A. I. A., Fathy M. M., Ali Z. Y., El-Garawany A. E. R. A., Mohamed E. K.*: Enhanced therapeutic benefit of quercetin-loaded phytosome nanoparticles in ovariectomized rats. *Chem. Biol. Interact.* 2017, 2271, 30-38.
13. *Gyamfi A. M., Ohtani I. I., Shinno E., Aniya Y.*: Inhibition of glutathione-S transferases by thoningianin A, isolated from the African medicinal herb, *Thonningia sanguinea*, in vitro. *Food Chem. Toxicol.* 2004, 42, 1401-1408.
14. *Habig W. H., Pabst M. J., Jakoby W. B.*: Glutathione S-transferases; The first enzymatic step in mercapturic acid formation. *J. Biol. Chem.* 1974, 249, 7130-7139.
15. *Haugen E., Nath K.*: The involvement of oxidative stress in the progression of renal injury. *Blood Purif.* 1999, 17, 58-65.
16. *Hunt C. D.*: The biochemical effects of physiologic amount of dietary boron in animal nutrition models. *Environ. Health Perspect.* 1994, 102, 35-43.
17. *Hunt C. D., Idso J. P.*: Dietary boron as a physiological regulator of the normal inflammatory response: a review and current research progress. *J. Trace Elem. Exp. Med.* 1999, 12, 221-233.
18. *Ince S., Kucukkurt I., Cigerci I. H., Fidan A. F., 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.
19. *Khalik H., Juming Z., Ke-Mei P.*: The physiological role of boron on health. *Biol. Trace Elem. Res.* 2018, 186, 1-21.
20. *Kireev R. A., Tresguerres A. C. F., Garcia C., Borrás C., Ariznavarreta C., Vara E., Tresguerres J. A.*: Hormonal regulation of pro-inflammatory and lipid peroxidation processes in liver of old ovariectomized female rats. *Biogerontology* 2010, 11, 229-243.
21. *Kucukkurt I., Ince S., Demirel H. H., Turkmen R., Akbel E., Celik Y.*: The effects of boron on arsenic-induced lipid peroxidation and antioxidant status in male and female rats. *J. Biochem. Mol. Toxicol.* 2015, 29, 564-571.
22. *Leśnikowski Z. J.*: Recent developments with boron as a platform for novel drug design. *Expert. Opin. Drug Discov.* 2016, 11, 569-578.
23. *Lowry O. H., Rosebrough N., Farr A. L., Randall R. J.*: Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 1951, 193, 265-275.
24. *Ltaif M., Manel G., Ahlem S.*: Protective effects of *A. sativa* against oxidative stress-induced liver damage in ovariectomized mice. *Biomed Res. Int.* 2021.
25. *Lu Shelly C.*: Dysregulation of glutathione synthesis in liver disease. *Liver Research* 2020, 4, 64-73.
26. *Mohora M., Boghianu L., Muscurel C., Duta C., Dumitrache C.*: Effects of boric acid on redox status in the rat liver. *Romanian J. Biophys.* 2002, 12, 77-82.
27. *Morrone S., Schnorr C. E., Behr G. A., Gasparotto J., Bortolin R. C., Moresco K. S., Moreira J. C. F.*: Curcumin supplementation decreases intestinal adiposity accumulation, serum cholesterol alterations, and oxidative stress in ovariectomized rats. *Oxid. Med. Cell. Longev.* 2016, 5719291.
28. *Naziroglu M., Gun H. B., Savas S., Çelik Ö., Sözbir E., Özkaya M. O., Köse S. A.*: Capparid ovata modulates ovariectomy induced-oxidative toxicity in brain, kidney and liver of aged mice. *Cell Membr. Free Radic. Res.* 2011, 3, 186-193.
29. *Osmanova S., Sezer E., Turan V., Zeybek B., Terek M. C., Kanit L.*: The effects of raloxifene treatment on oxidative status in brain tissues and learning process of ovariectomized rats. *Iran. J. Reprod. Med.* 2011, 9, 295-300.
30. *Ozdemir H., Yaren B., Oto G.*: Effect of dietary boron on learning and behavior in rats administered with boric acid. *Cell. Mol. Biol.* 2019, 65, 65-72.
31. *Paglia D. E., Valentine V. N.*: Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J. Lab. Clin. Med.* 1967, 70, 158-169.
32. *Pawlowski M., Harrison A., Piersiak T., Grabos D., Tymicki G., Pawlowski K., Puzio I.*: Effect of camelina oil on the structure of aortas in rats. *Med. Weter.* 2016, 72 (4), 240-246.
33. *Pizzorno L.*: Nothing boring about boron. *Integr. Med.* 2015, 14, 35-48.
34. *Radzki R. P., Bieńko M., Kapica M., Puzio I., Dobrowolski P., Filip R.*: Effect of β 2-adrenergic receptor agonist clenbuterol on the mineralization of tibiae in rats with established osteopenia. *Med. Weter.* 2006, 62 (7), 824-826.
35. *Schuller A. K., Mena Canata D. A., Hackenhaar F. S., Engers V. K., Heemann F. M., Putti J. S., Benfato M. S.*: Effects of lipoic acid and n-3 long-chain polyunsaturated fatty acid on the liver ovariectomized rat model of menopause. *Pharmacol. Rep.* 2018, 70, 263-269.
36. *Singh A. K., Kewalramani N., Mani V., Sharma A., Kumari P., Pal R. P.*: Effects of boric acid supplementation on bone health in crossbred calves under tropical condition. *J. Trace Elem. Med. Biol.* 2021, 63, 126647.
37. *Sogut I., Oglakci A., Kartkaya K., Ol K., Sogut M. S., Kanbak G., Inal M. E.*: Effect of boric acid on oxidative stress in rats with fetal alcohol syndrome. *Exp. Ther. Med.* 2015, 9, 1023-1027.
38. *Sogut I., Paltun S. O., Tunçdemir M., Ersoz M., Hurdag C.*: The antioxidant and antiapoptotic effect of boric acid on hepatotoxicity in chronic alcohol-fed rats. *Can J. Physiol. Pharmacol.* 2018, 96, 404-411.
39. *Tang J., Zheng X., Xiao K.*: Effect of boric acid supplementation on the expression of BDNF in African ostrich chick brain. *Biol. Trace Elem. Res.* 2016, 170, 208-215.
40. *Takeshima T., Usui K., Mori K., Asai T., Yasuda K., Kurodo S., Yumura Y.*: Oxidative stress and male infertility. *Reprod. Med. Biol.* 2021, 20, 41-52.
41. *Ulas M., Cay M.*: 17β -Estradiol and vitamin E modulates oxidative stress-induced kidney toxicity in diabetic ovariectomized rat. *Biol. Trace Elem. Res.* 2011, 144, 821-831.
42. *Virdis A., Ghiadoni L., Pinto S., Lombardo M., Petraglia F., Gennazzani A., Salvetti A.*: Mechanisms responsible for endothelial dysfunction associated with acute estrogen deprivation in normotensive women. *Circulation* 2000, 101, 2258-2263.
43. *Weir R. J., Fisher R. S.*: Toxicologic studies on borax and boric acid. *Toxicol. Appl. Pharmacol.* 1972, 23, 351-364.
44. *Wronski T. J., Dann L. M., Scott K. S., Cintron M.*: Long-term effects of ovariectomy and aging on the rat skeleton. *Calcif. Tissue Int.* 1989, 45, 360-366.
45. *Yoshioka T., Kawada K., Shimada T.*: Lipid peroxidation in maternal and cord blood and protective mechanism against active oxygen toxicity in the blood. *Am. J. Obstet. Gynecol.* 1979, 135, 372-376.

Corresponding author: Hakan Tekeli, Program of Pharmacy Services, Gölhisar Vocational School of Health Services, Burdur Mehmet Akif Ersoy University, 15030 Burdur, Turkey; e-mail: htekelim@mehmetakif.edu.tr