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 lipid peroxidation markers, such as MDA and ROS, and decreased concentrations of antioxidant enzymes, such as catalase (CAT), glutathione S-transferase (GST), glutathione peroxidase (GPx), 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 were 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

Beric acid supplementation affects antioxidant activity in ovariectomized female rats

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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 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
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), xylazine (10 mg/kg) and underwent a bilateral ovariec-
tomy. 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 (43). The abdomi-
nal 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 ovariec-
tomy 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 (44). 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 spectropho-
tometer (Shimadzu, Japan) by methods of Habig et al. (14) and Aebe 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 ovarieotomy-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 ovariec-
tomy, but there was no statistically significant change
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, deterioration 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,

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>C</th>
<th>OVX</th>
<th>OVX + BA$_5$</th>
<th>OVX + BA$_{10}$</th>
<th>BA$_5$</th>
<th>BA$_{10}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (µmol/g)</td>
<td>13.22 ± 5.57$^a$</td>
<td>34.62 ± 8.66$^a$</td>
<td>81.48 ± 10.83$^a$</td>
<td>71.83 ± 6.16$^a$</td>
<td>51.93 ± 4.42$^b$</td>
<td>46.14 ± 2.56$^b$</td>
</tr>
<tr>
<td>GSH (nmol/g)</td>
<td>3.36 ± 0.38$^a$</td>
<td>7.56 ± 1.17$^a$</td>
<td>6.24 ± 0.75$^a$</td>
<td>4.57 ± 0.47$^a$</td>
<td>5.22 ± 0.38$^b$</td>
<td>3.83 ± 0.24$^b$</td>
</tr>
<tr>
<td>GPx (U/g)</td>
<td>53.55 ± 3.25$^a$</td>
<td>156.75 ± 19.49$^a$</td>
<td>103.97 ± 8.57$^a$</td>
<td>63.46 ± 5.37$^a$</td>
<td>67.74 ± 3.20$^b$</td>
<td>49.22 ± 6.76$^b$</td>
</tr>
<tr>
<td>GST (nmol/g)</td>
<td>4125.92 ± 152.83$^a$</td>
<td>2481.61 ± 408.06$^a$</td>
<td>3129.90 ± 229.67$^a$</td>
<td>3728.71 ± 200.20$^a$</td>
<td>3846.32 ± 200.46$^a$</td>
<td>4677.66 ± 220.91$^a$</td>
</tr>
<tr>
<td>CAT (kat/mg)</td>
<td>72.24 ± 33.01$^a$</td>
<td>42.97 ± 13.67$^a$</td>
<td>37.81 ± 6.63$^a$</td>
<td>6.61 ± 2.03$^a$</td>
<td>25.05 ± 25.36$^b$</td>
<td>37.70 ± 15.36$^b$</td>
</tr>
</tbody>
</table>

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 ± SEM)

<table>
<thead>
<tr>
<th>Parameters</th>
<th>C</th>
<th>OVX</th>
<th>OVX + BA$_5$</th>
<th>OVX + BA$_{10}$</th>
<th>BA$_5$</th>
<th>BA$_{10}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (µmol/g)</td>
<td>16.53 ± 2.73$^a$</td>
<td>15.13 ± 2.84$^a$</td>
<td>94.57 ± 10.11$^a$</td>
<td>40.46 ± 3.65$^a$</td>
<td>39.45 ± 5.61$^a$</td>
<td>38.12 ± 5.00$^a$</td>
</tr>
<tr>
<td>GSH (nmol/g)</td>
<td>14.60 ± 0.86$^a$</td>
<td>14.88 ± 1.80$^a$</td>
<td>26.11 ± 3.19$^a$</td>
<td>26.01 ± 2.15$^a$</td>
<td>12.53 ± 0.90$^a$</td>
<td>10.48 ± 0.60$^a$</td>
</tr>
<tr>
<td>GPx (U/g)</td>
<td>111.87 ± 9.74$^a$</td>
<td>188.22 ± 11.81$^a$</td>
<td>258.30 ± 25.96$^a$</td>
<td>246.98 ± 15.16$^a$</td>
<td>108.07 ± 11.00$^a$</td>
<td>95.77 ± 5.78$^a$</td>
</tr>
<tr>
<td>GST (nmol/g)</td>
<td>288.44 ± 38.77$^a$</td>
<td>319.20 ± 23.12$^a$</td>
<td>167.53 ± 25.91$^a$</td>
<td>152.85 ± 20.99$^a$</td>
<td>515.78 ± 53.49$^a$</td>
<td>491.28 ± 34.98$^a$</td>
</tr>
<tr>
<td>CAT (kat/mg)</td>
<td>6.90 ± 2.60$^a$</td>
<td>13.02 ± 4.82$^a$</td>
<td>34.75 ± 7.40$^a$</td>
<td>52.22 ± 14.09$^a$</td>
<td>12.38 ± 2.74$^a$</td>
<td>20.52 ± 5.63$^a$</td>
</tr>
</tbody>
</table>

Explanation: as in Tab. 1.

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

<table>
<thead>
<tr>
<th>Parameters</th>
<th>C</th>
<th>OVX</th>
<th>OVX + BA$_5$</th>
<th>OVX + BA$_{10}$</th>
<th>BA$_5$</th>
<th>BA$_{10}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MDA (µmol/g)</td>
<td>15.89 ± 3.45$^a$</td>
<td>14.56 ± 2.00$^a$</td>
<td>17.67 ± 2.53$^a$</td>
<td>15.75 ± 2.57$^a$</td>
<td>30.45 ± 6.32$^a$</td>
<td>30.82 ± 2.87$^a$</td>
</tr>
<tr>
<td>GSH (nmol/g)</td>
<td>31.98 ± 2.74$^a$</td>
<td>26.09 ± 1.49$^a$</td>
<td>25.50 ± 0.83$^a$</td>
<td>34.27 ± 4.35$^a$</td>
<td>32.62 ± 6.99$^a$</td>
<td>30.38 ± 3.00$^a$</td>
</tr>
<tr>
<td>GPx (U/g)</td>
<td>54.48 ± 5.41$^a$</td>
<td>65.64 ± 6.64$^a$</td>
<td>56.62 ± 5.16$^a$</td>
<td>45.56 ± 7.01$^a$</td>
<td>64.19 ± 11.78$^a$</td>
<td>62.32 ± 4.27$^a$</td>
</tr>
<tr>
<td>GST (nmol/g)</td>
<td>96.35 ± 8.81$^a$</td>
<td>98.54 ± 11.19$^a$</td>
<td>100.16 ± 5.30$^a$</td>
<td>76.78 ± 9.66$^a$</td>
<td>66.78 ± 7.66$^a$</td>
<td>56.44 ± 3.54$^a$</td>
</tr>
<tr>
<td>CAT (kat/mg)</td>
<td>51.77 ± 6.14$^a$</td>
<td>33.62 ± 8.89$^a$</td>
<td>35.50 ± 2.89$^a$</td>
<td>48.88 ± 7.25$^a$</td>
<td>37.22 ± 6.55$^a$</td>
<td>46.53 ± 5.95$^a$</td>
</tr>
</tbody>
</table>

Explanation: as in Tab. 1.
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). Naziroğlu 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 tissue 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 vitro and in vivo studies at different doses.

References


