Cellular and Molecular Biology
E-ISSN : 1165-158X / P-ISSN : 0145-5680
www.cellmolbiol.org

Original Research

Effect of dietary boron on learning and behavior in rats administered with boric acid

Hulya Ozdemir*, Biljana Yaren, Gokhan Oto

Department of Pharmacology, School of Medicine, Van Yuzuncu Yil University, Van, Turkey

Correspondence to: hulyaozdemir39@gmail.com

Received April 5, 2018; **Accepted** January 16, 2019; **Published** January 31, 2019

Doi: http://dx.doi.org/10.14715/cmb/2019.65.1.12

Abstract: This study was designed to investigate the effect of dietary boron on spatial learning, anxiety, some vitamins and oxidative parameters in rats. Thirty-two Wistar albino male rats were used in the study. The rats were equally divided into four groups with 8 rats each: I control group: standard pellet diet only, II. group: 250 ppm boric acid, III. group: 500 ppm boric acid and IV. group: 1000 ppm boric acid added into standard pellet diet. Over a five-week period, elevated plus-maze test was used for anxiety assessment and Morris water maze test was used for evaluating spatial learning. Additionally, blood samples were obtained at the end of the experiment and were used to determine the serum levels of some vitamins and oxidative parameters. Dietary boron significantly increased weight gain (p<0.001) and food consumption in the 250 ppm and 500 ppm groups (p<0.05). Although boron supplementation had no significant effect on learning and anxiety-related behavior, it had beneficiary effects on memory retention in the 1000 ppm group (p<0.05). Biochemical analyses showed a significant decrease in the MDA levels (p<0.05) and an increase in vitamin D_3 levels (p<0.01) in the 500 ppm group, a significant increase in GSH-Px activity in the 250 ppm and 500 ppm groups (p<0.05), and a decrease in vitamin E levels in all the experimental groups (p<0.05). In conclusion, our study demonstrated that dietary boron can be beneficial for health when administered at appropriate doses.

Key words: Anxiety; Boron; Learning; Oxidative parameters; Vitamins.

Introduction

The role of microelements in brain development and cognitive performance is a major concern among researchers. Deficiency of vitamins, minerals, and trace elements may lead to poor brain development and function (1,2). Moreover, a diet low in minerals and vitamins may result in cognitive deficits at advanced ages (1,3). However, supplemental trace elements should be given at physiological doses when administered in parenteral nutrition since excess doses of these elements may lead to reduced cognitive performance (4). Trace elements particularly including copper, zinc, iron, iodine, and selenium are highly essential for human health.

Boron (B), atomic number 5, is a metalloid belonging to the Group IIIA on the periodic table and is used in the forms of borax, colemanite, boronate-calcite, and boric acid (5). Moreover, boron is commonly used in pharmaceutical products due to its antimicrobial effectiveness including disinfectants, toothpaste, eyewash solutions, mouthwashes, irrigation solutions, and antiseptics. In addition, boron is also used in a cancer treatment method known as Boron Neutron Capture Therapy (BNCT) (6). In body fluids and tissues, 98.4% of boron is found as boric acid (H3BO3) and 1.6% of it is in borate form (7,8). Therefore, boron is often administered in the form of boric acid in the studies investigating the physiological effects of dietary boron (9-11).

Literature indicates that there are several hypotheses explaining the effectivity of boron and boron-containing compounds. One of these hypotheses postulates that boron has key roles in the function, stability, or structure of cell membranes (12,13). Another hypothesis maintains that the probable effects of boron may emerge from its reaction with polysaccharides, AMP, pyridoxine, riboflavin, pyridine, and other cis-diol-containing biomolecules. In this way, according to this hypothesis, boron alters the functions of cis-diol-containing compounds by stabilizing these compounds regardless of their functions (14). A third hypothesis, on the other hand, suggests that boron functions as a negative regulator that affects some key enzymatic reactions in metabolic pathways through competitive inhibition (15).

Boron has recently been shown to be involved in various nutritional, metabolic, hormonal, and physiological processes in human development. In particular, boron has been reported to have antioxidant effects (8,13), to regulate immune system functions (16,17), to regulate the mineral metabolism (13,18), vitamin metabolism (19), and steroid hormone metabolism (20,21), to improve bone strength (22,23), to be effective in the treatment and prevention of arthritis (24,25), to have beneficiary effects on the treatment and prevention of cancer (26-28) and to improve brain and cognitive function (29,30).

Deficiency of dietary boron has been shown to result in an alteration in rat behavior. In addition, human studies have indicated that low-dose boron intake improves brain functions, whereas boron deprivation may have adversary effects on short-term memory, motor skills, and attention. Literature reviews show that there has been no study investigating the effects of boron on learning, memory, and anxiety. Therefore, the present study aimed to investigate the effects of boron on spatial

learning, memory retention, and anxiety and on some antioxidant parameters and vitamin levels in rats administered with boric acid at different doses.

Materials and Methods

Subjects

A total of 32 Wistar-albino male rats weighing 200±20 g were used for the experiment. All the rats were obtained from Yuzuncu Yil University Experimental Animals Research and Application Laboratory. The animals were kept at room temperature (22 °C) with a 12-h dark/light cycle. The study protocol regarding animal care and the procedure was approved by the local ethics committee.

Procedure

The study was performed over a period of 4 weeks. The rats were divided into four groups with eight rats each: (I) control group: standard pellet diet only, (II) 250 ppm: 250 ppm (0.025%) boric acid added into standard pellet diet, (III) 500 ppm: 500 ppm (0.05%) boric acid added into standard pellet diet, and (IV) 1000 ppm: 1000 ppm (0.1%) boric acid added into standard pellet diet.

Behavioral procedures

Elevated plus maze (EPM) test was performed on day 0 and day 28 during the experiment. Animals were brought to the laboratory at least 3 hours before the test for acclimatization. To minimize the anxiety of the animals, the maze was assembled in an isolated room away from any external interference of noises, scents, or movement. A plus-shaped maze elevated 50 cm from the ground consisting of two opposite open and two opposite closed arms (width 12 cm, length 50 cm) and a central square were used. Each rat was placed in the central square of the maze facing one of the open arms and was video recorded for 5 min using Noldus Ethno Vision Tracking System. The maze was cleaned after the testing of each animal. The proportion of entries into open arms/total entries and the percentage of time spent on the open arms/total time were calculated for each rat.

Morris water maze (MWM) test was performed using the spatial version of the MWM test utilized by Tuzcu and Baydaş (31). For pretraining orientation, the rats were made to swim in the platform-free maze for 2 min. Before the testing, environmental cues that aid the rats in spatial learning including high-contrast geometric patterns were placed on the walls visible to the animal from the water and platform throughout the duration of the experiment. Using a computer equipped with Noldus EthoVision Tracking System, the maze was divided into 4 equal quadrants. Care was taken to position the platform in the same quadrant throughout the test. Each rat swam for 4 times within four consecutive days. The time spent on locating the platform (reaction time) and the time spent in the platform quadrant were recorded for each rat.

Each rat was allowed to swim for a maximum period of 90 sec (32). The rats that could not locate the hidden platform during this period were guided to the platform by the researcher. For such cases, the reaction time was accepted as 90 sec. During both training and testing sessions, the rats were left on the hidden platform for a brief session of 30 sec to allow them to recognize the environmental cues. To avoid rote learning, the rats were placed in a different starting point prior to each testing session. Care was taken to use the same starting points for each rat in the same order (33).

On the fifth day of the experiment, each rat was made to swim in the platform-free maze for 1 min in order to determine reference memory. The percentage of the time spent in the platform quadrant was accepted as the indicator of memory retention.

At the end of the experiment, blood samples were collected into serum gel separator tubes and serum was separated after centrifugation at 3,000 rpm for 30 min. Serum levels of vitamin D_3 and E were measured by using the HPLC device, oxidative stress parameters were defined by a Shimadzu V-1800 spectrophotometer, and the differences among the groups were evaluated statistically.

Chromatographic analysis

Serum levels of vitamin A, D3, and E and serum MDA levels were measured using the high-performance liquid chromatography with ultraviolet detection (HPLC–UV) method (34,35).

Spectrophotometric analysis

Serum total antioxidant capacity (TAC) was measured spectrophotometrically by using a commercially available kit developed by Erel (36) (Relassay Diagnostics, Total Antioxidant Status Assay Kit) which allows the measurement of TAC in numerous body fluids. Glutathione peroxidase (GSH-Px) was measured using a commercially available kit (Randox Laboratories Ltd.). The experimental protocol was performed in accordance with the method developed by Paglia and Valentine (37). Using a Shimadzu UV-1800 spectrophotometer, enzyme activity was assessed by monitoring the changes in NADPH absorbance at a wavelength of 340 nm.

Statistical analysis

Data were analyzed using SPSS 13.0 for Windows (SPSS Inc. Co., Chicago, IL, USA). Descriptive statistics were presented as mean ± standard deviation (SD). Kruskal-Wallis test was used for comparing three or more groups. A p value of <0.05 was considered significant.

Results

The present study was conducted over a period of 4 weeks. Morris Water Maze (MWM) test was performed to evaluate the effects of boron on spatial learning and memory retention and the EPM test was performed to evaluate the effects of boron on anxiety in rats. In addition, the effects of boron on some vitamins and oxidative stress parameters were also investigated.

Morris Water Maze test

Morris Water Maze (MWM) test was performed for a period of four days to evaluate the effects of boron on learning. In the MWM test, the rats learned to escape from the water by locating the hidden platform. The re-

Table 1. Effect of boron on the time spent for locating the hidden platform (MWM test).

Day	Control group Mean±SD	250 ppm B Mean±SD	500 ppm B Mean±SD	1000 ppm B Mean±SD
1	49.68 ± 27.77 a	44.13 ± 30.33 a	39.13 ± 32.01 a	47.93 ± 30.24 a
2	34.14 ± 23.17 b	37.21 ± 29.19 a	30.47 ± 25.87 ab	31.00 ± 22.63 b
3	27.68 ± 21.93 bc	30.82 ± 24.93 ab	19.57 ± 14.90 bc	22.07 ± 22.63 b
4	17.14 ± 15.76 c	18.56 ± 15.99 b	15.68 ± 10.17 c	21.04 ± 15.94 b

The results with two letters indicate a significant difference between the days shown by the letters ($p<0.05$).

sults of the MWM test were expressed in seconds and were presented in Table 1 and Figure 1.

An analysis on the time spent on locating the hidden platform on each training day among the four groups indicated that the time length gradually decreased from day 1 to day 4 in all four groups. However, no significant difference was observed among the groups ($p>0.05$) (Table 1). Nevertheless, although no significant difference was found among the groups, administration of boron led to faster learning, particularly in the 500 ppm group (Figure 1).

Table 2 and Figure 2 presents the MWM test results regarding memory retention. In the MWM test, the time spent in the platform quadrant and the distance of the path traveled by each rat in one minute were recorded.

No significant difference was observed among the four groups with regards to the distance of the path traveled by the rats ($p>0.05$). However, the time spent in the platform quadrant significantly increased in the 1000 ppm group compared to the control group ($p<0.05$). Moreover, although no significant difference was found between the 500 ppm group and the control group, the rats in this group showed an important increase in the time spent in the platform quadrant compared to the control group.

No significant relationship was found among the parameters obtained by the Kruskal-Wallis test ($p>0.05$). In the 1000 ppm group, a tendency for the increase was observed although no significant increase was established.

Elevated plus maze test

First and 28 elevated plus maze test performed on the day of the results of the study are shown in Table 3, Figure 3 and Figure 4. Allowed the ratio of the total number of entries and time spent in the open arms, a number of entries into the open arms was observed as a percentage of the total time.

According to the Kruskal-Wallis test was no statistical difference between the parameters obtained (p>

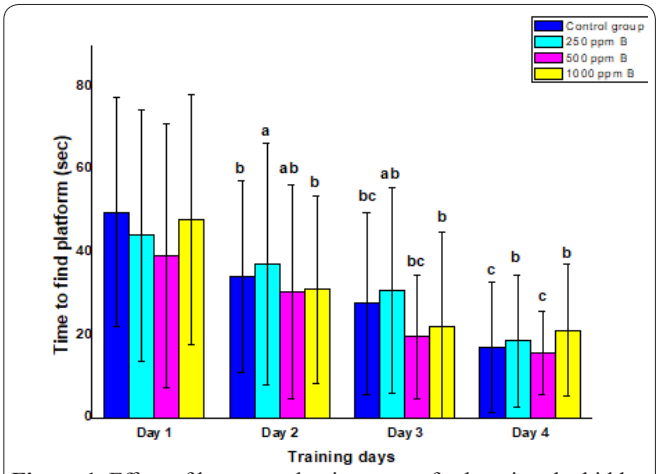

Figure 1. Effect of boron on the time spent for locating the hidden platform (MWM test). The results with two letters indicate a significant difference between the days shown by the letters ($p<0.05$).

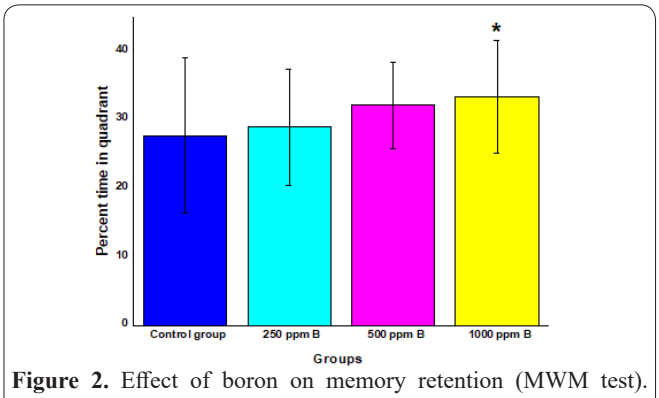

Figure 2. Effect of boron on memory retention (MWM test). *p<0.05 compared to the control group.

Table 2. Effect of boron on memory retention (MWM test).

Group	Time spent in the platform quadrant (%) Mean±SD
Control	27.65 ± 11.32
250 ppm	28.91 ± 8.44
500 ppm	32.07 ± 6.27
1000 ppm	33.34 ± 8.20*

*$p<0.05$ compared to the control group.

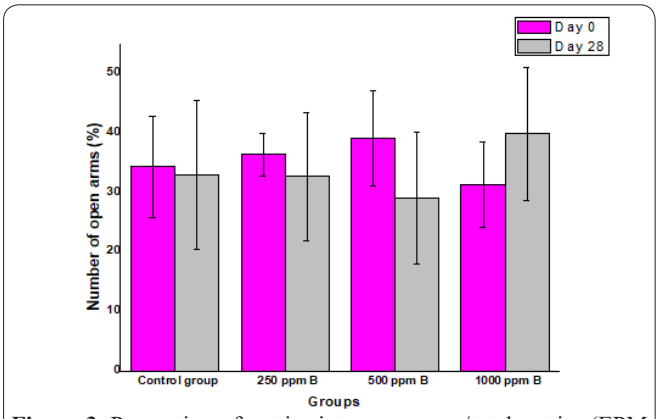

Figure 3. Proportion of entries into open arms/total entries (EPM test).

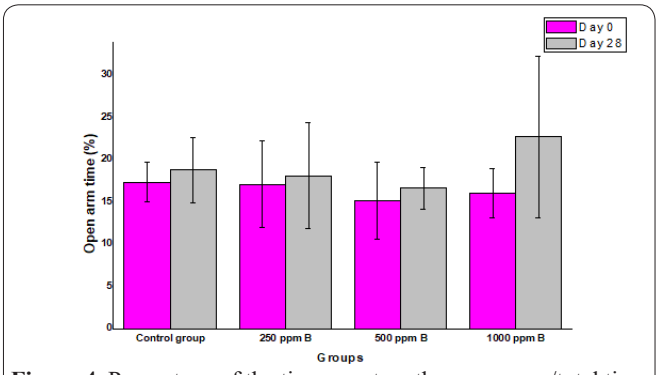

Figure 4. Percentage of the time spent on the open arms/total time (EPM test).

Table 3. Effect of boron on EPM test results

	Entries into open arms/total entries (%)		Time spent on the open arms/total time (%)	
	Day 0 Mean±SD	Day 28 Mean±SD	Day 0 Mean±SD	Day 28 Mean±SD
Control	34.29±8.42	32.90±12.52	17.37±2.36	18.80±3.89
250 ppm	36.38±3.62	32.67±10.82	17.14±5.15	18.11±6.29
500 ppm	39.09±7.92	29.05±11.13	15.17±4.52	16.63±2.49
1000 ppm	31.29±7.17	39.85±11.21	16.09±2.90	22.72±9.56

EPM results obtained on day 1 and day 28.

Table 4. Effect of different doses of boron on vitamin A, D_3 and E levels.

Group	Control Mean±SD	250 ppm Mean±SD	500 ppm Mean±SD	1000 ppm Mean±SD	*p*
Retinol (µg/ml)	0.861±0.111	0.857±0.112	0.943±0.226	0.924±0.220	>0.05
Alpha-tocopherol (µg/ml)	0.987±0.124	0.678±0.234*	0.724±0.267*	0.764±0.214*	<0.05
D_3 (ng/ml)	19.80± 2.55	22.09±3.78	28.94±1.29**	24.16±5.88	<0.01

* $p<0.05$, ** $p<0.01$ compared to the control group.

Table 5. Effect of different doses of boron on serum levels of MDA, GSH-Px and TAC.

Group	Control Mean±SD	250 ppm Mean±SD	500 ppm Mean±SD	1000 ppm Mean±SD	*p*
MDA (nmol/ml)	1.54 ± 0.39	1.47 ±0.23	1.12±0.11*	1.62±0.33	<0.05
GSH-Px (U/l)	179.99±30.05	254.19±64.64*	223.26±48.80*	167.37±57.70	<0.05
TAC (mmolTrolox Eq/L)	1.31 ± 0.47	1.09± 0.53	1.11±0.49	1.10±0.43	>0.05

*$p<0.05$ compared to the control group.

0.05). When 1000 ppm boric acid discussed the results of the applied rate was found to be statistically significant if the upward trend.

Vitamin levels

In the serum samples obtained after the experiment, serum levels of retinol (vitamin A) and alpha-tocopherol (vitamin E and D_3) were measured using the HPLC-UV method. Table 4 and Figure 5 (retinol), Figure 6 (alpha-tocopherol) and Figure 7 (vitamin D_3) present the results.

Serum levels of retinol established no significant difference among all four groups ($p>0.05$). However, vitamin D_3 levels increased significantly in the 500 ppm group ($p<0.01$) Moreover, serum levels of alpha-tocopherol decreased significantly in the experimental groups compared to the control group ($p<0.05$) although no significant difference was found among the experimental groups.

Oxidative stress parameters

In the serum samples obtained after the experiment, serum levels of malondialdehyde (MDA) were measured using the HPLC-UV method and serum levels of glutathione peroxidase (GSH-Px) activity and total antioxidant capacity (TAC) were measured spectropho-

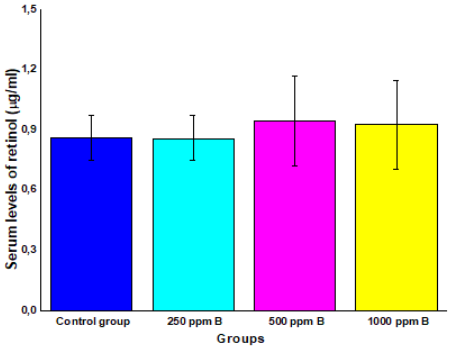

Figure 5. Effect of different doses of boron on serum levels of retinol.

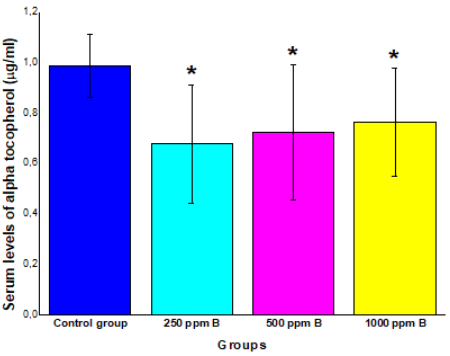

Figure 6. Effect of different doses of boron on serum levels of alpha-tocopherol. *p<0.05 compared to the control group.

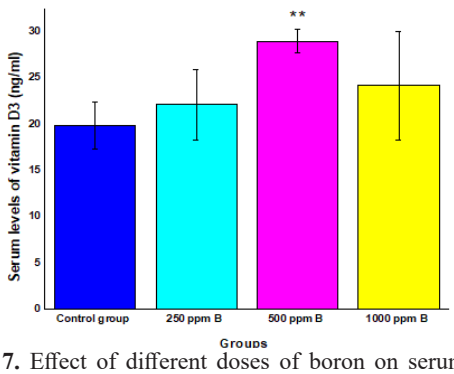

Figure 7. Effect of different doses of boron on serum levels of vitamin D_3. **p<0.01 compared to the control group.

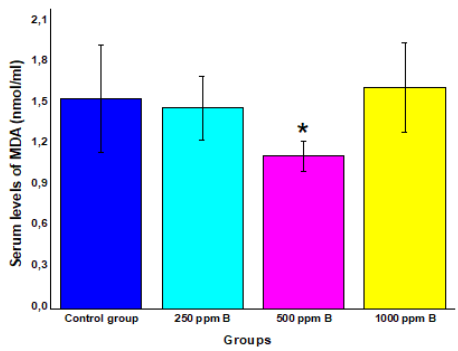

Figure 8. Effect of different doses of boron on serum levels of MDA. *p<0.05 compared to the control group.

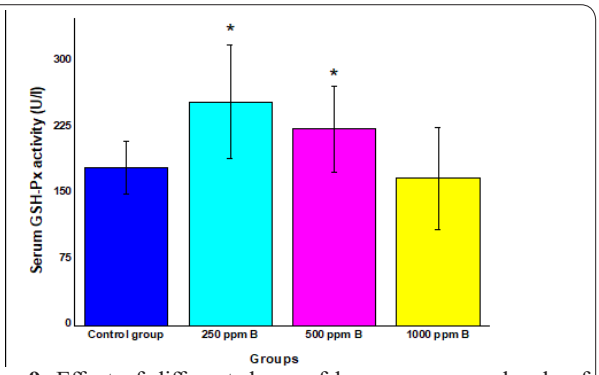

Figure 9. Effect of different doses of boron on serum levels of GSH-Px activity. *p<0.05 compared to the control group. *p<0.05 compared to the control group.

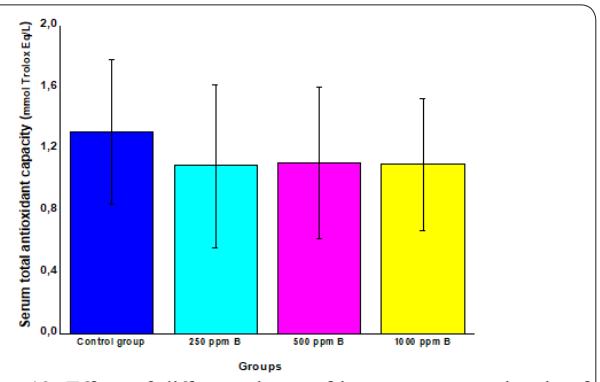

Figure 10. Effect of different doses of boron on serum levels of TAC.

tometrically. Table 5 and Figure 8 (MDA), Figure 9 (GSH-Px) and Figure 10 (TAC) present the results.

Serum levels of MDA decreased significantly in the 500 ppm group compared to the control group (p<0.05). However, although serum levels of TAC established no significant difference among the groups (p>0.05), serum levels of GSH-Px activity increased significantly in the 250 and 500 ppm groups compared to the control group (p<0.05).

Discussion

In the present study, the effects of dietary boron on anxiety, memory retention, oxidative stress parameters, and the serum levels of some vitamins in rats were examined. The rats in the experimental groups were administered with boron at different doses and were compared with the control rats that were fed standard pellet diet only.

Boron has been shown in numerous studies to be a key microelement for the sustainment of normal central nervous system function (8,13). Penland (29, 30) evaluated the effect of boron on brain function and cognitive performance in elderly subjects by investigating electrical brain wave activity on electroencephalography (EEG). Both studies concluded that boron decreased high-frequency activity and increased low-frequency activity. The studies also noted that the group administered with boron 3.25 mg/day had more favorable outcomes in manual dexterity, eye-hand coordination, attention, and short- and long-term memory compared to the group with low boron intake. The studies concluded that boron may have a role in human brain function and cognitive performance and is an essential nutrient for humans.

Morris water maze (MWM) test is a technique used for evaluating visual-spatial memory. In our study, an analysis on the time spent on locating the hidden platform among the four groups revealed that the time length decreased noticeably from day 1 to day 4 in all four groups. This finding suggests that the rats learned the location of the platform after repeated attempts and the method we used improved their spatial memory. However, although no significant difference was observed among all four groups, administration of boron led to faster learning in all the experimental groups, particularly in the 500 ppm group. On the other hand, the test on spatial memory indicated that the time spent on the platform increased in the 500 ppm and 1000 ppm groups and the increase in the 1000 ppm group were statistically significant.

Literature reviews indicate that there has been no study investigating the effect of dietary boron on spatial memory in animal models. In our study, the tendency for an increase in the time spent on the platform in the 500 ppm and 1000 ppm groups implies that the 4-week boron administration improved the cognitive functions used by the rats for locating the hidden platform such as storing and reinforcement. This finding is consistent with the findings presented by Penland (29, 30) and Nielsen (13). However, the absence of a difference in the time spent for locating the platform among all four groups suggests that low-term boron administration leads to no significant difference in locating a hidden item and learning in healthy rats.

Elevated plus maze (EPM) test is widely used for evaluating experimental anxiety although it is commonly believed that the animal models of anxiety cannot precisely represent the human models of anxiety. By examining the emotional activity of the subjects, the EPM test provides useful outcomes in the evaluation of behavioral, physiological, and pharmacological effects (38) as well as the anxiolytic and anxiogenic activities of a drug (39). In the present study, we investigated the effect of boron on rat behavior depending on the previous studies that reported that boron has various effects on brain activity.

Nielsen and Penland (11) investigated whether boron deprivation changes rat behavior and whether dietary long-chain omega-3 fatty acids play a role in the reduction of behavioral responses to boron deprivation. The authors found that boron-deficient rats (0.1 mg/kg) were less active and also showed lower performance on the EPM test compared to the boron-adequate rats (3.1 mg/kg). Although this study investigated the effect of boron deprivation on rat behavior, to our knowledge, the present study is the first report to investigate the relationship between dietary boron and rat behavior.

In our study, EPM was performed on day 0 and day 28 and the proportion of entries into open arms/total entries and the percentage of time spent on the open arms/total time were calculated for each rat. However, the results established no significant difference among the four groups, which suggests that the administration of boron at doses of 4.1, 8.2, and 15.0 mg/kg over a four-week period had no effect on the behavior and anxiety in our rats. Nevertheless, this and other findings of our study could be considered as the initial findings regard-

ing the anxiolytic and anxiogenic effects of dietary boron examined in our study.

Lipid peroxidation is a chain reaction initiated by free radicals, leading to the degradation of membrane lipids and cell membrane functions through the oxidation of polyunsaturated fatty acids in the cell membrane. Measurement of MDA level is commonly performed for identifying lipid peroxidation and this measurement correlates well with the degree of peroxidation (40). Türkez (41) evaluated the in-vitro effects of boric acid on the MDA levels in human erythrocyte and revealed that low-dose oral boric acid intake (5-50 mg/L) led to no significant change in the MDA levels while high-dose boric acid intake (500 mg/L) significantly increased the MDA levels. Similarly, Ince *et al*. (42) investigated the effect of supplemental boric acid and borax (100 mg/kg) added to standard pellet chow on lipid peroxidation, antioxidant activity, and DNA damage and reported that dietary boron supplementation decreased the lipid peroxidation and also significantly decreased the MDA levels and improved the antioxidant defence mechanism. Also, Acaroz *et al.* (43) investigated the ameliorative effects of boron (B) against Acrylamide (ACR) exposed rats . ACR causes the accumulation of reactive oxygen species and oxidative stress and it has neurotoxic, genotoxic, and carcinogenic effects..ACR-treatment significantly increased malondialdehyde levels,the activities of superoxide dismutase and catalase in erythrocytes and tissues whereas decreased glutathione levels in rat tissues and mRNA expression levels of NFκB, IFN-γ, IL-1β, and TNF-α in liver and brain of rats were increased under ACR treatment. B reduces acrylamide(ACR) induced toxicity. B inhibitis oxidative stres and restores LPO, GSH, SOD and CAT in rats. B attenuates inflammatory response and ameliorates biochemical alterations.

In our study, the MDA levels in the 250 ppm group were similar to those in the control group, whereas a significant decrease was found in the 500 ppm group compared to the control group. Moreover, the MDA levels slightly increased in the 1000 ppm group compared to the control group, which could be explained by the fact that this dose is close to the toxic threshold. These findings were consistent with those reported by Ince *et al.* (42) but were dissimilar to those found by Türkez (41) , which could be attributed by the administration of different methods; although Ince *et al.* added boric acid to standard pellet as we did, Turkez added boric acid to human erythrocyte at different doses *in vitro*.

Glutathione peroxidase (GSH-Px) is a key component of the antioxidant defense mechanism and is required for the normal functions of reduced glutathione (GH) that can deactivate reactive oxygen radicals. Reduced GSH-Px activity leads to the accumulation of hydrogen peroxide and cell damage (44). Boron has been shown to regulate NADPH production, thereby increasing the GSH concentration in the body (15). Also, Boron has been reported to increase IFN-γ, IL-1β, TNF-α, and NFκB mRNA expression levels in rat tissue. However, boron treatment improved arsenic-induced alterations in biochemical parameters and increases in DNA damage and proinflammatory cytokine gene expressions.At the same time it was determined higher TOS levels and lower TAS levels in the plasma of the arsenic-exposed rats than in the control rats. These increased oxidant levels may be due to overproduction of oxidant substances. Treatment of boron in a dose- dependent manner resulted in significantly reduced TOS and increased TAS levels compared with those of the arsenic-exposed rats. This results showed that the oxidant/antioxidant balance shifted toward antioxidant status with the boron treatment of the rats (45).

Türkez (41) reported that dietary boron compounds increased the selenium-dependent GSH-Px activity at low doses and decreased it at high doses and thus the determining role of boron on the GSH-Px activity could be dependent on selenium. Similarly, in the present study, GSH-Px activity increased in the 250 and 500 ppm groups and slightly decreased in the 1000 ppm group. Total antioxidant capacity (TAC) refers to cumulative action of the antioxidants available in plasma and other body fluids. In our study, serum TAC level established no significant difference among all four groups. This finding was consistent with those reported by Ince *et al.* (42), whereas it contradicted with the finding presented by Türkez (41) who demonstrated that low-dose boron intake increased the TAC levels in human erythrocyte. This contradiction could be explained by the fact that Türkez analyzed the TAC levels in human erythrocyte *in vitro*, whereas we analyzed the TAC levels in the serum samples. On the other hand, TAC enables not only the evaluation of the capacity of known and unknown antioxidants but the assessment of their synergistic and antagonistic interaction and the delicate balance *in vivo* between oxidants and antioxidants (46).

Literature shows that there has been no study reporting on the relationship between dietary boron or boric acid and vitamin levels. Ince *et al*. (42) reported that dietary boric acid intake at a dose of 100 mg/kg led to no significant change in vitamin A and beta carotene levels. In our study, we obtained similar findings. In addition, serum retinol levels increased slightly in the 500 ppm and 1000 ppm groups although no significant difference was observed, whereas alpha-tocopherol levels decreased significantly in all the experimental groups. Vitamin E is a frontline defense against membrane phospholipid peroxidation and protects the membranes and subcellular structures against oxidative damage, and a decrease in vitamin E is a key indicator of the onset of oxidative stress (47). Accordingly, we believe that the decrease in vitamin E levels induced by boron intake in our study could also be related to the onset of oxidative stress considering that the lowest dose of boric acid administered in our study (250 ppm) was 2.5 times greater than the dose administered by Ince *et al*.(42).

Boron has also been shown to have a role in the regulation of minerals in the body such as calcium and vitamin D_3 and to maintain bone integrity by preventing calcium and magnesium loss (13). Moreover, dietary boron has been reported to reduce the risk of growth disorders associated with vitamin D_3 deficiency and the risk of mineral imbalances (19). In addition, boron has also been reported to play a role in the metabolism of steroid hormones and to increase the half-life of vitamin D_3, thereby increasing bone strength and mineral content (8). In line with the literature, the serum vitamin D_3 levels in our study showed a tendency for an increase in the experimental groups, with a significant increase ob-

served in the 500 ppm group. This tendency implicates that dietary boron has an incremental role in vitamin D_3 concentration.

The results obtained in this study indicate that boron has numerous beneficiary effects on animals and humans. In particular, the results suggest that boron, due to its role in increasing vitamin D_3 concentration, can be used as a supplementary treatment in the prevention of rachitis and osteoporosis and other growth disorders associated with vitamin D_3 deficiency. However, the effect of boron on the antioxidant parameters that fight free radicals and protect the organism against oxidative stress remains controversial. On the other hand, our results also indicated that the doses of boron administered in our study decreased the lipid peroxidation, increased the GSH-Px activity, and significantly decreased the vitamin E levels in all four groups. Nevertheless, boron supplementation had no effect on vitamin A levels and serum TAC levels.

Among all three experimental groups in our study, the 500 ppm group had the most favorable outcomes, in which the MDA levels decreased significantly and the GSH-Px activity and vitamin D_3 levels increased. However, the only adverse outcome in this group was the decrease in vitamin E levels.

In conclusion, dietary boron was found to have beneficiary effects on rat behavior, memory retention, lipid peroxidation, GSH-Px activity, and vitamin D_3 levels in the rats administered with different doses of boric acid. We consider that boron can have beneficiary effects on the organism when administered at appropriate doses.

Acknowledgements
This study was supported by a grant from the Scientific Research Projects Presidency of Van Yuzuncu Yil University, Turkey (2010-SBE-YL137)

Conflicts of interest
There are no conflicts of interest.

References

1. Chandra RK. Effect of vitamin and trace-element supplementation on cognitive function in elderly subjects. Nutrition, 2001;17(9):709-712.
2. Huskisson E, Maggini S, Ruf M. The influence of micronutrients on cognitive function and performance. J Int Med Res. 2007;35(1):1-19.
3. Lam PK, Kritz-Silverstein D, Barrett Connor E, Milne D, Nielsen F, Gamst A *et al.* Plasma trace elements and cognitive function in older men and women: the Rancho Bernardo study. J Nutr Health Aging.2008;12(1):22-27.
4. Emsley CL, Gao S, Li S. Trace element levels in drinking water and cognitive function among elderly Chinese. Am J Epidemiol., 2000;151:913-920.
5. Warington K. The effect of boric acid and borax on the broad bean and certain other plants, Ann. Bot., 1923;37:629–672.
6. Yılmaz A. Her derde deva hazinemiz bor. Tübitak-Bilim ve teknik dergisi, Ankara, Mayıs 2002, 2002;38-48.
7. Sutherland B, Strong P, King JC. Determining human dietary requirements for boron. Biol Trace Elem Res., (1998);66(1-3):193-204.
8. Devirian TA and Volpe SL. The physiological effects of dietary boron. Crit Rev Food Sci Nutr.,2003;43(2): 219-231.
9. Price CJ, Strong PL Murray FJ, Goldberg MM. Blood boron concentrations in pregnant rats fed boric acid trough gestation. Reproductive Toxicology,1997;11(6): 833-842.
10. Sheng MH, Taper LJ, Veit H, Thomas EA, Ritchey SJ, Lau KH. Dietary boron supplementation enhances the effects of estrogen on bone mineral balance in ovariectomized rats. Biol Trace Elem Res., 2001;81(1):29-45.
11. Nielsen FH, Penland J. Boron deprivation alters rat behavior and brain mineral composition differently when fish oil instead of safflower oil is the diet fat source. Nutritional Neuroscience, 2006;9:105-112.
12. Eren M. Borun biyolojik önemi ve metabolizma üzerine etkileri. Erciyes Üniv Vet Fak Dergisi.,2004;55-59.
13. Nielsen FH. Is boron nutritionally relevant?. Nutrition Reviews, 2008;66(4):183-191.
14. Bolanos L, Lukaszewski K, I Bonilla I, Blevins D. Why boron? Plant Physiology and Biochemistry, 2004;42:907–912.
15. Hunt CD. One possible rol of dietary boron in higher animals and humans. Biological trace element research, 1998;66:205-225.
16. Hunt CD and Idso JP. Dietary boron as a physiological regulator of the normal inflammatory response: A review and current research progress. The Journal of Trace Elements in Experimental Medicine, 1999;12:221–233.
17. Bai Y, Hunt CD, Newman SM Jr. Dietary boron increases serum antibody (IgG and IgM) concentrations in rats immunized with human typhoid vaccine. Proc ND Acad Sci.,1997;51:81.
18. Hunt CD and Nielsen H. Dietary boron affects bone calcification in magnesium- and cholecalciferol deficient chicks. Trace Elements in Human and Animal Nutrition, 5th ed. New York: Academic Press, Inc.,1986;275–277.
19. Hunt CD, Herbel JL, Idso JP. Dietary boron modifies the effects of vitamin D3 nutriture on indices of energy substrate utilization and mineral metabolism in the chick. J. Bone Min. Res.,1994;9:171–182.
20. Samman S, Naghii MR, Lyons Wall PM, Verus AP. The nutritional and metabolic effects of boron in humans and animals. Biol Trace Elem Res.,1998;66(1-3):227-235.
21. 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. J Trace Elem Med Biol.,2010;25(1):54-58.
22. Naghii MR, Torkaman G, Mofid M. Effects of boron and calcium supplementation on mechanical properties of bone in rats. Biofactors,2006;28(3-4):195-201.
23. Chapin RE, Ku WW, Kenney MA, McCoy H, Gladen B, Wine RN, *et.al*. The effects of dietary boron on bone strength in rats. Fundamental and Applied Toxicology,1997;35:205-215.
24. Newnham RE. Essentiality of boron for healthy bones and joints. Environ Health Perspect.,1994;102(7):83-85.
25. Grajeta H. Nutrition in prevention and treatment of osteoporosis. Przegl Lek.,2003; 60(10):649-653.
26. Barranco WT, Huda PF, Echert CD. Evaluation of ecological and in vitro effects of boron on prostate cancer risk (United States). Cancer Causes Control.,2007;18(1):71-77.
27. Gonzalez A, Peters U, Lampe JW, White E. Boron intake and prostate cancer risk. Cancer Causes Control.,2007;18(10):1131-1140.
28. Korkmaz M, Uzgören E, Bakirdere S, Aydin F, Ataman OY. Effects of dietary boron on cervical cytopathology and on micronucleus frequency in exfoliated buccal cells. Environ Toxicol.,2007;22(1):17-25.
29. Penland JG. Dietary boron, brain function, and cognitive performance. Environ Health Perspect.,1994;102(7):65-72.
30. Penland JG. The importance of boron nutrition for brain and psychological function. Biol Trace Elem Res.1998; 66(1-3):299-317.
31. Tuzcu M ve Baydaş G. Effect of melatonin and vitamin E on

diabetes-induced learning and memory impairment in rats. Eur J Pharmacol.,2006;537(1-3):106-110.
32. Kuhad A, Sethia R, Chopra K. Lycopene attenuates diabetes-associated cognitive decline in rats. Life Sciences,2008;83:128–134.
33. Morris RG, Garrud P, Rawlins JN, O'Keefe J. Place navigation impaired in rats with hippocampal lesions. Nature,1982;297(5868):681-683.
34. Karatepe M. Simultaneous determination of ascorbic acid and free malondialdehyde on human serum by HPLC-UV. LGCG Asia Pafic.,2004;7(2):36-38.
35. Karataş F, Tuğ T, Konar V. Serum antioxidant vitamins (A, E, C), selenium and malondialdehyde levels in workers exposed to aerosol. Turk Toraks Derg.,2008;9(1): 13-16.
36. Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem.,2004;37(4):277-85.
37. Paglia DE and Valentine WN. Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. J Lab Clin Med.,1967;70:158.
38. Küçük A ve Göleli A. Deney hayvanlarında anksiyete modelleri ve anksiyetin değerlendirilmesi. Erciyes Sağlık Bilimleri Dergisi, 2005;14(3):209-217.
39. Dolu N ve Özesmi E. Anksiyetenin değerlendirilmesinde güncel olarak kullanılan bazı deneysel hayvan modelleri. Klinik psikofarmakoloji bülteni,2004;14:216-225.
40. Romero FJ, Bosch-Morell F, Romero MJ, Jareño EJ, Romero B, Marín N, *et.al.* Lipid peroxidation products and antioxidants in human disease. Environ Health Perspect.,1998;106:1229-1234.
41. Türkez H. Diyetteki borun vücuttaki oksidatif metabolizma üzerine etkileri değerlendirmek amacıyla Atatürk Üniversitesi, Fen Bilimleri Enstitüsü, Biyoloji Anabilim Dalı Doktora Tezi, Erzurum,2007.
42. İnce S,Küçükkurt İ, Ciğerci İH, Fidan AF, Eryavuz A. The effects of dietary boric acid and borax supplementation on lipid peroxidation, antioxidant activity and DNA damage in rats. Journal of Trace Elements in Medicine and Biology,2010;24:161-164.
43. Acaroz U, Ince S, Arslan-Acaroz D, Gurler Z, Kucukkurt I, Demirel HH, *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.
44. Thomas MJ. The role of free radicals and antioxidants. Critical Rew. Food. Sci. and Nutrit.,1995;35(1-2):21-39.
45. Ince S, Kucukkurt I, Acaroz U, Arslan-Acaroz D, Varol N**.** Boron ameliorates arsenic-induced DNA damage, proinflammatory cytokine gene expressions oxidant/antioxidant status and bichemical parameters in rats. J.Biochemical Mol. Toxicol,2018;28:e22252. https://doi.org/10.1002/jbt.22252.
46. Ghiselli A, Serafini M, Natella F, Scaccini C. Total antioxidant capacity as a tool to assess redox status: critical view and experimental data. Free Radic Biol Med.,2000;29(11):1106-1114.
47. Kayaalp O. Rasyonel tedavi yönünden tıbbı farmakoloji. Yağda çözünen vitaminler. Pelikan Yayıncılık, İstanbul,2009;1307-1321.