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

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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 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, anxiety, and some vitamins and oxidative parameters in rats.
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 EthoVision 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 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 EthoVision 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.

The rats were 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 D₃ 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 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.

Spectrophotometric analysis
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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-
The results 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 \((p>0.05)\). 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 open arms compared to the control group.

<table>
<thead>
<tr>
<th>Group</th>
<th>Time spent in the platform quadrant (%) Mean±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>27.65 ± 11.32</td>
</tr>
<tr>
<td>250 ppm</td>
<td>28.91 ± 8.44</td>
</tr>
<tr>
<td>500 ppm</td>
<td>32.07 ± 6.27</td>
</tr>
<tr>
<td>1000 ppm</td>
<td>33.34 ± 8.20*</td>
</tr>
</tbody>
</table>

*\(p<0.05\) compared to the control group.

![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 \((p<0.05)\).](image1)

![Figure 2. Effect of boron on memory retention (MWM test). *\(p<0.05\) compared to the control group.](image2)

![Figure 3. Proportion of entries into open arms/total entries (EPM test).](image3)

![Figure 4. Percentage of the time spent on the open arms/total time (EPM test).](image4)
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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 D₃) were measured using the HPLC-UV method. Table 4 and Figure 5 (retinol), Figure 6 (alpha-tocopherol) and Figure 7 (vitamin D₃) present the results.

Serum levels of retinol established no significant difference among all four groups (p>0.05). However, vitamin D₃ 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 spectrophotometrically.

Table 3. Effect of boron on EPM test results

<table>
<thead>
<tr>
<th>Group</th>
<th>Entries into open arms/total entries (%)</th>
<th>Time spent on the open arms/total time (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 0 Mean±SD</td>
<td>Day 28 Mean±SD</td>
</tr>
<tr>
<td>Control</td>
<td>34.29±8.42</td>
<td>32.90±12.52</td>
</tr>
<tr>
<td>250 ppm</td>
<td>36.38±3.62</td>
<td>32.67±10.82</td>
</tr>
<tr>
<td>500 ppm</td>
<td>39.09±7.92</td>
<td>29.05±11.13</td>
</tr>
<tr>
<td>1000 ppm</td>
<td>31.29±7.17</td>
<td>39.85±11.21</td>
</tr>
</tbody>
</table>

EPM results obtained on day 1 and day 28.

Table 4. Effect of different doses of boron on vitamin A, D₃ and E levels.

<table>
<thead>
<tr>
<th>Group</th>
<th>Control Mean±SD</th>
<th>250 ppm Mean±SD</th>
<th>500 ppm Mean±SD</th>
<th>1000 ppm Mean±SD</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retinol (μg/ml)</td>
<td>0.86±0.111</td>
<td>0.85±0.112</td>
<td>0.94±0.226</td>
<td>0.92±0.220</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Alpha-tocopherol (μg/ml)</td>
<td>0.98±0.124</td>
<td>0.67±0.234*</td>
<td>0.72±0.267*</td>
<td>0.76±0.214*</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>D₃ (ng/ml)</td>
<td>19.80±2.55</td>
<td>22.09±3.78</td>
<td>28.94±1.29**</td>
<td>24.16±5.88</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>

* 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.

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

*p<0.05 compared to the control group.

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 D₃. **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.
Boron has been shown in numerous studies to be a key microelement for the sustenance 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 trical brain wave activity on electroencephalography 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.

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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 regarding 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.

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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 boron acid on the MDA levels in human erythrocyte and revealed that low-dose oral boron acid intake (5-50 mg/L) led to no significant change in the MDA levels while high-dose boron acid intake (500 mg/L) significantly increased the MDA levels. Similarly, Ince et al. (42) investigated the effect of supplemental boron 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 inhibits 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 boron acid to standard pellet as we did, Türkez added boron 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 result 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 also 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 boron acid and vitamin levels. Ince et al. (42) reported that dietary boron 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 boron 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, 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 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 D3, thereby increasing bone strength and mineral content (8). In line with the literature, the serum vitamin D3 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 D₃ 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 D₃ concentration, can be used as a supplementary treatment in the prevention of rachitis and osteoporosis and other growth disorders associated with vitamin D₃ 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 D₃ 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 D₃ 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.

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Conflicts of interest
There are no conflicts of interest.

References

30. Tuzcu M ve Baydaş G. Effect of melatonin and vitamin E on...