The Effect of Borax on Hematological Parameters and Immunoglobulin Values in Rats
E Keklik\textsuperscript{1}, M Keklik\textsuperscript{2}, U Bakkaloglu\textsuperscript{1}, M Yuruk\textsuperscript{3}, B Coksevim\textsuperscript{1}

Affiliations:
\textsuperscript{1}Department of Physiology, Faculty of Medicine, Erciyes University, Kayseri, Turkey.
\textsuperscript{2}Department of Hematology, Kayseri Education and Research Hospital, Kayseri, Turkey.
\textsuperscript{3}Department of Parasitology, Faculty of Medicine, Erciyes University, Kayseri, Turkey.

Correspondence:
Dr E Keklik
Department of Physiology
Faculty of Medicine
Erciyes University
Kayseri
Turkey
Fax: +903524379348
E-mail: drkeklik@yahoo.com

Short title: Boron Supplementation in Animal and Human Nutrition

Synopsis: Boron supplementation in animal and human nutrition may have important effects on various metabolic, physiological systems of the organism. The aim of this study was to evaluate the effect of borax on hematological parameters and immunoglobulin values in the rats.
ABSTRACT

Objective: Boron is an essential metalloid which plays a key role in plants and animals metabolisms. In the last few years boron compounds became increasingly more frequent in some hematological diseases. The aim of this study was to evaluate the effect of borax as a boron source on hematological parameters and immunoglobulin (Ig) values in the rats.

Methods: In a prospective controlled study, we used two groups, consisting of each 16 healthy Wistar rats. Borax was given by adding to drinking water (704 mg/L) to borax group for a period of 28 days.

Results: It was found that the decrease the white blood cell count (WBC), hemoglobin (HGB), hematocrit (Htc), red blood cell (RBC) and platelet count (PLT) in borax group male rats. Likewise, a significant decrease in lymphocyte (L), neutrophil (N), monocyte (M) and basophil (B) counts were detected in this group. When female rats were evaluated, it was found that the significant decrease the WBC, HGB, Htc, RBC, PLT and M count in borax group. On the other hand, MCV was increased in this group. Additionally, while there was no significant difference between borax and control groups in terms of IgG, IgM and IgA values of the male rats, it was found that the statistically significant increase the IgG value in borax group female rats.

Conclusion: Results from this study indicate that supplemental borax had significant effects on hematological parameters of male and female rats, and did not affect immun function of rats only except female rats IgG values.

Keywords: borax, immunoglobulin, rat
INTRODUCTION

Boron is a trace mineral for plants, animals and humans (1). It was first isolated in 1808. Boron complexes with organic compounds containing hydroxyl groups, sugars and polysaccharides, adenosine-5-phosphate, pyridoxine, riboflavin, dehydroascorbic acid, and pyridine nucleotides (2, 3). Daily intake of boron is dependent on its concentration in water supplies and food sources. Studies in animals have shown that boron is readily absorbed following oral exposure in rats (4). Also, >90% of orally administered boron is absorbed from the gastrointestinal tract within 3 hours and that absorption is complete within 24 hours.

Some studies have demonstrated that boron has effects on the metabolism of minerals, enzymes, hormonal status and biochemical parameters (5-7). Also, the knowledge of the hematological changes caused by boron compounds is critical to the effective pharmacokinetic researches (8-11). On the other hand, boron has only recently been receiving due attention in medicinal chemistry and drug development (12-15).

The present study was planned to investigate the effect of borax (sodium tetraborate decahydrate) as a boron compound on the complete blood cell (CBC) count and immunoglobulin (Ig) levels in rats.

SUBJECTS AND METHODS

This experimental study was conducted between September 2015 and February 2016 at the Erciyes University Experimental Research and Application Center (DEKAM) obtaining the approval of the ethical committee of Erciyes University School of Medicine (Kayseri, Turkey).

Thirty-two healthy Wistar rats (16 males, 16 females), 60 days of age and weighing 150–300 g, were included in the study. Within seven days prior to the study, they were kept at
room temperature (24°C)) in 12-hour natural light-12-hour dark cycle. They were fed with standard rodent diet and given tap water. Water in the troughs was daily refreshed and the nests were cleaned every other day.

In the follow up, the rats were divided in two groups consisting of each 16 animals, and there were 8 female and male rats in each group. Normal diet and tap water were given to control and treated groups for a period of 28 days. Borax was given by adding to drinking water (704 mg/L borax= 79.8 mg/L boron) to borax group as a boron source for a period of 28 days. The borax used in the study was obtained from Sigma-Aldrich (USA). At the end of the work, all the rats were sacrificed under general anesthesia (80 mg/kg ketamine and 5 mg/kg xylazine ip). Just before they were sacrificed, 4-5 cc blood samples were obtained from each rat by intracardiac puncture and put in to EDTA tubes and no-additive Vacutainer tubes, respectively. All samples were tested for CBC count on the Advia 2120i Hematology system (Siemens Healthcare diagnostics Inc, tarrytown NY, USA) as the primary routine method in our laboratory. The other blood samples were centrifuged at 3000 rpm for 10 minutes and the serum was separated. Thereafter, the samples were stored at –20°C until the Ig G, M, A values were studied. Immunoglobulin measurement was performed by enzyme-linked immonosorbent assay (ELISA) method (Sunredbio, Shangai, China).

**Statistical analysis**

Before the analysis, the continuous variables were evaluated with Shapiro–Wilk test to determine whether the distribution was normal or not and data were expressed as the median (range) (for skewed data) or mean± standard deviation (SD) (normally distributed data).

The results were statistically evaluated according to the independent-samples Student’s t-test (normally distributed data) and Mann-Whitney U-test (for skewed data). A p value ≤0.05 was considered statistically significant. All data were analyzed using the Statistical Package for the Social Sciences (SPSS) computer program version 22.0.
RESULTS

In the course of the study, one female rat from the control group died on first week. As a result of the evaluation of the remaining 31 rats, there was no statistically significant difference between the groups in terms of weight and height. Table 1 shows that hematological analysis of the groups. At the end of the study, it was found that the decrease the white blood cell count (WBC), hemoglobin (HGB), hematocrit (Htc), red blood cell (RBC) and platelet count (PLT) in borax group male rats and this findings were statistically significant. Likewise, a significant decrease in lymphocyte (L), neutrophil (N), monocyte (M) and basophil (B) counts were detected in this group. Also, it was found that the significant increase the mean cell hemoglobin (MCH) and the mean cell hemoglobin concentration (MCHC) values in borax group male rats.

With regard to mean cell volume (MCV), eosinophil (E) and mean platelet volume (MPV) values, there was no significant difference between borax group and the control group in male rats. When female rats were eveluated, it was found that the significant decrease the WBC, HGB, Htc, RBC, PLT and M count in borax group. On the other hand, MCV was increased in this group. With regard to MCH, MCHC, L, N, E, B and MPV values, there was no difference between borax and the control group in female rats. Table 2 shows that Ig values of the groups.

While there was no significant difference between borax and control groups in terms of IgG, IgM and IgA values of the male rats, it was found that the statistically significant increase the IgG value in borax group female rats (Figure)(p=0.015). Also, with regard to IgM and IgA values, there was no difference between the female groups.
DISCUSSION

Several studies have been performed to investigate the effects of boron intake on animals. The rabbits were administered boron orally different doses boraks (10, 30, and 50 mg/kg of body weight) at 96-h intervals. Complete blood count was not affected by boron administration (16). Also, according to the Kurtoglu et al.’s study, HGB and Htc values were significantly increased by boron supplementation but there were no effects on WBC ratios such as E, B, M, L and PLT count in broiler chicks (17). On the other hand, Feng et al. showed that in rats exhibited different degree increases, the RBC numbers and HGB contents consuming 40 and 80 mg/L of boron significantly raised but the changes of the leucocyte percentages were more no significant (18).

In our study we found that borax reduces the HGB, RBC, WBC and PLT counts in rats. Also, we detected a significant decrease in L, N, M and B counts in male rats. On the other hand, in female borax group, while it was found that the decrease the M counts; L, N, B and E values didn’t change.

Several studies of boron with human subjects were not conducive to the study of its effect on blood parameters (19, 20). Following, in a human study, Nielsen et al. reported that boron supplementation to subjects who had previously followed a dietary regimen deficient in boron resulted in increases in blood HGB, MCH, and MCHC, and decreases in Htc, RBC count and PLT count (21). Also, Türkez et al.’study, peripheral blood cultures were exposed to various doses (5 to 500 mg/L) of boron com- pounds. According to their findings, various boron compounds at low doses were useful in supporting antioxidant enzyme activities in human blood cultures (22). Also, recent studies indicate that dietary boron compounds affects various immunological processes (23). In our study, it was found that significant increase the IgG value in borax group female rats. In general, females demonstrate greater antibody responses than males (24).
Estradiol, progesterone and testosterone affect the effector functions of immune cells. Also, in our study, there was no significant difference between borax and control groups in terms of IgG, IgM and IgA values of the male rats. Bai et al.’s study, physiologic amounts of boron added to a boron-low diet more than doubled serum total antibody concentrations to injected antigen (human typhoid vaccine) in rats (25). There are several lines of evidence that dietary boron exerts influence on immune function in humans and animal models. The mechanisms for these systemic effects have not yet been identified. The effect of dietary boron on immune responses may relate to the ability of boron to alter production of various cytokines (26).

CONCLUSION
Although several studies on the effects of boron and how it functions have been performed over the last decade, more information is needed to clarify both its effects and how it produces its action.

ACKNOWLEDGEMENTS
This study was supported by the Erciyes University Research Fund (TTU-2015-5952).

Author Contributions:
Ertugrul Keklik conceived paper, oversaw data collection, conducted data analysis, wrote manuscript and approved final version. Muzaffer Keklik participated in study design, data analysis and interpretation, critically revised manuscript and approved final version. Umut Bakkaloglu participated in study design, data analysis, and interpretation of data and revision of manuscript and approved final version. Merve Yuruk participated in study design,
interpretation of data and revision of manuscript and approved final version. Bekir Coksevim provided oversight to study, participated in data interpretation and revision of manuscript, and approved final version. The authors declare that they have no conflicts of interest.
REFERENCES


<table>
<thead>
<tr>
<th>Variables</th>
<th>Male (n=16)</th>
<th>Female (n=15)</th>
<th>p</th>
<th>Male (n=16)</th>
<th>Female (n=15)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Borax (n=8)</td>
<td>Control (n=8)</td>
<td></td>
<td>Borax (n=8)</td>
<td>Control (n=7)</td>
<td></td>
</tr>
<tr>
<td>HGB (g/dL)</td>
<td>13.92±2.15</td>
<td>16.18±0.87</td>
<td>0.022*</td>
<td>14.26±0.93</td>
<td>15.3±0.34</td>
<td>0.024*</td>
</tr>
<tr>
<td>Htc (%)</td>
<td>37.80±5.77</td>
<td>48.50±1.96</td>
<td>0.001*</td>
<td>42.65±3.94</td>
<td>47±1.19</td>
<td>0.017*</td>
</tr>
<tr>
<td>RBC (x10^{12}/L)</td>
<td>6.97±0.92</td>
<td>8.81±0.29</td>
<td>0.001*</td>
<td>7.50±0.57</td>
<td>8.18±0.18</td>
<td>0.171*</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>54.08±1.96</td>
<td>55±0.82</td>
<td>0.275</td>
<td>59±2.49</td>
<td>55.86±0.36</td>
<td>0.009*</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>19.95±1.31</td>
<td>18.34±0.43</td>
<td>0.010*</td>
<td>19.05±0.83</td>
<td>18.68±0.38</td>
<td>0.342*</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>36.93±1.77</td>
<td>33.35±0.66</td>
<td>&lt;0.001*</td>
<td>32.35±2.64</td>
<td>33.45±0.56</td>
<td>0.361*</td>
</tr>
<tr>
<td>WBC (x10^9/L)</td>
<td>6.22±1.78</td>
<td>8.99±1.34</td>
<td>0.005*</td>
<td>5.34±1.9</td>
<td>7.61±1</td>
<td>0.022*</td>
</tr>
<tr>
<td>L (x10^9/L)</td>
<td>4.44±1.14</td>
<td>6.08±1.03</td>
<td>0.013*</td>
<td>3.96±2.03</td>
<td>5.45±0.77</td>
<td>0.118*</td>
</tr>
<tr>
<td>N (x10^9/L)</td>
<td>0.06(0.05-0.21)</td>
<td>0.17(0.10-0.47)</td>
<td>0.014*</td>
<td>0.07(0.04-0.4)</td>
<td>0.07(0.05-0.20)</td>
<td>0.852*</td>
</tr>
<tr>
<td>M (x10^9/L)</td>
<td>1.12(0.77-2.24)</td>
<td>1.86(1.35-2.10)</td>
<td>0.021*</td>
<td>0.74(0.58-1.37)</td>
<td>1.35(1.04-1.79)</td>
<td>0.005*</td>
</tr>
<tr>
<td>E (x10^9/L)</td>
<td>0.01±0.00</td>
<td>0.02±0.01</td>
<td>0.103</td>
<td>0.01±0.00</td>
<td>0.01±0.00</td>
<td>0.931*</td>
</tr>
<tr>
<td>B (x10^9/L)</td>
<td>0.01±0.00</td>
<td>0.03±0.01</td>
<td>0.001*</td>
<td>0.02±0.03</td>
<td>0.02±0.00</td>
<td>0.760*</td>
</tr>
<tr>
<td>PLT (x10^9/L)</td>
<td>562±65</td>
<td>993±61</td>
<td>&lt;0.001*</td>
<td>726±103</td>
<td>945±67</td>
<td>0.001*</td>
</tr>
<tr>
<td>MPV (fL)</td>
<td>7.15(6.90-7.40)</td>
<td>7.30(7.10-7.40)</td>
<td>0.094</td>
<td>7(7-7.60)</td>
<td>7.05(6.80-7.20)</td>
<td>0.573*</td>
</tr>
</tbody>
</table>

Table 2: Immunoglobulin values of the groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Borax (n=8)</th>
<th>Control (n=8)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male (n=16)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgG (mg/ml)</td>
<td>9.91(4.13-13.41)</td>
<td>11.10(9.93-12.32)</td>
<td>0.310</td>
</tr>
<tr>
<td>Mg (µg/ml)</td>
<td>77.07(63.26-83.43)</td>
<td>72.13(66.19-74.97)</td>
<td>0.310</td>
</tr>
<tr>
<td>IgA (µg/ml)</td>
<td>395.65(388.83-425.80)</td>
<td>395.03(323.72-431.62)</td>
<td>0.841</td>
</tr>
<tr>
<td></td>
<td>Borax (n=8)</td>
<td>Control (n=7)</td>
<td></td>
</tr>
<tr>
<td>IgG (mg/ml)</td>
<td>8.93(8.22-9.81)</td>
<td>7.73(4.25-9.38)</td>
<td>0.015*</td>
</tr>
<tr>
<td>Female (n=15)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IgM (µg/ml)</td>
<td>60.08(11.59-70.62)</td>
<td>61.78(52.51-70.96)</td>
<td>0.699</td>
</tr>
<tr>
<td>IgA (µg/ml)</td>
<td>330.54(249.71-432.24)</td>
<td>293.36(118.64-340.46)</td>
<td>0.240</td>
</tr>
</tbody>
</table>

Ig: Immunoglobulin. all Ig values median (range), *statistically significant
Figure 1: Box-plot graph of the IgG values of female groups.