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Investigation of the Effects of Boron Addition to Drinking Water of Rats on Hematologic Parameters and Serum Boron, Calcium, Phosphorus and Magnesium Levels

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ABSTRACT

Objective: In this study, it was aimed to determine the effect of boron added to the drinking water of rats on serum boron levels, calcium, magnesium and phosphorus concentrations and the changes that may occur in hematological parameters. **Materials and Methods:** A total of 12 250-350 g Wistar Albino male rats, 6 in the control group and 6 in the experimental group, were used in the study. The rats were fed ad libitum and 2 mg/day/rat/25 ml borax decahydrate (Sigma) was added to the drinking water. At the end of 14 days, the rats were decapitated. Serum boron analysis by ICP-OES was performed according to Tokay and Bagdat (2022). In addition; Ca, Mg, P concentrations were determined with a biochemical analyzer device. **Results:** When the serum boron levels of the rats were analysed, those in the experimental group were significantly higher (p:0.0107) than those in the control group. However, there was no effect on haematological parameters or serum calcium, magnesium and phosphorus levels. **Conclusion:** As a result of this study, it was determined that boron given to rats with drinking water had no effect on the minerals examined. However, it was concluded that more information could be obtained with more comprehensive studies on this subject.

Keywords: Boron, Drinking Water, Rat.

Sıçanların İçme Suyuna Bor İlavesinin Hematolojik Parametreler ile Serum Bor, Kalsiyum, Fosfor ve Magnezyum Seviyelerine Etkilerinin Araştırılması

ÖZ

Amaç: Bu araştırmada; sıçanların içme suyuna katılan borun, serum bor düzeyleri, kalsiyum, magnezyum ve fosfor konsantrasyonlarına etkisi ve hematolojik parametrelerde oluşturabileceği değişikliklerin belirlenmesi amaçlanmıştır. **Gereç ve Yöntem:** Araştırmada, 6 adet kontrol, 6 adet deney grubu olmak üzere toplam 12 adet 250-350 gr Wistar Albino erkek sıçan kullanıldı. Ad libitum olarak beslenen sıçanların içme suyuna, 2 mg/gün/sıçan/25 ml boraks dekahidrat (Sigma) ilave edildi. 14 günün sonunda sıçanlar dekapite edildi. ICP-OES ile serum bor analizi Tokay ve Bagdat'a (2022) göre yapılmıştır. Ayrıca; Ca, Mg, P konsantrasyonları biyokimyasal analizör cihazı ile belirlendi. **Bulgular:** Sıçanların serum bor düzeyi incelendiğinde deney grubu hayvanların seviyesi kontrol grubuna göre anlamlı bir şekilde yüksek (p:0.0107) bulundu. Ancak, hematolojik parametreler ile serum kalsiyum, magnezyum ve fosfor seviyeleri arasında anlamlı bir ilişki olmadığı tespit edildi. **Sonuç:** Bu çalışmanın sonucunda, sıçanlara içme suyu ile verilen borun, incelenen minerallerin üzerine etkisinin olmadığı tespit edildi. Ancak, bu konu hakkında yapılacak daha kapsamlı çalışmalarla daha fazla bilgi sahibi olunabileceği sonucuna varıldı.

Anahtar Kelimeler: Bor, İçme Suyu, Sıçan.

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INTRODUCTION

Boron (B), first discovered by the French chemist Gay-Lussac in 1808, is a semiconductor substance in group 3A in the periodic table. It is not found alone in nature and forms many compounds. The compounds formed with the elements calcium (Ca), magnesium (Mg) and sodium (Na) are called natural compounds (Oganov and Solozhenko, 2009). It is thought that the combination of metal and non-metal properties, as well as the tendency to bond with oxygen, are the main reasons for the existence of more than two hundred derivatives. This diversity has enabled the usage areas to increase day by day. From past to present, boron has been utilized in different fields such as agriculture, cleaning, metallurgy, insulation and energy, especially in glass and textile industry (Bilgic, 2024; Halvacı et al., 2024). Boron compounds, which are known to be taken mainly through food, intake the living body through air, water and soil, especially in areas where they are extracted and processed (Buluttekin, 2008). Following intake, the substance is converted into boric acid. Thereafter, it is transported via the bloodstream (Buluttekin, 2008).

In terms of animal and human biology, boron is a crucial trace element that is becoming more and more significant. In the body, digestive, respiratory, skin, skeletal, brain and immune systems are affected by boron (Kuru et al., 2019). It has also been reported to have immunomodulatory, anti-inflammatory, antineoplastic, anticoagulant and lipid-lowering effects. However, the mechanism of its effects on human and animal biology has not been clearly established (Kuru et al., 2019).

In recent years, the field of health has seen an increased focus on boron. There are many studies evaluating its use as a biomaterial after surgical operations, its contribution to osteogenesis, its potential for use in burn, wound treatments and weight loss, and its antioxidant and antimicrobial properties (Bitmez ve Balbal, 2024). In addition, research aiming to utilize boron compounds in the treatment of diseases such as cancer and Alzheimer's disease, the treatment of which is still unclear today, are being carried out (Celebi ve ark., 2024). Boron deficiency and excess can have metabolic effects in the body. Especially its effects on hormonal and mineral metabolism are very important. It can cause changes in the concentration of many substances that have important roles in enzyme reactions and cell membrane functions. In deficiency, research has shown that the absorption of minerals such as phosphorus (P), calcium (Ca) and magnesium (Mg) is reduced. These minerals have been shown to play a regulatory role in the metabolism of other minerals, including aluminium (Al), molybdenum (Mo) and others (Abdik et al., 2019; Yünlü, 2016).

Considering the usage areas of boron mineral and its effects on health, the aim of this study was to determine its level in rats. For this purpose, healthy

rats were given boron to determine the boron levels in their serum. In addition, when its interaction with many parameters and minerals was evaluated, it was aimed to determine its effect on hematological parameters and serum Ca, P and Mg levels.

MATERIALS AND METHODS

In the study, 12 male, 2-3 months old, male, Wistar albino rats weighing 250-350 grams were used. The rats were obtained from Balıkesir University Experimental Animal Production, Care, Application and Research Centre. Rats were housed in individual cages. In order to determine the amount of water consumed per animal more accurately, 12 rats were housed in a 4-storey system with 3 individual cages on each floor (12 hours light/12 hours dark, 40%-60% relative humidity and 20-24 °C ambient temperature) without any change or restriction (except water) (Figure 1). Rats were fed ad libitum (using pellet feeds) and 2 mg/day/rat/25 ml borax decahydrate (Sigma) was added to the drinking water (Aksoz, 2020; Environmental Protection Agency (EPA), 2004). Rats were decapitated at the end of 14 days. Blood samples were collected in K3EDTA tubes for hematologic analysis and in biochemical gel tubes for Ca, P and, Mg analysis. Hematological parameters were analyzed on hemogram device (HASVET VH3 Veterinary Blood Count Device) without waiting. Blood was centrifuged at 4000 rpm for 5 minutes to obtain serum samples. Serum samples were analyzed on Erba Lachema Biochemical analyzer XL 200. Boron concentrations were measured by ICP-OES device.

ICP-OES analysis

Serum boron analysis by ICP-OES was performed according to Tokay and Bagdat (2022). 0.5 ml serum samples were placed in Teflon containers. 2.5 ml HNO₃ and 1 ml H₂O₂ solution were added. The Teflon containers were placed in a microwave oven set at 150°C T1 temperature for 15 minutes and 190°C T2 temperature for 15 minutes. After degradation, 5 ml of ultrapure water was added, and dilution was performed. Following the filtration of the samples through a 0.45 µm filter, they were introduced into the ICP-OES apparatus. For each sample, three repeated measurements were obtained and subsequently averaged.

Before the samples were fed to the ICP-OES device, a calibration graph was drawn using 0.1-5 ppm doses and LOD: 0.979, LOQ: 2.967. (Figure 2).

Statistical analysis

In the study, Kolmogorov-Smirnov test was applied for normality assumptions and $p > 0.05$ was determined. Independent t-test, one of the parametric tests, was applied to determine whether there was a significant difference between the groups. The data obtained were analysed using SPSS 25.0 (IBM Corp., Armonk, NY, USA). In case of a significant difference between the groups, post-Hoc test was used to determine between which groups there was a

significant difference. $p < 0.05$ was considered statistically significant.

Ethical considerations

This study was carried out with the permission of the Animal Experiments Local Ethics Committee of Balıkesir University (BAUN-HADYEK) (permission no: 2024/4-5 of April 25, 2024).



Figure 1. Housing conditions of the animals used in the study.

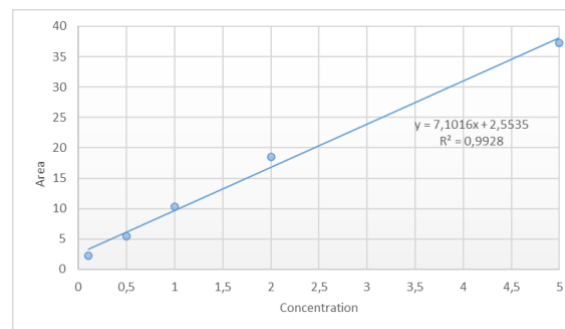


Figure 2. Concentration area plot for the analysis of boron.

RESULTS

It was determined that the administration of 2 mg/day/rat/25 ml borax decahydrate in the drinking water of rats for 14 days did not cause any adverse health effects.

Hematological parameters results are presented in Table 1. According to Table 1, it was determined that there was no statistically significant difference between the control and experimental groups ($p > 0.05$).

Table 1. Results of analysis of hematological parameters of rats.

	Experimental Group		Control Group		U	p
	Mean±SD	Median (Min-Max)	Mean±SD	Median (Min-Max)		
WBC ($10^9/L$)	13.87±3.87	13.00 (10.33-18.73)	12.35±4.51	10.83 (7.8-18.02)	0.801	0.423
LYM# ($10^9/L$)	8.07±2.96	7.96 (4.92-11.49)	7.88±3.99	6.90 (4.2-12.94)	0.480	0.631
MID# ($10^9/L$)	1.93±0.71	1.66 (1.35-3.28)	1.22±0.44	1.17 (0.79-1.91)	1.925	0.054
GRAN# ($10^9/L$)	3.88±0.88	4.03 (2.39-4.7)	3.24±0.80	3.48 (2.17-4.06)	1.441	0.150
LYM% (%)	0.57±0.07	0.57 (0.46-0.65)	0.61±0.12	0.62 (0.47-0.76)	0.962	0.336
MID% (%)	0.14±0.04	0.14 (0.09-0.2)	0.10±0.03	0.10 (0.07-0.16)	1.761	0.078
GRAN% (%)	0.29±0.07	0.26 (0.23-0.41)	0.28±0.10	0.26 (0.18-0.43)	0.480	0.631
NLR	0.53±0.20	0.43 (0.38-0.88)	0.51±0.28	0.43 (0.23-0.9)	0.480	0.631
PLR	120.18±56.83	104.61 (60.1-223.4)	157.66±98.04	107.40 (79.5-289.3)	0.320	0.749
RBC ($10^{12}/L$)	8.99±1.26	9.03 (6.86-10.67)	8.67±0.52	8.73 (8.04-9.2)	0.641	0.522
HGB (g/L)	136.33±16.22	139.00 (106-151)	135.00±6.54	136.50 (127-141)	0.482	0.630
HCT	0.50±0.06	0.51 (0.39-0.56)	0.49±0.03	0.49 (0.46-0.52)	0.722	0.470
MCV (fL)	55.77±1.46	56.40 (52.9-56.7)	56.45±0.90	56.55 (55.3-57.5)	0.484	0.629
MCH (pg)	15.22±0.55	15.45 (14.1-15.5)	15.55±0.23	15.55 (15.2-15.8)	1.309	0.191
MCHC (g/L)	272.83±3.31	273.00 (268-278)	275.67±3.39	276.00 (270-279)	1.532	0.126
RDW-CV (%)	0.16±0.00	0.16 (0.15-0.16)	0.16±0.01	0.16 (0.15-0.17)	1.046	0.295
RDW-SD (fL)	32.03±0.57	32.20 (30.9-32.5)	33.07±1.19	33.05 (31.8-34.7)	1.143	0.253
PLT ($10^9/L$)	861.00±202.39	897.00 (599-1099)	989.50±258.74	1022.00 (533-1245)	0.961	0.337
MPV (fL)	5.95±0.30	5.90 (5.6-6.4)	5.72±0.15	5.70 (5.6-6)	1.398	0.162
PDW-CV (%)	0.13±0.00	0.13 (0.13-0.14)	0.13±0.00	0.13 (0.13-0.13)	0.966	0.334
PDW-SD (fL)	9.38±0.97	9.35 (8.2-10.9)	8.60±0.53	8.45 (8.2-9.6)	1.457	0.145
PCT (%)	5.12±1.07	5.40 (3.4-6.2)	5.65±1.45	5.80 (3-7)	0.722	0.470
P-LCC ($10^9/L$)	68.83±13.11	69.00 (49-88)	69.67±17.21	74.00 (37-88)	0.486	0.627
P-LCR (%)	0.08±0.02	0.08 (0.06-0.11)	0.07±0.01	0.07 (0.06-0.08)	1.125	0.261

SD: Standart deviation, Min: Minumum, Max: Maximum, WBC: White blood cell, LYM: Lenfosite, MID: Monosite, GRAN: Granulosite, NLR: Neutrophil lymphocyte ratio, PLR: Platelet-lymphocyte ratio RBC: Red blood cell, HGB: Haemoglobin, HCT: Hematocrit, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, RDW-CV: Red blood cell distribution width, PLT: Platelet count, MPV: Mean platelet volume, PDW: Platelet distribution width, PCT: Procalcitonin, P-LCR: Platelet large cell ratio, P-LCC: Platelet large cell coefficient

Serum Ca levels

Figure 3 shows mean levels of serum Ca for the experimental and control groups. The results showed

no obvious statistically significant difference between the experimental and control groups ($p > 0.7991$).

Serum Mg levels

Figure 4 shows the average levels of serum Mg for the experimental and control groups. There was not observed statistically significant difference between the experimental and control groups ($p:0.3137$).

Serum P levels

Figure 5 shows the average serum levels of P for the control and experimental groups. There was not observed distinction between the experimental and control groups that was statistically significant. ($p:0.5430$).

Serum Boron levels

The mean serum boron levels of the control and experimental groups are given in Figure 6. A statistically significant difference was observed between the control and experimental groups ($p:0.0107$).



Figure 3. Mean Serum Ca Levels.

Values are given as mean±standard error ($n=6$, $p<0.05$).

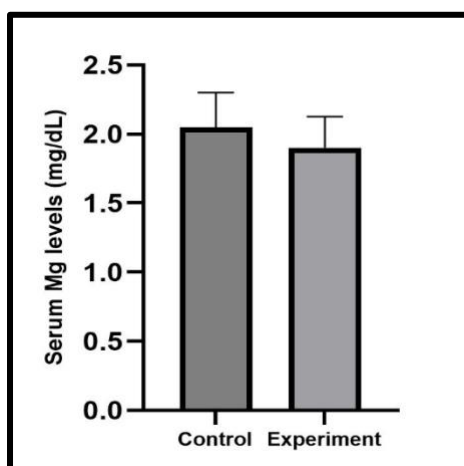


Figure 4. Mean Serum Mg Levels.

Values are given as mean±standard error ($n=6$, $p<0.05$).

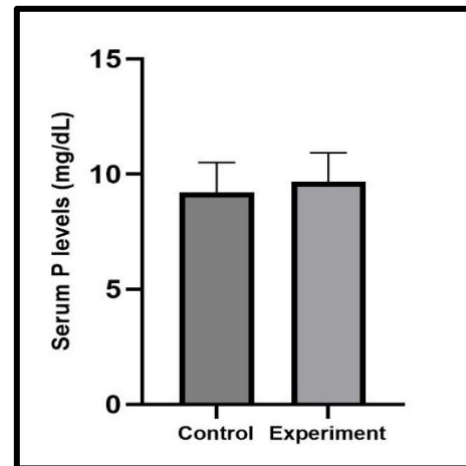


Figure 5. Mean Serum P Levels.

Values are given as mean±standard error ($n=6$, $p<0.05$).

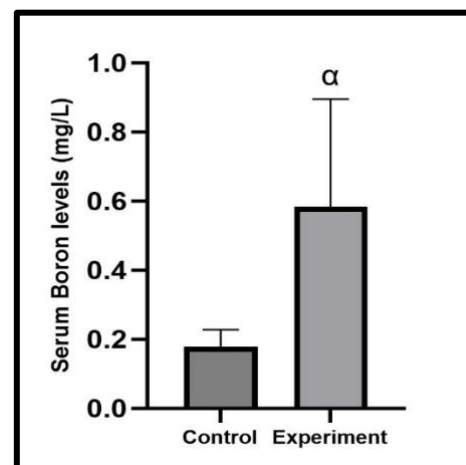


Figure 6. Mean serum boron levels.

α: Indicates significance according to the control.

Values are given as mean±standard error ($n=6$, $p<0.05$).

DISCUSSION

In this study, the effect of boron-supplemented drinking water on serum calcium, phosphorus and magnesium levels in rats was investigated and no statistically significant difference was found between the experimental and control groups ($p:0.7991$ for Ca, $p:0.5430$ for P, $p:0.3137$ for Mg).

These results indicate that boron intake does not cause a direct change in serum mineral levels. This may be due to the fact that boron is an element that may affect the storage and mobilisation processes of these minerals in bone, rather than having a direct regulatory role in mineral metabolism (Kurtoğlu et al., 2001).

A statistically significant difference was found between the experimental group and the control group

($p:0.0107$ for B) when the serum boron levels of rats drinking boron-supplemented water were evaluated. This finding was due to boron intake.

Hematological and biochemical parameters are routinely measured in cases such as disease, metabolic disorders and detection of side effects of drugs, and changes in the physiological process can be determined by the results. Changes in hematological and biochemical parameters are important sources of information for physicians in the differential diagnosis of clinical and subclinical diseases, their severity, follow-up of the course and success of treatment. When boron is taken as a dietary or food supplement, 84-85% of it is excreted from the body through urine and the amount of boron in plasma is mainly kept under control by renal excretion (Kuru and Yarat, 2017; NseAbasi et al. 2014). Durmuş et al., (2018) investigated the effects of boron administration on hematological parameter levels in rats given gentamicin. They found that only 20 mg/kg boron administration decreased the leukocyte count at a statistically significant level, but other low boron levels had no effect on the parameters examined. They found that gentamicin-induced decrease in leukocyte count could not be corrected by any of the boron levels, decrease in HCT level was prevented by B, and boron applications had no effect on erythrocyte count and hemoglobin levels. Hoffman et al. (1991), a decline in blood haemoglobin levels and haematocrit values was observed in ducks fed a diet that was deficient in protein and rich in boron. Conversely, Sisk et al. (1990) reported an increase in haemoglobin levels in goats afflicted with experimental boron toxicity. Yildirim et al., (2018) in the study investigating the protective effects of lithium borate on blood and histopathological parameters in rats acutely administered cadmium, erythrocyte count, white blood cell (WBC), neutrophil % and C-reactive protein (CRP) levels increased statistically significantly in cadmium and lithium borate + cadmium groups compared to the control group, while lymphocyte % and monocyte % levels decreased. Başoğlu et al., (2010) stated that erythrocyte count, hemoglobin amount, hematocrit value and platelet count were not affected in rabbits given borax. Kuru et al., (2018) conducted a retrospective study on the evaluation of dietary boron intake in terms of health. They stated that they did not find a significant relationship between daily boron intake and biochemical and hematological parameters. The fact that there was no difference in hematological parameters between the experimental and control groups in the study is similar to the studies in the literature. The reasons for obtaining different results may be due to the difference in the level and source of boron mineral used in the studies and the animal species used in the experiment. Considering that people are exposed to boron even with daily diet, it is important that there is no negative

effect (Durmuş et al., 2018; Kuru et al., 2018).

Studies have shown that boron interacts with various minerals. It affects transmembrane signaling and alters cell membrane integrity in experimental animals deficient in K, Ca, Mg and cholecalciferol. Boron deficiency may increase stress due to vitamin deficiency (Kurtoğlu et al., 2001; Bakken and Hunt, 2003). Karabulut, (2006) found that boron added to quail feed at different doses decreased serum Ca, P and Mg levels in the experimental groups compared to the control after 35 days of feeding. Armstrong and Spears, (2001) reported that the addition of boron to pig feed and King et al., (1993) reported that in ovo injection of boron into turkey eggs did not affect serum Ca, P and Mg levels. Chapin et al., (1997) found that boron added to the diets of rats decreased serum Ca, P and Mg levels, while Dupre et al., (1994) found that it increased them. In the present study, the fact that there was no statistically significant difference in serum Ca, P and Mg levels is consistent with the studies in the literature. It is thought that the different results may be related to the amount of boron given to the animals.

Boron is one of the trace elements for animals and is generally reported as essential in very small amounts in their diet. Living organisms usually ingest boron through food and/or water (Uçkun, 2013; Yakıncı and Kök, 2016).

Yiğit et al., (2013) administered 31.25, 62.5, 125 mg/kg boric acid to rabbits. It was stated that serum boron levels of animals in the experimental groups 31.25 mg/kg (1.80 ± 0.19 mg/L), 62.5 mg/kg (2.71 ± 0.25 mg/L), 125 mg/kg (4.36 ± 0.30 mg/L) increased compared to the control group (1.25 ± 0.10 mg/L). Bintaş, (2013) reported that when laying hens were fed with 555 mg boric acid/kg, serum boron levels were 509.90 µg/L, which increased serum boron levels compared to the control group. Korkmaz et al., (2007) conducted a study to determine the amount of boron in the urine of males living in a region rich in B in those who used water containing at least 2 mg/l boron and those who did not. They calculated the mean daily B exposure value as 6.77 mg in the study group and 1.26 mg in the controls. Başaran et al., (2012) conducted a study on reproductive toxicity in workers exposed to boron in Bandırma, Turkey. They found that the average blood boron concentration of the workers in the high exposure group was higher than the control. In this study, the fact that the amount of boron in the blood serum of rats receiving B-containing water was higher than those who did not is in accordance with the studies in the literature.

CONCLUSION

Boron is an essential trace element for humans and animals. Studies have shown that it should be taken in certain amounts daily. It was concluded that boron mineral, which was stated to have many benefits in the studies, did not have a negative effect on

hematological and biochemical parameters, serum Ca, P and Mg levels. In order to obtain more detailed information on the health parameters of boron, it was concluded that studies should be carried out in different animal species and doses.

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None.

Conflict of Interest

The author declares no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

Author Contributions

Plan, design: HS, CC, MC, OR, PD, IK; **Material, methods and data collection:** HS, CC, MC, OR, PD, IK; **Data analysis and comments:** HS, CC, MC, OR, PD, IK; **Writing and corrections:** HS, CC, MC, OR, PD, IK

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Ethical Approval

Institution: Animal Experiments Local Ethics Committee of Balıkesir University (BAUN-HADYEK).

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