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Hyperglycemic Effect of Dietary Boron in Rats with Experimental Diabetes Mellitus Induced by Streptozotocin

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ABSTRACT In this study, the effect of boric acid (BA) on blood sugar levels, vitamins and minerals in Streptozotocin (STZ)-induced diabetes in rats was investigated. In the study, 48 male Wistar albino rats (200-220 g) were divided into 6 groups, no special treatment was administered to Group1, experimental diabetes was induced by administering STZ (45 mg/kg) through intraperitoneal (IP) injection to other groups. Group 2 diabetes; Group 3 (6 U/kg insulin) insulin; Group 4; 250 ppm, group 5; 500 ppm and group 6 1000 ppm BA mixed with their feed. Blood glucose levels of all groups were quantified from blood taken from the tail vein every week. At the end of study, the rats were sacrificed and their blood was taken. The serum levels of vitamins A, E, and minerals were studied. When compared with other groups, blood glucose levels of groups 4, 5 and 6 were found to be increased ($p<0.05$). When compared to the baseline values, it was found that Vitamin A in the group 2 and 4, vitamin E in the group 4, Cu in the group 4, 5, 6, Zn, Mg and Na in the group 2 decreased and Fe in the group 2 increased. While Ca and P decreased in groups 5 and 6, no change was observed in Al in all groups. As a result, it has been observed that boron has a hyperglycemic effect when evaluated together with vitamins and minerals in diabetic rats. Whether boron is a suitable agent in the treatment of diabetes should be evaluated with further studies.

Keywords: *Boron, Diabetes mellitus, Rat, Mineral, Streptozotocin, Vitamin.*

ÖZ

Streptozotosin ile Deneysel Diyabet Oluşturulan Ratlarda Diyetteki Borun Hiperglisemik Etkisi

Bu çalışmada ratlarda Streptozotosin (STZ) ile oluşturulan diyabetes mellitüste borik asidin (BA)'in kan şeker, vitamin ve mineral düzeyleri üzerine etkisi araştırıldı. Sunulan çalışmada 48 adet erkek Wistar albino rat (200-220 g) 6 gruba ayrıldı, Grup 1'e özel bir tedavi uygulanmadı, diğer gruplara intraperitonal (İP) 45 mg/kg STZ uygulanarak deneysel diyabet oluşturuldu. Grup 2 diyabet grubu; Grup 3 (6 U/kg insülin) insülin grubu; Grup 4, 5 ve 6'nın yemlerine sırasıyla 250, 500 ve 1000 ppm BA katıldı. Tüm grupların kan glikoz seviyesi haftalık olarak ölçüldü. Çalışmanın sonunda sıçanlar anestezi altında kan örnekleri alındıktan sonra sakrifiye edildi. Elde edilen serum örneklerinden A ve E vitamin ile bazı mineraller ölçüldü. Kan glikoz düzeylerinin 4, 5 ve 6. gruplarda arttığı belirlendi ($p<0,05$). Başlangıç değerleri ile karşılaştırıldığında 2 ve 4. grupta A vitamini, 4. grupta E vitamini, 4, 5 ve 6. gruplarda Cu, 2. grupta Zn, Mg, Na ve Fe düzeylerinin azaldığı tespit edildi. 5. ve 6. gruplarda Ca ve P azalırken, tüm gruplarda Al'de değişiklik gözlenmedi. Sonuç olarak, borun diyabetik sıçanlarda vitamin ve mineral düzeyleri ile birlikte değerlendirildiğinde hiperglisemik etkiye sahip olduğu gözlendi. Bor'un diyabet tedavisi için uygun bir ajan olup olmayacağı ileri çalışmalarla değerlendirilmelidir.

Anahtar Kelimeler: *Bor, Diabetes mellitus, Mineral, Streptozotosin, Sıçan, Vitamin.*

INTRODUCTION

Diabetes Mellitus (DM) is a chronic metabolic disorder that affects more than 400 million people across the world, causing impairments in carbohydrate, fat and protein metabolism due to absolute or relative insufficiency of insulin hormone secretion and/or insulin action (Regazzi 2018; Sacan et al. 2021). DM is an illness that requires lifelong continuous monitoring and treatment, impairs the quality of life of the patient due to its acute and chronic complications, and causes the death of thousands of people every year across the world due to chronic complications

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such as hypoglycemia, ketoacidosis, hyperglycemic hyperosmolar nonketotic coma, nephropathy, neuropathy, myocardial infarction, and atherosclerosis throughout the course of the disease (Domingueti et al. 2016). Although the underlying cause of DM complications is not fully elucidated, a great many studies have reported role of oxidative stress and lipid peroxidation. Lipid peroxidation and oxidative stress damage tissues by causing dysfunction of pancreatic β cells (Coban et al. 2015; Singh et al. 2022). Since free radicals increase and radical-binding systems decrease in DM, diabetic patients need antioxidants more. Natural vitamins (A, E, C) are used as antioxidants to hinder the detrimental effects of lipid peroxidation and oxidative stress (Asmat et al. 2016). Changes in antioxidant status in DM may be a result of nutritional status due to inadequate intake or excessive loss or may cause excessive consumption of these antioxidant vitamins in an environment where the oxidative load may be high (Zatalia and Sausi 2013).

Trace elements are essential for the body functions, growth, and physiology of the organism. Studies show that there is a relationship between glucose homeostasis and trace elements. Chronic hyperglycemia significantly changes the level of certain trace elements. Besides, the change in the level of trace elements increases the oxidative stress in DM and increases the occurrence of diabetes complications (Bahtıyar and Hacıoglu 2019; Krol et al. 2016). In a study conducted by the American Diabetes Association in 2007, the estimated cost of diabetes in the world was determined to be 174 billion dollars (Khalil 2017). Since diabetes is a socioeconomic burden in many countries and the medications used in its treatment cause multiple adverse effects in patients, the discovery and research of alternative hypoglycemic agents (herb, mineral, vitamin) play a vital role (Cakir et al. 2018; Tan et al. 2019).

Recent in vitro and epidemiological studies show that boron applications have significant effects on human health. Boron is found in nature as borax, colemanite, and boric acid, which is in group III-A in the periodic system with atomic number 5. Boron is an element that plays key roles in hormonal metabolism (insulin, estrogen, testosterone, calcitonin), cell membrane functions, minerals (Ca, P, Mg), lipid and carbohydrate metabolism, energy use, immune system functions, and enzymatic reactions (Bolt 2020; Wang et al. 2021; Ozel et al. 2022). Boron changes the permeability of the cell membrane to bioactive substances, the functions of membrane enzymes, and the affinity of receptors by forming complexes with polysaccharides, sugars, adenosine-5-phosphate, riboflavin, pyridoxine, pyridine nucleotides, phosphoinositides, dehydroascorbic acid, glycolipids and glycoproteins from organic compounds carrying cis-hydroxyl groups (Nielsen 2014; Hunter et al. 2019). It has also been reported that B compounds may have an inhibitory effect on important cellular components such as the proteasome, protease, and peptidase, by binding electron carriers such as NAD and hydroxyl group to the macromolecule side chain such as serine residues in protein structures (Cebecı et al. 2022). It has been suggested that boron is effective in the central nervous system and endocrine system functions by affecting vital structures for the organism such as magnesium, calcium, nitrogen, copper, glucose, reactive oxygen, triglyceride and estrogen in the life cycle of humans (Breydo 2013). Boric acid makes up 98.4% of the boron in the blood, and boron was mentioned as boric acid in most of the studies and pure boric acid (17.5% boron) was used as a boron source (Karimkhani et al. 2021). The lowest observed adverse effect level (LOAEL) and no-observed-adverse-effect level (NOAEL) for boric acid were found to be 78 and 55 mg BA/kg/day (13 and 10 mg/kg/day for boron), respectively. Higher doses cause developmental toxicity in mice, rats, and rabbits (Bolt et al. 2020; Sevım and Kara 2022).

In this study, the impact of boron administered as boric acid (4.1, 8.2, and 15.0 mg boron/kg) on weekly blood glucose levels, some vitamins and minerals in rats with diabetes mellitus induced by STZ (streptozotocin) was researched.

MATERIAL AND METHODS

Experimental Animals

In the study, 48 male 2-3 months old Wistar albino rats, with a bodyweight of 200-220 g were used which were obtained from the experimental animal unit of Van Yuzuncu Yil University. The rats were fed with standard or boric acid-enriched pellet feed in rooms at 22±2 ℃ illuminated with a 12-hour light, and 12-hour dark cycle. Before inducing experimental diabetes (except for the control group), the rats were fasted for 12 hours with free water intake, then released and housed in standard plastic cages with standard or boric acid-enriched feed and water intake. Before launching the study, approval was obtained from YYU Animal Experiments Local Ethics Committee (YUHADYEK) (adopted with its decision dated April 19th, 2012, and numbered 2012/03-04).

Preparation of Boric Acid Enriched Feed

Boric acid feed material to be used in the feeding of rats during the experiment was obtained from YYU Medical Faculty Health Research and Education Center Experimental Research Unit as standard pellet feed (Bayramoglu Feed Industry, Erzurum). Feed content is given in table 3. The feeds were ground in Ika Werke 2000 electric mill and passed through a 2 mm sieve in the laboratory of the Department of Animal Nutrition and Animal Nutrition Faculty of Veterinary Medicine, Van YYU. The amount of boric acid used in the experiment was prepared as 250, 500, and 1000 ppm (0.025%, 0.05%, 0.1% respectively) per 1 kg feed. Boric acid solutions of different concentrations were prepared in deionized water with the help of a magnetic stirrer and mixed homogeneously with the pellet powder. It was made into pellets and dried in the laboratory.

Induction of Diabetes in Rats with STZ

To induce diabetes, 45 mg/kg STZ (Vargas et. al 2023) solution prepared phosphate in citrate buffer (0.1 M. Ph: 4.5) was injected intraperitoneally (IP) into all rats except the control group. Before launching the study, rats in the other groups, except the control group, were fasted for 12 hours with free water intake. One week after STZ injection, the blood glucose levels of rats were determined by tail vein using a glucometer. Those with a level of >200 mg/dl were considered diabetes and included in the experiment.

Experiment Protocol

They were divided into 6 groups as follows, with 8 experimental animals in each group. On the 7th, 14th, 21st, 28th, 35th, and 42nd days of the experiment, blood was drawn from the tails of the rats with the help of a scalpel, and the blood glucose levels in the rats were quantified using a Glucometer.

The rats in Group 1 were the control group and fed with normal water and standard pellet feed.

The rats in Group 2, the diabetic group in which diabetes was induced by STZ, were fed with normal water and standard pellet feed.

The rats in Group 3, whose diabetes was induced by STZ and treated with insulin, were fed with normal water and standard pellet feed, and daily insulin at a dose of 6 U/kg was administered as IP.

The rats in the Group 4 were given normal water and pellet feed containing 250 ppm boric acid for 28 days.

The rats in the Group 5 were given normal water and pellet feed containing 500 ppm boric acid for 28 days.

The rats in the Group 6 were given normal water and pellet feed containing 1000 ppm boric acid for 28 days (Fail 1991; Scialli et al. 2010).

Blood Tests

Blood was drawn from the heart on the first and last day of the experiment, in accordance with the technique, by administering 50 mg/kg ketamine and 10 mg/kg xylazine IP for anesthesia. Blood samples were taken into gel biochemistry tubes, kept for 30 minutes, and centrifuged at 3000 rpm to separate the serum. The obtained material was stored in a refrigerator at -80 °C until analysis. In the subsequent stage, vitamin A and E levels were determined in Thermo Finnigan HPLC, and mineral levels were determined in AAS (Atomic Mass Spectrometry) and ICP-MS device, and the change between groups was analyzed statistically.

Chromatographic Analysis

Quantification of vitamins A and E: First, 200 µl of 5% sodium chloride, then 400 µl of ethanol was added to 200 µl of serum to precipitate the proteins in the sample and mixed in a vortex. 700 µl of n-hexane was added to this mixture and centrifuged at 3500 rpm for 10 minutes. In this way, fat-soluble vitamins A and E were extracted into the n-hexane phase. The combined n-hexane phases obtained by repeating this extraction process twice were evaporated to dryness under nitrogen gas. Residual methanol in the tube was dissolved into a mixture of DCM (50:50) ready for Chromatographic identification. For the determination of vitamins, A and E, C18 ODS-2 column, a mobile phase consisting of a mixture of methanol and water (97:3) degassed in an ultrasonic water bath was used. The flow rate of the mobile phase was set as 1.05 ml/min and the injection amount was set as 20 µl. A more sensitive fluorescent detector (excitation: 325 nm, emission: 480 nm) was preferred for the quantification of vitamins A and E) (Karatas et al., 2008).

Mineral Levels Analysis

Blood mineral analyzes were performed at YYU Central Research Laboratory Training and Research Center with ICP-MS and AAS devices. For analysis, serums were diluted with deionized water (100-fold diluted for ICP-MS and 10-fold for AAS). Results were presented in ppm.

Statistical Analysis

Descriptive statistics for the features studied were expressed as mean and standard deviation. Regarding these characteristics, a Repeated Measured Analysis of Variance was performed to determine whether there were differences between the study groups in terms of weeks and times (day zero - last day). Tukey's test was used to identify different groups and weeks following the Analysis of Variance. The statistical significance level was taken as 5% in the calculations and the SPSS statistical package software was used for the calculations.

RESULTS

Blood glucose results

There was no difference between the groups in the 1st week blood sugars ($p>0.05$). The blood glucose levels and standard errors of the study groups are shown in Table 1. Animals injected with STZ (groups 2, 3, 4, 5, 6) had blood glucose levels > 200 mg/dl one week later (week 2). It was determined that blood glucose levels increased (group 2, 3, 4, 5, 6) compared to the control group ($p<0.05$). It was determined that upon giving different doses of boric acid-containing feed (3rd, 4th, 5th, and 6th weeks) the blood glucose levels of the animals in Groups 4, 5, and 6 increased compared to the Groups 1, 2, and 3 ($p<0.05$) (Table 1).

Variation Between Vitamin and Mineral Levels

In our study, at the end of the experiment, it was observed that the serum vitamin A level of the 2 and 4 groups decreased significantly compared to the baseline values, and the serum vitamin E level of the Group 4 was lower than the baseline values ($p<0.05$). A significant decrease was observed in Cu levels compared to baseline values in Groups 4, 5, and 6, and in serum Zn, Mg, and Na levels in Group 2 ($p<0.05$), whereas increase was observed in serum Fe levels of group 2 ($p<0.05$). Besides, a significant decrease was observed in serum Ca and P levels in Groups 5 and 6, while no significant change was observed in serum Al in all groups (Table 2).

DISCUSSION AND CONCLUSION

Considering the fact that it affects many people, its treatment, complications, the rapid increase in the number of diabetes patients and the burden it brings to the national economy, DM is considered one of the most important problems in the health world. Intense efforts are being made to develop new strategies to achieve better metabolic control in diabetes and reduce diabetes-related complications (Domingueti et al. 2016; Darenskaya et al. 2021).

Vitamins and minerals are involved in biological processes, which are essential for the physiological functions of the body, as cofactors in the structure of enzymes, and in the formation of proteins and enzymes. Some trace elements control vital biological events by binding to receptor sites in the cell membrane or changing the shape of the receptor to prevent certain molecules from entering the cell. Trace elements and vitamins play a key role in DM and its complications as antioxidants (Kaur et al. 2021). It is well known that some inorganic trace elements such as chromium, zinc, copper, iron, sodium, potassium and nickel play a significant role in the maintenance of normoglycemia by activating the β-cells of the pancreas (Narendhirakannan et al. 2005). It has been reported that a change occurs in the metabolism of some minerals (Mg, Cu etc.) in DM and there is a relationship between this change and the progression of the disease (Tamrakar and Kachhawa 2016). Boron has notable effects on carbohydrate, lipid, protein, and enzyme metabolism, vitamins, trace elements, and immune and hormonal systems in humans and animals. Moreover, studies on the effects of boron on oxidative stress, which is of great importance in the complications of DM, have increased in recent years. Physiological amounts of boron regulate both energy substrate usage and mineral metabolism (Ozyarim and Coban 2021; Rahman et al. 2021).

Table 1: Descriptive statistics for glucose and comparison results of groups.

	1. group	2. group	3. group	4. group	5. group	6. group	*p*
1st week	93.00±8.00 a	93.57±11.80 a	93.57±11.80 a	104.25±13.37 a	100.00±13.26 a	95.14±12.15 a	0.359
2nd week	100.00±14.00 b	384.38±71.42 a	349.00±98.28 a	369.13±35.00 a	317.50±101.60 a	400.90± 69.27 a	0.001
3rd week	104.50±11.43 d	414.13±54.00 c	112.43±23.55 d	536.14±66.08 ab	447.00±148.29 bc	600± 0.01 a	0.001
4th week	95.13±7.31 d	438.80±64.16 c	104.00±24.30 d	500.00±85.45 b	371.43±97.00 bc	600 ± 0.01 a	0.001
5th week	90.25±5.00 d	414.17±54.41 c	130.43±29.01 d	536.57±59.22 ab	456.00±123.53 bc	600 ± 0.01 a	0.001
6th week	98.00±12.00 c	471.00±100.00 b	97.43±11.23 c	536.57±59.22 ab	491.43±125.49 b	600 ± 0.01 a	0.001

In the weeks with $P<0.05$, the difference in glucose change between the groups is significant. (a, b, c →) The difference between the group averages with different letters in the same line is significant.

1. group (control group) was fed with normal water and standard pellet feed.
2. group (the diabetic group with 45 mg/kg STZ, in whom diabetes occurred) was fed with normal water and standard pellet feed.
3. The group (DM+ insulin group in which 6 U/kg insulin was administered every day as IP) was fed with normal water and standard pellet feed.
4. group (DM + 250 ppm boric acid) was given normal water and pellet feed containing 250 ppm boric acid for 28 days.
5. group (DM+500 ppm boric acid) was given normal water and pellet feed containing 500 ppm boric acid for 28 days.
6. The group (DM+1000 ppm boric acid) was given normal water and pellet feed containing 1000 ppm boric acid for 28 days.

Table 2: Mineral and vitamin levels of the groups on the first and last day.

	1. group		2. group		3. group		4. group		5. group		6. group	
	Day Zero	The last day	Day Zero	The last day	Day Zero	The last day	Day Zero	The last day	Day Zero	The last day	Day Zero	The last day
Vitamin A	0.15±0.07	0.13±0.04	0.17±0.06	0.07±0.01 #	0.12±0.04	0.10±0.01	0.12±0.03	0.08±0.02 #	0.13±0.01	0.11±0.02	0.11±0.02	0.10±0.01
VitaminE	0.07±0.03	0.06±0.02	0.08±0.03	0.08±0.04	0.07±0.03	0.06±0.02	0.09±0.02	0.05±0.04 #	0.08±0.02	0.04±0.01	0.04±0.01	0.03±0.03
Cu	21.00±7.00	21.00±11.00	15.30±4.00	14.00±4.00	16.43±5.00	12.38±2.19	19.00±4.00	11.62±1.70 #	16.35±2.26	11.21±1.24 #	17.39± 4.11	12.14±2.04 #
Zn	24.00±6.02	23.48±5.00	33.36±6.00	23.27. ±8.00 #	15.31±3.10	26.18±2.00 #	24.00±.5.55	21.00±2.00	24.15±4.00	22.00±2.00	23.31±2.00	21.00±2.21 #
Fe	110.28±10.46	110.24±8.34	99.00±5.00	113.00±10.00#	111.56±14.15	112.00±14.27	111.00±11.00	107.00±11.36	110.00±13.44.	111.00±14.32	109.00±12.00	109.00±13.21
P	464.00±121.26	428.51±53.40	437.00±59.51	400.16±53.00	460.00±30.27	454.30±11.20	458.24±34.00	442.37±34.51	438.31±121.1	367.29±142.00#	423.00±±35.00#	370.00±37.31#
Na	100.24±3.44	97.65±4.00	98.32±5.40	93.00±3.00 #	96.47±5.00	95.00±4.00	99.31±4.00	96.13±4.00	97.00±5.00	94.50±3.52	97.00±4.50	95.29±4.00
Ca	15.19±4.07	16.09±2.19	16.00±0.08	14.60±3.00	16.00±2.00	16.00±1.00	17.00±0.09	15.60±2.41	17.00±0.06	14.35±1.48 #	17.00±1.01	14.11±1.10 #
Mg	11.00±1.48	10.22±2.00	11.02±2.22	7.80±4.29 #	10.00±2.00	10.45±1.39	11.00±3.00	8.00±3.00	10.46±3.00	9.41±1.37	10.00±2.00	8.38±1.42
Al	19.00±1.11	19.43±0.24	19.07±0.26	19.09±0.17	19.15±0.43	19.00±0.70	19.00±0.43	19.26±0.71	19.00±1.10	19.51±0.17	19.28±1.11	19.38±0.10

#: The difference from day zero is significant ($p<0.05$)
1. group (control group) was fed with normal water and standard pellet feed.
2. group (the diabetic group with 45 mg/kg STZ, in whom diabetes occurred) was fed with normal water and standard pellet feed.
3. The group (DM+ insulin group in which 6 U/kg insulin was administered every day as IP) was fed with normal water and standard pellet feed.
4. group (DM + 250 ppm boric acid) was given normal water and pellet feed containing 250 ppm boric acid for 28 days.
5. group (DM+500 ppm boric acid) was given normal water and pellet feed containing 500 ppm boric acid for 28 days.
6. The group (DM+1000 ppm boric acid) was given normal water and pellet feed containing 1000 ppm boric acid for 28 day

In the study, the impact of boron administered as boric acid on blood glucose levels, some vitamins (A, E), and minerals (Cu, Zn, Fe, P, Na, Ca, Mg, Al) in STZ-induced (45 mg/kg) DM was investigated.

It is well-documented that boron has an effect on insulin and serum glucose levels, and it is reported that boron can form a complex with the hydroxyl group in the structure of glucose and alter the blood glucose level (Muz et al. 2022). Although it was revealed in some studies that boron administered as gavage to diabetic rats decreased blood glucose levels compared to the control group (Cakır et al. 2018; Coban et al. 2015). In other studies, it was emphasized that boron added to the diet in rats decreased the insulin concentration without changing the plasma glucose concentration (Bakken and Hunt 2003; Kucukkurt et al. 2015). Hunt et al. (1997) reported that boron intake in chickens with vitamin D deficiency increased plasma glucose concentration, Dissordi et al. (2017) reported that the amount of physiological boron added to the normal water of diabetic rats increased blood sugar (Hunt et al. 1997; Dessordi et al. 2017). In this study, our results were like those of Hunt et al. (1997) and Dissordi et al. (2017). In the present study, blood glucose levels of the rats were measured one week after 45 mg/kg STZ was given to the other groups, except for Group 1, and because all rats' blood glucose levels were above 200 mg/dl (except for the control group), all of them were considered diabetic in the 2nd week and work started. When the feed containing boric acid was given to Groups 4, 5, and 6, the blood glucose level increased compared to Groups 1, 2, and 3. We think that the fact that boron raises the blood glucose level in diabetic rats is caused by the fact that boron triggers polyphagia by forming a complex with the cis-hydroxyl group in the glucose structure and inhibits the glycolytic pathway by reversibly reducing the activity of NAD or FAD, which are main substrates of glycolysis (Hunt et al. 1997; Geyıkoglu and Turkez 2007).

Dubey (2020) and Rad (2022) stressed that in the relationship between diabetes and its complications with reactive oxygen species, the antioxidant defense system is impaired to protect beta cells in the pancreas and glucose tolerance might also change. Studies have revealed a decrease in vitamin A and E levels in plasma in DM (Yuztaş and Degeryoruk 2014; Dubey et al. 2020; Rad et al. 2022). Thus, in our study, it was found that vitamin levels decreased compared to baseline values in all groups fed with boric acid, but only vitamin A in Group 2 and 4, and vitamin E in Group 4 decreased significantly ($p<0.05$). It can be suggested that there is a decrease in vitamin E and A levels due to the increase in free radical production and decrease in radical binding systems in long-term impaired glucose tolerance since diabetics need more antioxidants in oxidative stress. In minerals, serum copper (Cu) levels were significantly lower in Groups 4, 5 and 6 compared to the baseline values. Cu is an essential element for the activity of antioxidants in long-term impaired glucose tolerance. It has been reported that the decrease in plasma Cu level in DM is due to denaturing of antioxidant enzymes and oxidation of free oxygen radicals (Cho et al. 2007; Martirosyan et al. 2020; Sonkar et al. 2021). We are of the opinion that the decrease in mineral Cu level in Groups 4, 5, and 6 is due to denaturation or inhibition of antioxidant enzymes, given the fact that boric acid also exacerbates hyperglycemia in DM. Zinc (Zn) is required for insulin production in the pancreas and storage of insulin in vesicles. Moreover, it mediates insulin-induced glucose uptake into these cells by increasing the expression of Zn finger protein 407 and Zn-alpha-2-glycoprotein, glucose transporter type 4 (GLUT 4) protein in adipocytes and skeletal muscles (Bahtıyar and Hacıoğlu 2019). Bahtiyar and Hacıoğlu reported that serum Zn levels decreased in DM patients compared to the control group. When the data of our study were examined, it is noticed that although there was a decrease in serum Zn levels in Groups 2, 4, 5, and 6, the decrease in Groups 2 and 6 was statistically significant. It is seen that there is a significant increase in Group 3. We consider that the data we obtained support the view that a decrease in Zn level may occur due to a decrease in gastrointestinal absorption and/or hyperzincuria (Sanjeevi et al. 2018; Sonkar et al. 2021) while the increase in Group 3 is due to the daily administration of 6 U/kg insulin and the Zn in the structure of insulin. It has been reported that serum Fe levels increase in DM (Sha et al. 2021). In the presented study, serum Fe levels increased in groups 3, 5 and 2 compared to the initial values, but the increase in group 2 was significant. This increase is thought to be since Fe electrons act as electron donors and acceptors in biological systems and that Fe plays a role in the pathogenesis of diabetes as a prooxidant (Hansen et al. 2017; Skalnaya et al. 2017; Martirosyan et al. 2020). Although serum Na level decreased in groups 4, 5 and 6, it was not found to be statistically significant ($p>0.05$). At the end of the experiment, serum Na levels in group 2 decreased significantly compared to the initial values. Hyperglycemia has a diluting effect on electrolyte concentrations by regulating the internal environment for osmotic diuresis. A decrease in serum Na levels has been observed in DM due to osmotic diuresis (Siddiqui et al. 2014). Hakkı et al (2013) reported that boron increases the calcium and phosphorus levels in the bones in rabbits, Naghii and Samman (1996), and Khaliq et al. (2018) reported that the urinary excretion of calcium and phosphorus decreases with the addition of boron to the diet in menopausal women, and phosphorus and calcium are stored in the bones to reduce the possible bone loss, while Dupree et al. (1994) reported that the addition of boron to the diet in rats with vitamin D deficiency increased the absorption of calcium and phosphorus balance from the serum, and Dessordi et al.(2017) found that boron supplementation in the diet of diabetic rats decreased the serum Ca level of boron compared to rats with normal diet and control group (Dupree et al. 1994; Naghii and Samman 1996; Hakkı et al. 2013; Khaliq et al. 2018). In the study, a significant decrease was found in the serum Ca and P levels in Groups 5th and 6th compared to the baseline values. The serum P and Ca levels might have decreased because of the decrease in urinary excretion of B Ca and P and the contribution of these minerals to bone storage. Dessordi et al. (2017) Cakır et al. (2018) and Wang et al. (2021) observed a decrease in serum magnesium (Mg) levels in diabetes due to polyuria (Dessordi et al. 2017; Çakır et al. 2018; Wang et al. 2021). In the presented study, serum Mg levels decreased in groups 2, 4, 5 and 6 compared to the baseline values, but the decrease was found to be significant only in Group 2. The decrease in the 2nd group was due to polyuria and, as Abdelnour et al. suggested, the decrease in the 4^{th}, 5^{th} and 6^{th} groups with boron was not significant due to the storage of boron in the bones by decreasing urinary Mg excretion (Abdelnour et al. 2018).

Considering all the data obtained, in conclusion, it was determined that boron mineral did not lower blood sugar in rats with DM, on the contrary, it had a hyperglycemic effect by increasing it. It has been determined that boric acid reduces serum vitamin levels in diabetics, decreases

bone loss that may occur by increasing the absorption of calcium and phosphorus balance from serum in mineral levels, and the decreases in Cu, Zn, Na, and Mg levels resulted from the long-term impaired glucose tolerance. Besides, it was determined that the increased Fe level was caused by the decrease in the insulin level of iron stores in diabetes, and no change occurred at the mineral Al level. In future studies, it is recommended to investigate the hyperglycemic effect of boron by considering its impact on vitamins and minerals.

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest for this study.

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AUTHOR CONTRIBUTIONS

Idea / Concept: NAA, HÖ, GO
Supervision / Consultancy: HÖ, GO
Data Collection and / or Processing: NAA
Analysis and / or Interpretation: NAA, HÖ, GO
Writing the Article: NAA, HÖ, GO
Critical Review: NAA, HÖ, GO

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