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Investigation of the Effect of Boric Acid on Antioxidant System, HDL Levels and PON Activity in Rats Fed with High Fat Diet

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Öz

Son yıllarda yapılan araştırmalar, bu çağın en tehlikeli ve karmaşık sağlık sorunlarından biri olduğu bilinen obezitenin tedavisinde bor bileşiklerinin etkili olabileceğini ortaya koymuştur. Bu çalışma da diyetteki bor moleküllerinin obezite üzerindeki tedavi edici etkisi araştırılmıştır. Bu amaçla, yüksek yağlı diyetle beslenen farelerde bor bileşiklerinin Toplam Antioksidan Seviye (TAS), Toplam Oksidan Seviye (TOS), Yüksek Yoğunluklu Lipoprotein (HDL) seviyeleri ve Paraoksonaz (PON) aktivitesi üzerine etkisini incelenmiştir. Çalışma materyali, ortalama canlı ağırlığı 226,95 ± 5,75 g olan 4-5 aylık 40 Sprague Dawley sıçanıydı. Hayvanlar Grup I (normal diyet), Grup II (Yüksek yağ), Grup III (Yüksek yağ+ Borik Asit) ve Grup IV (Borik Asit) olmak üzere 4 gruba ayrıldı. Denemeden altı hafta sonra hayvanlardan kan örnekleri alındı ve örneklerden TAS, TOS, HDL seviyeleri ve PON aktivitesi ölçüldü. Ayrıca hayvanların canlı ağırlık değişimleri kaydedildi. Çalışma sonunda içme suyuna ek olarak verilen borik asidin TAS düzeyini ve PON aktivitesini azalttığını ancak istatistiksel olarak anlamlı olmadığını, TOS ve HDL düzeylerini yükselttiğini belirledik. Grup 3'de kontrol grubu dışında ki gruplardan daha fazla PON aktivitesi saptandı. Ayrıca borik asit uygulanan grubun ağırlık ortalaması da düşürdüğü gözlendi. Sonuç olarak borik asit ve yüksek yağlı diyet antioksidan sistem üzerine olumlu etki göstermedi fakat HDL düzeyinin artmasına ve PON aktivitesinin azalmasına neden oldular. Ratlar borik asit ve yüksek yağlı ile birlikte beslendiğinde PON aktivitesinde bir nispi artış gözlendi. PON ve HDL biribirinden bağımsız hareket ettiler. Ayrıca borik asit vücut ağırlığını düşürmede etkili olabilir

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Abstract

Recent studies have revealed that boron compounds can be effective in the treatment of obesity, which is known to be one of the most dangerous and complex health problems of this age. In this study, the therapeutic effect of boron molecules in the diet on obesity was investigated. For this purpose, the effects of boron compounds on Total Antioxidant Status (TAS), Total Oxidant Status (TOS), High Density Lipoprotein (HDL) levels and paraoxonase (PON) activity were investigated in mice fed a high-fat diet. The study material was 40 Sprague Dawley rats 4-5 months old with a mean live weight of 226.95 ± 5.75 g. Animals were divided into 4 groups as Group I (normal diet), Group II (High fat), Group III (High fat + Boric Acid) and Group IV (Boric Acid). Six weeks after the experiment, blood samples were taken from the animals and TAS, TOS, HDL levels and PON activity were measured from the samples. An increase in PON activity was observed when rats were fed a high-fat diet with boric acid. In addition, the live weight changes of the animals were recorded. At the end of the study, we determined that boric acid given in addition to drinking water decreased TAS level and PON activity, but it was not statistically significant and increased TOS and HDL levels. In Group 3, more PON activity was detected than the groups other than the control group. In addition, it was observed that the weight average of the group treated with boric acid decreased.

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As a result, boric acid and high-fat diet did not have a positive effect on the antioxidant system, but they caused an increase in HDL level and a decrease in PON activity. A relative increase in PON activity was observed when rats were co-fed with boric acid and high fat. PON and HDL acted independently of each other. In addition, boric acid may be effective in lowering body weight.

1. INTRODUCTION

Obesity which is defined as high and abnormal deposition of fat that may endanger adipose tissues is a complex and multifactorial disease in which social-behavioral disturbances are seen in addition to deformation in metabolic systems (Forster et al., 1988). Its etiology includes higher energy intake than expenditure, impaired lipid metabolism, stress, genetic vulnerability, low activity, and social factors. Considering the age we live in speed and change are influential in every aspect of our lives, including our nutrition and lifestyle habits. Many studies have reported that fast, excessive or irregular feeding by reducing feeding time triggers obesity (Bentley et al., 2018; Liu et al., 2015; Murakami et al., 2016).

Obesity is defined as a disease that decreases lifespan of living beings because it causes several health problems including cancer and cardiovascular disturbances. World Health Organization (WHO) reports that obesity is among the most risky diseases of our time and threatens our future (Forster et al., 1988).

Research has shown that increased workload on many tissues and increased metabolism which parallels excessive weight gain associated with obesity increase oxidative stress level. In addition, inadequate antioxidant intake and increased lipid content are thought to be effective in the development of obesity by leading to an increase in the amount of free radicals (Moylan & Reid, 2007; Tabur et al., 2010) . In short, oxidative stress in living organisms due to obesity is also seen as an effective factor in the progression of obesity.

Various methods are used to prevent obesity. Reducing body weight can be considered as the main purpose of these methods. Some studies on weight loss have shown that boric acid may be effective in reducing body weight by using supplemented boron compounds (Fail et al., 1998; Yildiz et al., 2013). Furthermore, healing the deteriorating lipid profile may be effective in struggle obesity(Doğan et al., 2017).

Boron is a semi-metal located at 3A in the periodic table and symbolized by B (Bolaños et al., 2004). Boron is generally found in human and in animals in the form of boric acid. It can be found in transparent crystal structure which is colorless, odorless and easily soluble in water as well as it can take the form of white granular powder (Sutherland et al., 1998; Woods,

1994). In addition to industrial use boron takes place in many processes in the metabolism of living beings like mineral, lipid and energy metabolism, and immune and endocrine systems. In addition, it has been reported that, it is also effective in the activity of many enzymes although the mechanism has not been fully appreciated (Armstrong et al., 2001; Dupre et al., 1994; Hunt, 1998; Hunt et al., 2009; Kurtoglu et al., 2018).

Boron enters the human body exogenously through respiration, skin, food and beverages; absorbed from mucosal surfaces at low concentrations and is converted to boric acid at appropriate pH values. It is distributed to various tissues of the body (nails, hair, teeth, liver, spleen, etc) and most of it (90-95%) is excreted in 24 hours with urine. It is accumulated in small amounts in bone tissue (Murray, 1998; Shuler, 1990; U.S. Environmental Protection Agency, n.d.). Inadequate boron intake by the organism has been stated to cause a decrease in functions of many biomolecules, such as calcium (Ca), magnesium (Mg), and vitamin D (Chapin et al., 1998).

In some studies with boric acid, enhancing effects of this mineral on antioxidant system were mentioned (Ince et al., 2010; Türkez et al., 2007). In studies using high fat diets, elevation in the oxidant system, reduction in High Density Lipoprotein (HDL) level which is a lipoprotein, and inhibition of HDL-dependent paraoxonase (PON) enzyme activity were reported (Olusi, 2002; Piva et al., 2011; Sorenson et al., 1995).

PON is a calcium-dependent ester hydrolase (Durrington et al., 2001) which can hydrolyse paraoxon, a potent inhibitor of cholinesterases (Türkez et al., 2007). It is defined as a glycoprotein because about 15.8% of the PON enzyme consists of carbohydrates. It has a cyclic structure due to interactions of disulfide bonds in the protein structure (Gan et al., 1991; La Du et al., 1999). PON enzyme, is integrated to HDL_2 and HDL_3 and hydrophobic nature of N-terminal of PON enzyme causes easy binding of HDL to lipids. The PON gene family has 3 members as PON_1, PON_2, and PON_3 (Primo-Parmo et al., 1996). PON_1 has antioxidant effects due to its LDL (low density lipoprotein) protective property from oxidation and the ability to neutralize many radicals. PON_1 has also been shown to play an important role in the metabolism of lipid peroxides by HDL (Gan et al., 1991).

There is no conclusive evidence that boron and its compounds, which are known to have many benefits in terms of protecting people's health, are essential elements. But due to the important role boron has in the metabolism and in regulation of many enzymes, bor element is included in trace elements and it is thought to be a probable essential element.

(Bauer & Pettersson, 1974; Cui et al., 2004; Hunt, 1998; Kettner & Shenvi, 1984; Tanaka & Fujiwara, 2008).

The aim of this study was to investigate the effects of boron supplements on some biochemical parameters such as Antioxidant System, Enzyme and HDL, levels and PON activity as an alternative treatment method in obesity.

2. MATERIAL AND METHOD

Material: Study material was 4-5 months old 40 Sprague Dawley rats with a mean live weight of 226,95 ± 5,75 g which were grown in Experimental Animals Laboratory of Kafkas University. The study was approved by the Ethics Committee of the Animal Experiments of Kafkas University (Decision no: KAÜ-HADEK: 2012.67).

Experimental Design**:** Animals were divided into 4 groups each containing 10 rats as follows; Group I (control; normal food and drinking water), Group II (high-fat diet), Group III received a high-fat diet and boric acid (5.72 mg/L H_3BO_3 (Sigma:B6768) dissolved in distilled water) containing 1mg/L boric acid in drinking water according to previously reported (Aysan et al., 2011). Group IV was fed with a normal diet and boric acid (H_3BO_3 in drinking water) containing a final concentration of 1 mg/L of boron. At the end of 6-week period, the intracardiac blood samples were collected into the serum tubes under ether anesthesia. Blood samples were centrifuged at 3000 rpm for 10 minutes and the serum samples were obtained. The samples were maintained at -20 °C until further analyses.

Biochemical Analyses**:** Serum total antioxidant status (TAS), total oxidant status (TOS) and HDL levels were determined using commerical kit respectivly (RelAssay, Gaziantep-Türkiye, Roche 657621). Serum PON activity was determined by a method which has previously been reported (Eckerson et al., 1983).

Statistical Analysis

Parametric tests were performed after it was determined that the data were parametric as a result of the Kolmogorov-Smirnov test. Statistical analyses were done using SPSS Windows 16.0 package program. Variance analysis (ANOVA) and Duncan test were used for statistical analysis of the data. The results are presented as mean ± standard error. Results with $P < 0.05$ were considered significant.

3. FINDINGS

Table 1. Content of feed used in the experiment of diets

Ingredients	(%)	The calorie composition of the experimental diets (%)		
Corn	60.50	**Contents of Nutrients**	**Normal**	**High-fat**
Gluten Meal	33.45	**Dry matter**	89.6	83.8
Vegetable fat	3.30	**Crude protein**	23.90	24.30
Marble dust	1.0	**Crude fat**	20.63	53.83
Dicalcium phosphate	0.5	**Crude cellulose**	7.17	7.12
Sodium chloride	0.5	**Crude ash**	8.43	8.37
DL-Methiyonine	0.10	**ME (kcal/kg^{-1})**	2600	5616
Lizin	0.15			
Vitamin-mineral premix[a]	0.5			

Mix supplied per 1.0 kg: 20,000,000 IU Vitamin A, 3000,000 IU Vitamin D3, 25 g Vitamin E, 4 g Vitamin B1, 8 g Vitamin B2, 5 g Vitamin B6, 20 mg Vitamin B12, 20 g nicotinamid, 12 g calcium-o-pantothenic acid, 200 g choline chloride, 50 g manganese, 50 g iron, 50 g zinc, 10 g copper, 0.8 g iodine, 0.15 g cobalt, 0.15 g selenium.

Table 2. The levels of TAS, TOS, HDL and PON activity in experimenteal groups

Parameters	Group I	Group II	Group III	Group IV	P
TAS (mmol TroloxEqv./L)	1,31±0,08^{a}	0,78±0,05^{b}	0,88±0,06^{b}	0,88±0,04^{b}	0,001
TOS (µmol H_2O_2Eqv./L)	3,5±0,24^{b}	4,74±0,60^{ba}	5,94±0,49^{a}	5,34±087^{ba}	0,063
PON (U/L)	50,43±7,45^{a}	26,96±5,21^{bc}	41,49±6,54^{ab}	21,66±5,17^{c}	0,009
HDL (mg/dL)	34±3,8^{b}	36±1,9^{ab}	44±2,61^{a}	43±2,48^{a}	0,036

Different superscript letters (a,b,c) within the same row indicate significant differences. Group I: Control, Group II :High-fat diet (**HF**), Group III: High-fat diet + Boric acid (**HF**+**BA**), Group IV: Boric acid (**BA**)

Table 3. Weight average of the groups by week (grams)

Weeks	Group 1	Group 2	Group 3	Group 4
1. week	230,8	232,8	223,4	220,8
2. week	240,1	367,5	258,7	230,1
3. week	230,4	266,3	257,8	220,4
4. week	217,7	270	265	207,2
5. week	222,1	272	284,87	212,1
6. week	225,8	285,44	288,88	215,8

Feed and water of the animals were given in equal amounts per hour at 24 hour intervals. Group weights were weighed weekly.

4. DISCUSSION AND CONCLUSION

The effects of boron on antioxidant oxidative stress is one of the current controversial issues. In addition, the lowering effects of boron compounds on body weight are promising for the problems caused by excessive weight(Atakisi et al., 2019; Fail et al., 1998). Increased oxidative stress with increased weight gain leads to an increase in the amount of free radicals and deterioration of homeostasis between free radicals and antioxidants in the body against antioxidants. Increased metabolic and mechanic workload on tissues increases oxygen consumption of these tissues and this increased oxygen consumption increases reactive oxygen species (ROS) and therefor amount of free radicals (Moylan & Reid, 2007; Roh & So, 2017).

In a study conducted by Ozata et al. (2002), there was a significant decrease in activity of erythrocyte selenium-dependent glutathione peroxidase (GSH-Px) and copper-zinc superoxide dismutase (Cu-Zn SOD) in obese subjects and erythrocyte TBARS (thiobarbituric acid reactive substance level) was increased significantly compared with the control group(Ozata et al., 2002; Roh & So, 2017). Our study showed increase in ROS amount and reduction in antioxidant system in the groups that had high-fat diet.

Farshad et al (2012) studied Cu-Zn SOD, GSH-Px, and catalase (CAT) activities in women with abdominal obesity and found decreased activities of Cu-Zn SOD, GSH-Px, and CAT activities compared with women who had normal body weights (Amirkhizi et al., 2014). Cu-Zn SOD, GSH-Px, and CAT enzymes act as first-line defense mechanisms in the body against ROS in the organism. Decreased activity of these enzymes is believed to be due to increased amount of oxidants or prooxidants. Decreased TAS amount in our study is thought to be due to increased TOS level due to oxidative stress produced by prooxidant or oxidant levels mediated by obesity.

Research about effects of boron and boron compounds on oxidative system has gained momentum during recent years. However this studies investigating the effect of boron supplements on the antioxidant system are often contradictory. (Garcia-Gonzalez et al., 1991; Ince et al., 2010; Türkez et al., 2007). A study about the effects of boron and boric acid on blood samples exposed to heavy-metals revealed that malonil dialdehyde (MDA) levels which were increased after exposure to heavy metals decreased with addition of 5 ppm boric acid. In addition, the authors reported increases in SOD, CAT, and GSH-Px activities which were decreased due to effects of heavy metals and boric acid might have antioxidant effects (Türkez

et al., 2007). Similarly, Kucukkurt vd. demonstrated that boron applications have positive results on the antioxidant system in mice showing arsenic toxicity(Kucukkurt et al., 2015). Sharma et al. reported that boron supplements have positive effects on Vitamin D level and antioxidant system(Sharma et al., 2020). Another study which explored effects of boric acid and borax on antioxidative system of rats reported that boron compounds decrease MDA level, which is a product of lipid peroxidation and increase GSH amount and CAT and SOD activities (Ince et al., 2010). TAS and TOS can be evaluated as cumulative markers of oxidative stress (Ghiselli et al., 2000) Türkez et al. showed in their studies that boron containing compounds increase the amount of TAS and decrease the amount of TOS(Türkez et al., 2007). Similar results were observed in the study by Cengiz et al. They reported that boron supplementation increased the levels of GSH, TAS, and decreased the levels of MDA and TOS(Cengiz et al., 2020).

In contrast to Ince (2013) could not find any effect of borax dehydrate on antioxidant system in the study which examined acute effects of different doses (100 mg/kg and 200 mg/kg) of borax dehydrate on antioxidant system (Ince et al., 2010). Gonzalez et al. studied *Anabaena sp.* PCC 7119[1] and found increased amounts of SOD and CAT enzymes in erythrocytes in boric acid insufficiency(Garcia-Gonzalez et al., 1991). Karabal et al. studied Anatolian and Hamidiye barleys by adding 5 mM and 10 mM boric acid to water. Compared with the control group MDA amount was higher in the study groups and MDA amount increased with increasing boric acid amounts. (Karabal et al., 2003).

Findings from our study demonstrated that antioxidant capacity decreased and oxidant capacity did not change after boric acid addition. This difference is thought to be due to dosage given to the patients and duration of the study.

Recent studies have demonstrated that boric acid might be effective in decreasing body weight (Atakisi et al., 2019; Fail et al., 1998; Yildiz et al., 2013). A study reported that addition of various doses (1200, 2500, 5000, 10000, 20000 ppm) of boric acid to drinking water decreased body weight and this decrease was positively correlated with dosage (Fail et al., 1998). Results of aforementioned studies are in parallel with our study. Weight of the group which took 1mg/L boric acid added to drinking water decreased during the study. But addition of boric acid with high-fat diet did not change body weight. These results support the notion that boric acid when used solely may decrease body weight.

This suggests that boric acid might have a direct or indirect effect on fat metabolism and boron compounds have been administered effectively in the control of obesity. β-catenin are positive effectors for Wnts. Wnt-10b-mediated Wnt signaling acts as a molecular switch that governs adipogenesis. By inhibition of Wnt signaling, differentiation of preadipocytes is inhibited through the adipogenic transcription factors CCAAT/enhancer binding protein a (C/EBPa) and peroxisome proliferator-activated receptor (PPARϕ). It has been reported that it can inhibit adipogenesis in this way(Prestwich & MacDougald, 2007; Ross et al., 2000). Studies have shown that boron can inhibit genes and proteins related to adipogenesis by inhibiting Wnt / β -catenin, Akt and other extracellular pathway (Doğan et al., 2017). In studies on lipid profile, it was reported that boron-containing diet caused a significant decrease in LDL and total colesterol (TC) levels, and a nonsignificant increase in HDL levels was observed. (Kuru et al., 2019) In another study in which similar results were obtained, it was emphasized that boron supplementation decreased LDL level and increased HDL level (Hall et al., 1989). It has also been shown by Başoğlu that borax applied in addition to foods affects the lipid profile. Insulin, serum glucose, and apolipoprotein-B levels decreased and lipidemia decreased in dogs over the 4 week of the study (Basoglu et al., 2000). Some studies show that boron has negative effect on the lipid profile. In a study on this subject 0.45 g/L boric acid solution was given to animals by adding 2 mg boron to drinking water. After 2 weeks, serum levels of HDL and triacylglycerol were decreased (Naghii & Samman, 1997). In our study, addition of boric acid increased HDL level.

PON is an enzyme responsible for the hydrolysis of organophosphate and statins found in HDL dependent. Recent developments have revealed its LDL and cholesterol lowering effect in cardiovascular disease (CVD). In addition, it has the ability to specifically reduce oxidized phospholipids. (Meneses et al., 2019). An in vitro study by Aviram A et al demonstrated that inhibition of PON enzyme by copper sulfate ($CuSO_4$) increased oxidation of HDL and caused elevation of MDA. In addition, HDL level was positively correlated with PON activity(Aviram et al., 1998). These findings suggest that PON enzyme might have protective effects like an antioxidant molecule.

Aslan et al. as a result of the study conducted by, it was reported that HDL and PON activity decreased when oxidative stress increased in obese individuals (Aslan et al., 2011). It shows a decrease in the activation of the PON enzyme, which is a strong antioxidative agent, with the oxidant load and advanced glycation reactions that occur in obesity(Cakir et al., 2018;

Cervellati et al., 2018; Yu et al., 2017). This may explain the decrease in PON activity measured in the high-fat diet group in our study. PON is a Ca^{+2} dependent enzyme. The thiol groups in its structure are the target site of many metal ions and can be inhibited especially by metals with high concentrations(Debord et al., 2003; Deveci et al., 2015; Gonzalvo et al., 1997). Inhibition of metal ions can explain the purpose of PON activity in boric acid days in our study. However, with the combination of boric acid and high fat diet, PON activity increased compared to the obese group. Alak et al. They reported that PON level and antioxidant effect of boric acid decreased at high doses, but increased PON level and antioxidant level at low doses. In the same study, they reported that myeloperoxidase (MPO) showed an inverse correlation with the PON enzyme(Alak et al., 2020). MPO, another HDL-dependent enzyme, is responsible for LDL oxidation and may lead to impaired activation of the PON enzyme(Y. Huang et al., 2013). co-execution of boric acid with a high-fat diet may also reduce boric acid absorption. In this case, the antioxidant effect of low-dose boric acid may cause increased PON activation, decreased MPO level, and decreased heavy metal load. This information may help explain the increase in PON observed in Group 3 compared to Group 2 and Group 4.

Although PON is an HDL-dependent antioxidant enzyme(Kunutsor et al., 2016), there are many parameters that affect its expression. therefore, it is not entirely correct to directly link its activation to HDL(Costa et al., 2005). further, some studies suggest that PON1 activity behaves independently of HDL(Cervellati et al., 2018; Mackness et al., 2003). In particular, the increase in BMI (body mass index) deteriorates the stabilization between PON and HDL. In this regard, Thomàs-Moyà et al. reported in their study that the main reason for the decrease in PON1 in obese individuals was not the decrease in HDL. instead, it suggests that the resulting obesity and oxidative stress have an effect on the PON level(Thomàs-Moyà et al., 2008). Morever most of the PON activity is found in HDL-3. In this case, another theory is that PON activity may be dependent on lower fractions of HDL(Pérez-Méndez et al., 2014). This information may help us to explain the PON enzyme's HDL independent results.

Changes in lipoprotein levels which have roles in body homeostasis may be seen by excessive weight gain. Olusi et al. showed that while MDA levels of people with high BMI increased, Cu-Zn SOD activity and HDL levels decreased.(Olusi, 2002). These results are consistent with our study. Similarly a study on 48 volunteers showed decrease in HDL with increasing BMI (Piva et al., 2011). Huang et al (2013) studied rats and gave normal forage to the control group and fatty diet (430 kcal/100 g) (%10 protein, %15 bacon, %10 sucrose) for

176 days. No difference was found in HDL levels between the control and the study groups (W. Huang et al., 2013). Despite previous studies that have shown decreased HDL levels we found increased HDL levels compared with the control group ($P<0.05$). HDL lowering effect of boric acid suggests that butter will be a better explanation for this subject. Studies on butter demonstrated that butter with high cis-9 and cis-11 conjugated linoleic acids increase HDL levels (de Almeida et al., 2014; Roy et al., 2007). This studies suggest that butter produced with traditional methods as a source of high-fat diet will not decrease HDL level and may even increase HDL level by trials using different amounts of butter.

In addition studies have shown a positive correlation between PON activity and HDL level (Aviram et al., 1998; Deveci et al., 2015). But, our findings suggest that HDL values increase and PON activity decrease in the groups taking high-fat diet and boric acid diet compared with the control group in contrast with the previous studies.

5. SUGGESTIONS

As a result, boric acid and high-fat diet did not have a positive effect on the antioxidant system, but they caused an increase in HDL level and a decrease in PON activity. A relative increase in PON activity was observed when rats were co-fed with boric acid and high fat. PON and HDL acted independently of each other. In addition, boric acid may be effective in lowering body weight. This study will provide up-to-date information to the literature in terms of a more comprehensive evaluation of the effect of boric acid on obesity and the antioxidant system.

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