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The Effect of Boric Acid on Tissue and Enzyme Activity in *Eisenia Fetida*

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Abstract

Our country is the leader in terms of boron richness in the world and boron compounds are used in numerous industrial and agricultural purposes. Although it has such a vast area of use, studies on its effects in living things are limited. The present experimental study was planned composing of 10 groups (each group contained 10 Eisenia fetida earthworms). The study included two control groups; negative control group (Group I), and positive control groups (Groups II, III and IV). Positive controls were administered three different doses of cadmium nitrate tetrahydrate. Groups V, VI and VII were exposed with three different doses of boric acid. At the end of the applications, conducted for 7 days at experimental habitats, intestinal epithelium and chlorogenic tissue's histopathology were evaluated by using light microscope. In transmission electron microscope (TEM) examinations, the cells taken form these tissues were assessed regarding to both mitochondrial and smooth endoplasmic reticulum (ER) structural changes with glycogen and lipid droplet accumulations. Activities of catalase (CAT) and glutathione peroxidase (GPx) enzymes were also examined by using native gel electrophoresis technique. The obtained findings revealed that the toxic effects of tested boron compound were higher than that of cadmium when only used in high amount. Boric acid was not toxic as much as cadmium.
Key words: *Boric acid, Earthworms, Histopatology, TEM, Antioxidant enzyme*

Özet

Ülkemiz bor zenginliği açısından dünyada lider konumdadır ve bor bileşikleri çok sayıda endüstriyel ve tarımsal amaçla kullanılmaktadır. Bu kadar geniş bir kullanım alanına sahip olmasına rağmen canlılar üzerindeki etkileri ile ilgili çalışmalar sınırlıdır. Mevcut deneysel çalışma 10 grup (her grupta 10 adet Eisenia fetida toprak solucanı) içerecek şekilde planlanmıştır. Çalışma iki kontrol grubunu içeriyordu; negatif kontrol grubu (Grup I) ve pozitif kontrol grupları (Grup II, III ve IV). Pozitif kontrollere üç farklı dozda kadmiyum nitrat tetrahidrat uygulandı. Grup V, VI ve VII, üç farklı dozda borik asit ile maruz bırakıldı. Deneysel habitatlarda 7 gün süreyle gerçekleştirilen uygulamaların sonunda ışık mikroskobu kullanılarak bağırsak epiteli ve klorojenik dokunun histopatolojisi değerlendirildi. Transmisyon elektron mikroskobu (TEM) incelemelerinde bu dokulardan alınan hücreler hem mitokondriyal hem de düz endoplazmik retikulum (ER) yapısal değişiklikleri ile glikojen ve lipid damlacıkları birikimleri açısından değerlendirildi. Katalaz (CAT) ve glutatyon peroksidaz (GPx) enzimlerinin aktiviteleri de doğal jel elektroforez tekniği kullanılarak incelendi. Elde edilen bulgular, test edilen bor bileşiğinin sadece yüksek miktarda kullanıldığında toksik etkilerinin kadmiyumdan daha yüksek olduğunu ortaya koymuştur. Borik asit, kadmiyum kadar zehirli değildi.
Anahtar keimeler: *Borik asit, Toprak solucanı, Histopatoloji, TEM, Antioksidant enzim*

INTRODUCTION

Boron element, not found freely in nature and generally oxygen-bound, is mostly found in the form of borate compounds (Çöl and Çöl, 2003). Boron compounds are widely distributed in various forms on earth (Yazbeck, et al., 2005). For it's broad usability area both as a raw material and product, investigating effects of boron compounds on living things have become necessary. The amount of boron required for the metabolism of each living species is different (Apostol and Zwiazek, 2004). It has been reported that boron compounds, which is an essential element for the growth, flowering and development of plants (WHO, 1998c; Armstrong, et al., 2002), may adversely affect the development when taken in excess (Nable, et al., 1997). Later, considering the studies on its effects on animals and humans, boron was included in the class of essential elements for human health by the World Health Organization (WHO) (WHO 1996). Today, the World Health Organization (WHO) have stated the daily safe boron intake to be between 1-13 mg and should not exceed 28 mg/day (Tepedelen, et al., 2016).

Boron intake in humans and animals occurs via consuming high boron content foods, and through drinking water or breathing (Yazbeck, et al., 2005). Almost all of the boron compounds are absorbed through the digestive and respiratory systems (Huel, et al., 2004). Various studies have shown boron to play an important roles in bone development (Aydın, et al., 2018), regulation of mineral and hormone metabolisms (Kurtoglu, et al., 2002), lipid metabolism, cell membrane functions (Şaylı, et al., 1998), defense system and enzyme activity (Korkmaz, et al., 2007).

Earthworms are considered to be indicator organisms when proving the bio-accumulation of chemical substances from within their living environments and determining its possible effects on other living things (OECD, 1984). Any heavy metal in the soil upon reaching a certain level, causes histopathological effects primarily on the skin and intestine of the earthworm (Lukkari, et al., 2004). Since these effects appear immediately in the Eisenia fetida species, they are used more in ecotoxicological studies (Reddy and Rao, 2008).

Scientific studies on the dose-dependent effects of boron compounds, highly found in some regions of our country and used in various important sectors, on living organisms are scarce. For this reason, the effects of boric acid in a dose-dependent manner compared to a heavy metal compound with known toxicities on the Eisenia fetida type earthworm, were investigated in our study.

MATERIALS AND METHODS

All experimental animals were collected from a thought to be pollution-free and far from traffic forested area with humus soil in Çanakkale Esenler.

Experimental Protocols

In our study, aqueous solutions of boric acid and Cd(NO3)2.4H2O (cadmium nitrate tetra hydrate) compounds were used. The doses used during the experiment were designed as given in Table 1, according to the OECD 207 "Soil Worm Acute Toxicity" (1984) protocol. Homogeneous distribution of the solutions was achieved by adding 20 mL each into 750 g soil in glass containers in accordance with the protocol. Only 20 mL of distilled water was added for negative control group. Ten (n=10) healthy-looking and of similar body sizes earthworms, each weighing (1.8±0.2) g, were randomly selected. Earthworms were then placed in glass containers and allowed to complete the 7-day acute toxicity experiment at room temperature.

Table 1.*Experimental groups and applied amounts of doses (n=10).*

Groups \ Doses (ppm)		--	1000	2000	3000	5000	6000	7000
Negative control	I	√						
Positive control $Cd(NO_3)_2.4H_2O$ (Cadmium nitrate tetrahydrate)	II		√					
	III			√				
	IV				√			
H_3BO_3 (boric acid)	V					√		
	VI						√	
	VII							√

Collection and Evaluation of Tissue Samples

After the experimental applications, the experimental animals belonging to all groups were kept in an environment containing moist filter paper for 24 hours in order to draw out their intestinal contents. At the end of the period, the experimental animals were divided into parts for studies on the determination of enzyme activity by light microscope and TEM examinations.

Histological and TEM Studies

After chemical fixation of the body part of the earthworms in the midgut, paraffin blocks were prepared. 5µ thick transverse histological sections were taken and stained with haematoxilin-eosin (H&E). It was examined under an Olympus brand CH40 light microscope. The sample cross-sections were photographed using the Spot Insight 3.2.0 model camera and the Spot Advanced 4.0.6 program.

Tissue samples were fixed in 4% glutaraldehyde and osmium tetroxide for TEM applications. After the dehydration processes, the tissues were embedded in pure araldite and blocked, and 60 nm thick sections were taken. Thin sections (Gao, et al., 2007) treated with 2% uranyl acetate and lead acetate were examined under a JEOL-JEM-1220 Transmission Electron Microscope and photographed with the aid of Olympus Megaview G2 camera.

Histopathological and cytological TEM findings in the form of "1; none availability, 2; less availability, 3; availability and 4; more availability" scores were used to compare all groups.

Determining Isozyme Activity

Tissues reserved for studies to determine enzyme activity were lysed with a homogenizer in 2-3 volumes/gram PO4 buffer. After centrifugation of homogenates at 5000g for 60 minutes in Eppendorf 5804 R centrifuge, the supernatant obtained was separated into polyethylene tubes and stored in a deep freezer at -80°C. Before starting the analysis, the protein content of the supernatants was determined by Lowry, et al. (1951) method.

Native gel electrophoresis

In determining the CAT activity, Woodbury et al. (1971) method was used in the study. After running the protein samples, the polyacrylamide gel was kept in 5 mM H2O2 substrate for enzyme activity and the gel was stained in a solution mixture consisting of 1% potassium ferric cyanide and 1% ferric chloride.

The method of Lin et al. (2002) was used to determine GPx activity. After running the protein samples on a polyacrylamide gel, 0.2 g of L-Glutathione and 8 µL of 30% H2O2 were put into 50 mL Tris-HCl buffer (pH 8.0) and kept in the prepared substrate. At the end of the enzyme activity, the gel was stained with a prepared dye solution containing 50 mL Tris-HCl buffer (pH 8.0), 0.025 g NBT (nitrobluetetrazolium) and 0.025 g PMS (phenosine metasulphate).

At the end of experiment, the visualization of the bands revealing the CAT and Gpx enzyme activities in the gel was performed using the Kodak Gel Logic 1500 Imaging System Gel imaging system, with the help of the Kodak Molecular Imaging Software package program, and the sectional areas formed due to the enzyme activities calculated.

RESULTS

Histopathological Evaluations

In our study, no pathology was observed in the histological sections of the non-treated negative control group (Group I) animals. It was determined that the skin, muscle and intestinal tissues of Group I animals were intact, and the microvilli and chlorogen tissue in the intestinal lumen were normal histologically (Figure 1.A).

The histological sections of the positive control groups, in which three different doses of cadmium nitrate tetrahydrate were applied, showed different appearances compared to Group I. In the intestinal epithelium of the earthworms belonging to Group II and Group III, disruptions in various areas of the intestinal epithelium, continuous interruptions in the villi and partial cell death were observed. The integrity of the intestinal epithelial cells that had partially lost their villi, and its surrounding chlorogen tissue was determined to be partially lost (Figure 1.B). Histologically, Group IV earthworm samples were observed to have impaired tissue integrity, vacuolization of the intestinal epithelial cells, lysis of the villi and increased membrane fragmentation. It was determined that the chlorogen tissue was damaged at a high rate (Figure 1.C).

Histological sections of earthworms belonging to Groups V, VI and VII, treated with 3 different doses of boric acid, were examined at the light microscope level. Tissue integrity was determined to be continuous in Group V except in some places in the intestinal epithelium. No significant damage was observed in the chlorogen tissue (Figure 1.D). In Group VI, disruption in the intestinal epithelium integrity, formation of evident spaces between the epithelial cells, villi fragmentation, and little damage to the chlorogen tissue were detected (Figure 1.E)., it was observed in Group VII, that most of the intestinal epithelial cells were atrophied, the cellular borders were lost and cell death occurred. But unlike in Group IV with the highest treated dose of cadmium nitrate tetrahydrate, lysis of the villi was less likely. Vacuolization appearances detected in Group IV were not observed in any of the boric acid groups. In addition, the chlorogen structure was undamaged in Group VII sections where the highest dose of boric acid was applied (Figure 1.F).

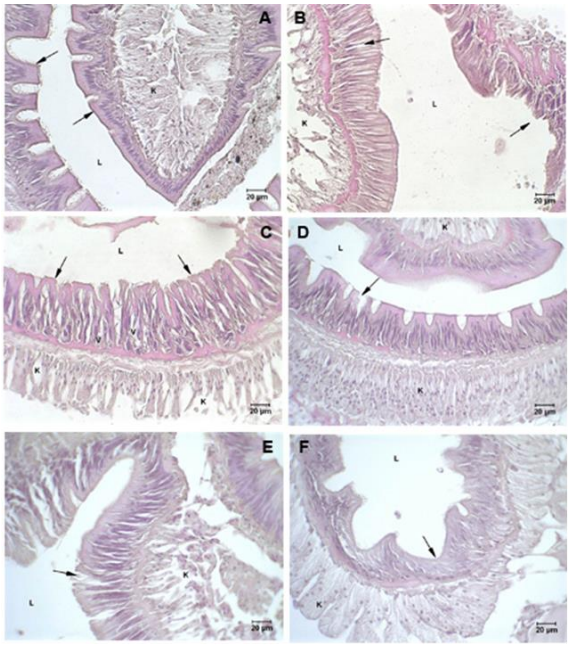

Figure 1. *Chlorogen tissue (K) and villi of the epithelial tissue (arrow) surrounding the intestinal lumen (L) in the transversal section of the experimental groups (V) (A: Group I; B: Group II-III; C: Group IV; D: Group V; E: Group VI; F: Group VII.*

TEM Evaluations

In the chlorogen tissue cells of Group I animals, it was determined that the inner and outer membrane structures of the mitochondria and ER appeared to be normal, and that glycogen particles were densely stored in their cytoplasm (Figure 2.A). It was determined that lipid droplets were also dense in the cytoplasm of the chlorocytes forming the chlorogen tissue (Figure 2.A).

Although normal mitochondrial membrane in intestinal epithelial cells and chlorocytes of Group II earthworms was seen, some of the mitochondria were morphologically elongated. Enlargement was detected in some regions of the ER lumens (Figure 2.B). A decrease was observed in the amount of lipid and glycogen accumulated in chlorocytes compared to Group I.

TEM examinations of animals belonging to Group III, showed morphological swelling and decrease in crystals despite the integrity of the mitochondria outer membrane (Figure 2.C). The enlargements observed in the ER lumen, lipid droplets, and the amount of glycogen were found to be similar to Group II.

In the TEM examinations of the earthworms belonging to Group IV, structural damage in the mitochondria was more detected than in Group II and III. the mitochondrial elongation and deformation of the crystals in some regions was also determined (Figure 2.D). ER was found to have a reticulated appearance (Figure 2.D). The amount of lipid droplets and glycogen in the chlorocytes was decreased compared to the applied doses of Groups II and III.

It was observed that the mitochondrial structures in the chlorogen tissue cells of Group V animals were generally compatible with normal morphology. And although the outer membrane integrity was preserved, there were regional losses in the cristae structures in some mitochondria (Figure 2.E). No deformations were found in the ER structures of animals belonging to this group. It was determined that while the content of lipid droplets remained the same as seen in Group I, the amount of glycogen particles was less.

Deformations in the mitochondria similar to that of Group III, were observed in the cells of Group VI and Group VII (Figure 2.F). However, cristae structure was determined to be preserved generally. The enlargements of the ER lumen were less common in both Groups VI and VII compared to cadmium nitrate tetrahydrate-treated groups. Glycogen content in the chlorocyte was found similar to Group IV, but the amount of lipid droplet was quite high (Figure 2.G).

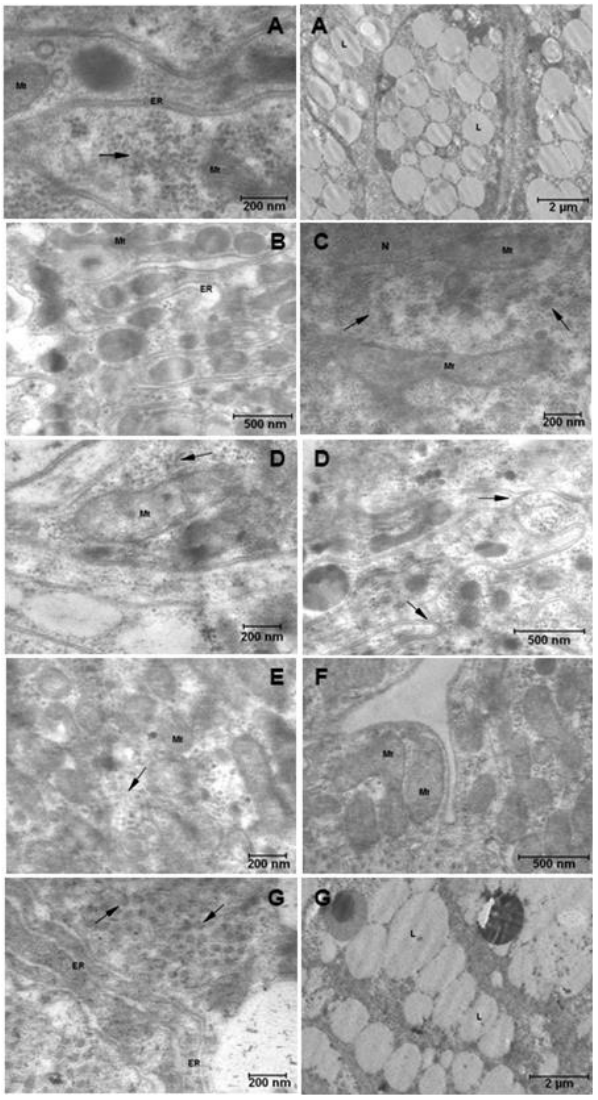

Figure 2. Glycogen particle (arrow), mitochondria (Mt), lipid (L), nucleus (N) and smooth endoplasmic reculum (ER) appearances in TEM micrographs of chlorogen tissue cells (chlorocyte) belonging to the experimental groups (A: Group I; B: Group II; C: Group III; D: Group IV; E: Group V; F: Group VI; G: Group VII).

Enzyme Activity Assessments

CAT enzyme activities in tissue samples were determined by calculating the band areas formed on the polyacrylamide gel (Table 2). CAT enzyme activities detected in Groups II, III and IV increased in a dose-dependent manner compared to Group I. Dose-dependent increase was also observed in the CAT enzyme activities of boric acid-treated experimental animals. It was found that CAT enzyme activity values increased compared to Group I, and Group VII had the widest band area with Groups V and VI, being very close to each other.

Activity bands of 5 isoforms of the GPx enzyme, named GPx1, GPx2, GPx3, GPx4 and GPx5, were determined (Table 2). It was determined that the band areas in Groups II, III and IV were similar to each other and considerably increased compared to Group I. A dose-dependent increase in GPx

enzyme activities was observed in the groups treated with boric acid.

Table 2. *Activity band area values of CAT and GPx enzymes isolated from earthworm samples belonging to experimental groups on polyacrylamide gel.*

Groups \ Band Areas (mm^2)		CAT	GPx1	GPx2	GPx3	GPx4	GPx5
Negative control	I	24,96	11,63	11,63	8,87	10,47	10,07
Positive control $Cd(NO_3)_2.4H_2O$ (Cadmium nitrate tetrahydrate)	II	30,36	18,27	18,20	13,41	7,72	16,55
	III	40,36	19,86	18,12	14,90	10,56	18,93
	IV	42,94	18,00	22,86	13,11	12,00	14,47
H_3BO_3 (boric acid)	V	35,63	18,77	19,58	12,38	10,81	13,90
	VI	35,73	19,96	22,24	12,74	17,21	13,41
	VII	45,32	21,45	18,30	12,89	20,36	20,11

DISCUSSION

Turkey ranks first among the countries in terms of abundant boron deposits in the world. With the wide use of boron compounds both as raw materials and products in our country and the world at large, our study intended to compare its effects on human health and other living things with that of heavy metal compounds. During the 7-day acute toxicity period, Eisenia fetida type earthworms were used in our study to investigate the possible dose-related histological, cytological and enzyme activities effects of cadmium nitrate tetrahydrate (1000, 2000 and 3000 ppm respectively Group II, III, IV), a heavy metal with known toxic effects and boric acid (5000, 6000, 7000 ppm respectively Group V, VI, VII) compounds as against the negative control group (Group I). Lukkari et al. (2004) and Gao, et al. (2007) also reported that earthworms are a biological indicator organisms that can give physiological and biochemical responses to metals or other chemicals. Therefore, it is also used as a bioindicator in chemical environmental pollution research (Mali, 2019).

Metal pollution in the soil has been reported to cause histopathological effects primarily on the skin and intestines of earthworms (Lukkari, et al., 2004). In our study, intestinal epithelial and chlorogen tissue damages were found to have increased in a dose-dependent manner in histological sections of the earthworm samples belonging to the positive control groups, in which three different doses of cadmium compound were applied. Amaral, et al., (2006) in their study of earthworms living in volcanic soils relating accumulation of zinc and cadmium in the soil to intestinal epithelium and chlorogen tissue damage, showed parallelity to our. Elyamine, et al., (2018) also showed that cadmium accumulation was associated with worm mortality in a study on the reduction of cadmium toxicity in earthworms by phenanthrene. Muthukaruppan and Gunesekaran (2010) observed that in a dose-dependent increase of butachlor, a herbicide, the intestinal villi of earthworms become lysed, chlorogen tissues deformed and cavities occurred in the tissue. The cadmium compound used in our study also caused tissue damage in a similar fashion (dose-dependent manner). The application times of the test substances were kept constant and different dose trials were made in some of the researches, as in our study too. On the other hand, it has been stated that damage to the intestines of earthworms (Morowati, 2000; Sharma and Satyanarayan, 2010) and muscle layer (Reddy and Rao, 2008) occur depending on time. Morowati (2000) detected partial membrane damage, nucleus lysis, cell death and cytoplasmic vacuolization in intestinal epithelial cells of earthworms in the first and second weeks of the application of glyphosate, a herbicide, but reported a decrease of the said damage in question via regeneration as the application continues into the third and fourth weeks.

Acute toxicity tests are usually the first step to assess the potential risk of chemicals on worms (Yu, et al., 2019). In our study, which was planned to detect acute toxicity, the administered doses of our test substances were close to the lethal dose, and are of the view that parallelism of their chronic effects with the aforementioned study could only be possible if the doses we applied were at much lower levels. Zhou et al. (2014) reported that both acute and subacute exposures of cadmium in invertebrates resulted in significant toxic effects on worms, which may be associated with increased concentrations in intracellular organelles with essential metabolic roles. On the other hand, we believe that the chemical structure of the test substances applied to earthworms and their relationship with biological macromolecules are also important. Sharma and Satyanarayan (2010), in their research on the effects of various heavy metals on earthworms at different application times and doses, detected tissue damage in the intestinal epithelium and villi as a result of applying a lower dose of cadmium salt for 100 days than used in our study. While these reported pathological cases were similar to Groups IV, VI and VII of our 7 days periodic study, the damage findings of the 60-day and 80-day experiment period were similar to Groups II, III and V in our study.

Özen, et al., (2009) investigated the effects of boric acid and sodium borate compounds and some heavy metal salts at fixed doses and different application times, similar to our study, on an aquatic indicator species, Limnodrilus hoffmeisteri. They emphasized that the degeneration increased over time in the intestinal epithelial cells and glandular cells of the living thing at the end of the research, and the cell damage was irreversible. The histopathological findings of our study are similar to this study. Although applied at the same times in our experiments, the degree of tissue damage caused by the cadmium compound was obserced at higher doses of the boron compounds. In the histopathological examinations, the pathological findings observed in Group V were observed to be parallel to Group II, and partial tissue and intestinal epithelial deformation, and losses in some regions of the villi occurred. In Groups VI and VII, a dose-dependent increase was observed to be directly proportional to the degree of these damages. On the other hand, tissue and cell damage caused by 3000 ppm cadmium compound was also found at 7000 ppm, the highest dose of boric acid. Since both intestinal epithelium and chlorogen tissue damage are much less, it can be said that the toxic effects of boric acid are less in terms of histopathology.

Living organisms use two ways to protect themselves from heavy metals and similar toxic chemicals in their living environments. The first of these is to prevent toxic components from entering the body by reducing food consumption, and the second is to ensure the detoxification of toxic substances entering the body (Maryański, et al., 2002). Mitochondria and the SER responsible for detoxification are the leading organelles affected by a chemical substance taken into the body in the organism (Gao, et al., 2007). Mitochondria are involved in all essential metabolic processes such as electron transport, oxidative phosphorylation, degradation of fatty acids, and amino acid metabolism (Reichert and Neupert 2002). Therefore, mitochondria are considered to be the most sensitive organelles against chemical poisoning, and it is mentioned that the damage that may occur in their structure or functions will cause the energy required for the cell to not be met and eventually lead to the death of the cell (Yongcan, et al., 1998).

In our study, in the TEM examinations of animals belonging to Group II and Group III to which cadmium nitrate tetra hydrate compound was applied, it was determined that the structural deteriorations in the mitochondria of intestinal epithelial cells and chlorocytes of earthworms increased more in Group IV and crystals were lost. Having studied the effects of metal pollution on intestinal cells of earthworms, Yongcan, et al. (1998), similar to our findings, reported that the space

between the inner and outer membranes of mitochondria increased, the cristae structures deteriorated, and the mitochondria completely lost their cristae structure with the increase in pollution. In another study, Hu, et al. (2010) examined the toxic effects of titanium and zinc heavy metal compounds in earthworms and reported the abnormalities seen in the mitochondria of our study. See, et al. (2010) reported that boric acid damages some parts of the mitochondria, and that the damage and the absence of metabolites required for ATP may have a detrimental effect on cell life and functions. In our study, it was determined that mitochondrial damage increased in the positive control and boric acid groups in a dose-dependent manner.

Testing two different doses of albendazole in earthworms, Gao, et al. (2007) stated that they observed a dose-dependent gradual degeneration in ERs as well as mitochondria. These researchers mentioned that high doses of DERs exhibit a pathological appearance with a reticulated appearance, as well as enlargement of the ER lumens. In our study, the reticulate appearance of the ER in the TEM examinations of the earthworms belonging to Group IV reveals the toxic feature of the cadmium compound. In addition, the expansions detected in some parts of the SER lumens of Group II and III animals were not observed in boric acid groups. Gao, et al. (2007) showed advanced detoxification events as the cause of the widening they observed in the SER lumen and argued that the extraordinary biochemical processes resulting from this were accompanied by mitochondrial damage.

While organisms use most of their energy in growth, reproduction and basic metabolic pathways, they also use it to remove toxic substances from their bodies or for detoxification. Thus, changes occur in energy metabolism. It has been reported that the energy stores of organisms are glycogen, fat and protein (Maryański, et al., 2002; Holmstrup, et al., 2011), and that glycogen is especially abundant in the chlorogen tissue, where basic detoxification processes take place, in studies conducted in earthworms (Adamowicz, 2005). Holmstrup et al. (2011) reported a link between metal compounds and a reduced amount of glycogen in the body of earthworms taken from areas contaminated with these compounds. Consistent with the previous studies, in our study, there was a simultaneous decrease in the amount of lipid and glycogen, which accumulates especially in chlorocyte, in Groups II, III and IV, respectively, compared to Group I. It is possible to think that detoxification accelerates according to the SER damage observed in these groups. With the knowledge that organisms primarily use carbohydrates and then fat and proteins as energy sources, it is important that both glycogen and fat catabolism increase in positive control groups in parallel with mitochondrial damage. There may also be problems in energy production as a result of mitochondrial damage. In this case, cells trying to survive may have used the anaerobic phosphorylation pathway independently of mitochondria to generate energy. Considering the epithelial tissue and villi damage, perhaps food consumption may be reduced, as stated by Maryański, et al., (2002). However, it would be more accurate to associate these possibilities with the detoxification of toxic substances entering the body, not with the tissue damage in our study. Because the decreased nutrient intake associated with epithelial tissue and villi damage and on the other hand, the increased energy requirement for detoxification may have resulted in a decrease in the amount of lipid and glycogen, and it can also be considered as the reason for the decrease in body weights of the positive control groups at the end of the experimental period.

The fact that the amount of lipid droplets was similar to Group I and the amount of glycogen decreased in a dose-dependent manner in all boric acid-treated groups (Groups V, VI, VII), showed that the toxic effect was less than that of the cadmium compound. It can be thought that the energy required during detoxification can only be met from glycogen stores and therefore there is no need for fat catabolism. Geyikoğlu and Turkez (2007) suggested in their study that boric acid reduces the production of ATP in the muscles and causes a decrease in the concentrations of some metabolites such as glucose, glycogen and lactate.

Earthworms are in constant contact with all chemicals or heavy metals in their environment. Therefore, it has been reported that there is an important relationship between the poisoning of earthworms by substances and the formation of free radicals, the development of defense systems against radicals and the glutathione mechanism (Laszczyca, et al., 2004). Valko, et al. (2006) explained that free radicals can cause damage to all important elements of cells such as nucleic acids, lipids, proteins, enzymes and carbohydrates. Hu et al. (2010) emphasized that enzymes that protect the cell against the negative effects of free oxygen radicals and take part in the antioxidant defense system are a good determinant parameter. In recent studies on earthworms, CAT and GPx activities of metals have been emphasized and it has been suggested that these two enzymes can be used as potential markers in antioxidant defense systems (Saint-Denis, et al., 1998).

In our study, in which different doses of cadmium nitrate tetra hydrate and boric acid compounds were applied, a dose-related increase was detected in the positive control and boric acid groups, per the activities of CAT and GPx enzymes of Group I animals. Zhang, et al. (2009) reported an increased in a dose-dependent manner of CAT activity in earthworms treated with cadmium for 48 hours. In another study conducted on an aquatic species, Tubifex tubifex, CAT enzyme activity at the 48th hour, reportedly increased by heavy metals cadmium and iron metals, but when a mixture of iron and cadmium metals was given to the experimental animals, the enzyme activity increased after 2 days (Dhainaut and Scaps, 2001). In another study using tubifex species, an increase in the activity of CAT and glutathione reductase enzymes was observed with increasing fenhexamid dose (Mosleh, et al., 2005). In one study, attention was drawn to the increase in GPx enzyme activity towards heavy metal-containing regions (Laszczyca, et al. 2004), and further stated that CAT and GPx enzyme activities change depending on seasonal changes, temperature and pH, as well as environmental pollution (Laszczyca, et al., 2004).

According to Mercan (2004), metal ions can react with superoxide anions and H2O2 to form OH· radical in cells through to the Fenton and Haber-Weiss reactions. Additionally, free oxygen radicals occurring in the cells may be the main cause of intestinal epithelial damage, villi damage, and damage caused by increased intercellular spaces, histopathologically detected in the experimental groups of our study. Flora, et al. (2008), in emphasizing the resulting effect of the free radicals and metal toxicities in disrupting antioxidant defense systems and damaging macromolecules within the cell, also supports this idea of ours.

The findings obtained within the scope of the materials and methods used in our experimental study showed that the boric acid compound cannot be as toxic as a heavy metal compound, on earthworms when in contact within the habitat. For this reason, in the future planning of similar studies, investigation of boron compounds at comparatively different doses and in different species of living organisms using more molecular level parameters, is foreseen to be key to the more efficient and productive use of this substance and for our boron-rich country.

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