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Histopathological Changes on Testes, Liver, Kidney and Brain Tissues in Acute Boric Acid Administration

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Introduction: In recent years as a result of the observation that the toxic effects of boron and its products have increased intensive studies have been initiated in our country and in the world regarding its effects, especially in the central nervous system, digestive system and reproductive system. The aim was to determine the histopathological changes caused by boric acid in rat testis, liver, kidney and brain tissues by light microscopy after oral administration of toxic dose of acute boric acid.
Material and Methods: In the study, 1000 mg/kg/day boric acid was given orally for 7 days to 12-week-old 30 male albino SpragueDawley rats in total with an average weight of 285 g. Twelve male albino Sprague-Dawley rats of approximately the same weight and age were used as controls. At the end of the seventh day testes, liver, kidney and brain tissues were isolated from the animals.
Results: At the end of the experiment, it was determined that the experimental group had significant body weight loss compared to the control group. Likewise, testicular, liver and kidney

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weights of the experimental group were decreased compared to the controls. In the histopathological examination performed with light microscopy in the testis, liver, kidney and brain tissues taken, congestion in the vascular bed of the testicular tissue and cellular degeneration at different rates were observed in paraffin sections and semi-thin sections.
Conclusion: It was observed that acute boric acid administration, together with its widespread toxic effect, caused histopathological changes by inhibiting spermatogenesis, especially in testicular tissue.

Keywords: Acute toxicity; boron; boric acid; tissue; testis.

1. INTRODUCTION

Boron which important compounds are boric acid and borax is a nonmetallic element that occurs in nature bound to oxygen. Its simplest compounds are boron oxide (B_2O_3) and boric acid (H_3BO_3) [1-5].Although it is known that boron and its compounds play an important role in biological processes and are necessary for the growth functions of plants animals and humans, the net effects of boron and its compounds on the human organism are still controversial today [1].

In studies on animals, borax and boric acid produces an acute toxicity schedule with depression, ataxic movements, convulsions and death [2,3]. As a result of histopathological examination of tissues exposed to toxic doses of boric acid; as the first effect, spermiogenesis is halted, followed by loss of germ cells, then Sertoli cell loss in the following stage, and results reaching testicular atrophy in a short period of 10-14 days [4,5].

The subacute and chronic effects of boric acid were mostly studied on mice and rats. As a result of oral subacute effect in mice, hyperplasia in the fore stomach, extramedullary hyperplasia in the spleen, testicular degeneration and atrophy; in addition as a result of the chronic effect, it has been observed that it causes growth and development anomalies and tumors in the hepatic and subcutaneous tissue [1,6]. When boron-containing compounds are added to daily diet and drinking water (500-5000 ppm boron), it has been shown to cause reproductive toxicity in the male rat in the subchronic and chronic stages [7,8]. Acute oral toxicity of boric acid in rats develops at the level of 3.5-4g/kg [5-7].

Similarly, it has been reported that boron and its compounds lead to decreased ovulation in females [9,10]. It has been pointed out that the use of boric acid as a topical antimicrobial agent in pregnant women may cause many congenital anomalies, especially congenital cataracts. Anomalies were also observed in rat embryos, especially in the eyes, brain ventricles, central nervous system, cardiovascular system and axial skeleton [9,11]. In this study, it was aimed to determine the histopathological changes caused by boric acid in rat testis, liver, kidney and brain tissues under light microscope after acute oral boric acid administration.

2. METHODS

2.1 Study Place

This randomized controlled study was carried out in Eskişehir Osmangazi University Faculty of Medicine Medical and Surgical Experimental Research Center and pathology laboratories.

2.2 Procedures

In this study, 42 Sprague-Dawley type adult male albino rats weighing approximately 285g and 12 weeks old were used. The animals to be treated were brought to the animal room at least one week before the experiments. For the experiments, the animals were kept in groups of three in comfortable, glass-enclosed cages. The rats in the control group were also exposed to the same conditions as the treatment group. 1000mg/kg/day boric acid was added to the drinking water of 30 rats in the application group and dissolved in water, and they were allowed to drink for 7 days [12].

Drinking water was replenished at the same time every morning. The daily weight loss of the animals in the treatment group during daily boric acid administration was taken into account. At the end of the specified time, the rats included in the control and administration groups were stunned under ether anesthesia and tissue isolation was performed. Histopathological

examination was performed on the tissues taken, following the measurement of their weight.

Testicular tissue samples from the taken tissues were kept in Bouin's Solution for 48 hours, then rinsed in 70% alcohol for 5 hours, and the brain tissue was kept in 10% Neutral Buffer Formalin (NBF) for 48 hours; liver and kidney tissues were determined by keeping them in 10% NBF for 24 hours and were included in the follow-up method. Tissues were embedded in plastic cassettes. 2-3µm thick sections were taken from the prepared paraffin blocks in the microtome device. The sections taken were placed on slides, opening in a water bath (37 0 C).Tissue sections on the slide were stained with Hematoxylin Eosin stain [13]. Eosin was used to stain the cytoplasm, and Hematoxylin to stain the nuclei. Since the fixation and staining times of the tissue samples were different, the tissue color tones were also different. The tissue was closed with a coverslip by dripping 2 drops of etellane on the tissue. The obtained preparations were examined under a photomicroscope and their photographs were taken.

2.3 Statistical Analysis

The data were determined in SPSS 12.0. Fisher's exact chi square, Yates chi square, Independent symptoms t test, paired symptoms t test AND Mean±SD were used for statistical analysis. A value of $p<0.05$ was considered statistically significant.

3. RESULTS

At the beginning of the experiment, there was no difference between the weights of the control (311.833±49.696) and treatment groups (305.266±22.578) ($p>0.05$). At the end of the experiment, a statistically significant difference was found between the control (320.333±50.931) and the treatment groups (265.533±29.192) in terms of mean body weights (**p<0.01**). At the end of the experiment, a statistically significant difference was found between the control (3.033±0.362) and treatment groups (2.426±0.623) in terms of testicular weight (**p<0.01**). At the end of the experiment, a significant difference was found between the liver weights of the control (8.144±0.873) and treatment groups (7.428±0.719) (**p<0.01**). At the end of the experiment, a significant difference was found between the kidney weights of the control (1.956±0.185) and treatment groups (1.708±0.317) (**p<0.01**). At the end of the experiment, a statistically significant difference was found between the control (2.042±0.171) and the treatment groups (1.846±0.277) in terms of brain weight (**p<0.01**). As a result of the comparison of the weights of the control group at the beginning (311.833±49.696) and the end of the experiment (320.333±50.931), a very high difference was found (**p<0.001**). In the application group, a very high difference was found as a result of the comparison of the weights at the beginning (305.266±22.578) and the end (265.533±29.192) of the experiment (**p<0.001**). The results are summarized in Table 1.

The clinical findings of boric acid exposure in the rat at the end of the experiment are summarized in Table 2. While water intake increased in the first 1-2 days, it gradually decreased in the other days. While the desire to drink water did not change in the first 1-2 days, it increased in the 3-4th days, decreased in the 5-6th days, and showed a severe decrease in the 7th day. While nutrient intake increased in the first 1-2 days, it decreased on the 3-4th day, and after the 5th day it gradually increased. While physical activity did not change in the first 1-2 days, it increased in the 3-4th days, decreased in the 5-6th days and decreased significantly on the 7th day. While body weight did not change in the first 1-2 days. After the 3rd day, it gradually decreased. Congestion in the nasal-oral mucosa and conjunctiva, yellowing of the nape hair, and postural change were absent in the first 4th day, but increased from the 5th day. Ataxic movements were not observed in the first 6 days, but started to be seen on the 7th day.

The histopathological changes observed in rat testis, liver, kidney and brain tissue at the end of the experiment are given in Table 3 and Fig. 1. In the application group, edema in the interstitial area in the testis tissue, increase in Leydig cells, basal membrane thickening in the seminiferous tubules; inflammatory cell infiltration in the Interstitium in kidney tissue; edema rate in the brain tissue was statistically higher than the control group (**p<0.001**). Apoptotic cells in testicular tissue were higher in the treatment group than in the control group (**p<0.05**). Other histopathological findings did not differ between the treatment and control groups ($p>0.05$).

Table 1. Body, testis, kidney, liver, and brain weights [(mean ±standard deviation (SD)] of the control and treatment groups at the end of the experiment

Measured parameter	Control group (n=12) Mean±SD	Application group (n=30) Mean±SD	Statistical analysis (p)
Body weight	320.333±50.931	265.533±29.192	**<0.01**
Testicular weight	3.033±0.362	2.426±0.623	**<0.001**
Liver weight	8.144±0.873	7.428±0.719	**<0.01**
Kidney weight	1.956±0.185	1.708±0.317	**<0.01**
Brain weight	2.042±0.171	1.708±0.317	**<0.01**

Table 2. Clinical findings of boric acid exposure in the rat

Clinical findings	Exposure days			
	1-2	**3-4**	**5-6**	**7**
Water intake	↑	↓↓	↓↓	↓↓↓
Desire to drink water	N	↑	↓	↓↓↓
Nutrient intake	↑	↓	↓↓	↓↓↓
Physical activity	N	↑	↓	↓↓↓
Body weight	N	↓	↓↓	↓↓↓
Congestion in the nose, oral mucosa and conjunctiva	-	-	↑	↑↑
Yellowing of nape hair	-	-	↑	↑↑
Posture change	-	-	↑	↑↑
Ataxic movements	-	-	-	↑

Table 3. Histopathological changes observed in rat testis, liver, kidney and brain tissue at the end of the experiment

Histopathological findings observed in rat testis tissue	Control group	Application group	Statistical analysis (p)
Fibrosis in the interstitial space	No	No	-
Inflammatory cell infiltration in the interstitial space	No	No	-
Edema in the interstitial area	No	Yes in 97%	**<0.001**
increase in Leydig cells	No	Yes in 67%	**<0.001**
Increase in Sertoli cells	No	No	-
Basal membrane thickening in the seminiferous tubules	No	Yes in 44%	**<0.001**
Degeneration in seminiferous tubules	No	Yes in 87%	**<0.001**
Stopping in spermatogenesis	No	Yes in 43%	**<0.01**
Apoptotic cells	No	Yes in 33%	**<0.05**
Multinuclear giant cells	No	No	-
Cell debris in the seminiferous tubules	No	No	-
Debris in epididymal ducts	No	No	-
focal atrophy	No	Yes in 53%	**<0.01**
Diffuse atrophy	No	Yes in 3%	>0.05
Histopathological findings observed in rat kidney tissue			
Acute tubular necrosis	No	Yes in 30%	>0.05
Tubular atrophy	No	No	-
Glomerular pathology	No	No	-

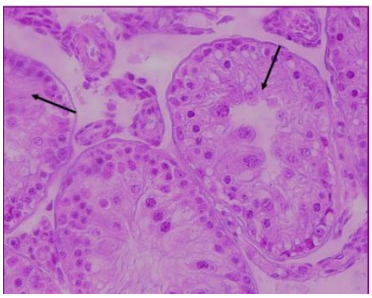
Histopathological findings observed in rat testis tissue	Control group	Application group	Statistical analysis (p)
Vascular pathology	No	No	-
Inflammatory cell infiltration in the interstitium	No	Yes in 67%	**<0.001**
Interstitial edema	No	Yes in 27%	>0.05
Interstitial fibrosis	No	No	-
Histopathological findings observed in rat liver tissue			
Congestion	No	No	-
Hydropic degeneration in hepatocytes	No	No	-
Sinusoidal dilation	No	Yes in 27%	>0.05
Single cell necrosis	No	Yes in 27%	>0.05
focal necrosis	No	Yes in 20%	>0.05
Diffuse necrosis	No	No	-
Inflammatory cell infiltration in the portal area	No	Yes in 17%	>0.05
Histopathological findings observed in rat brain tissue			
Congestion	No	Yes in 27%	>0.05
Edema	No	Yes in 87%	**<0.001**
Degeneration in neurons	No	Yes in 33%	>0.05
Apoptosis in neurons	No	Yes in 27%	>0.05
Necrosis	No	No	-

4. DISCUSSION

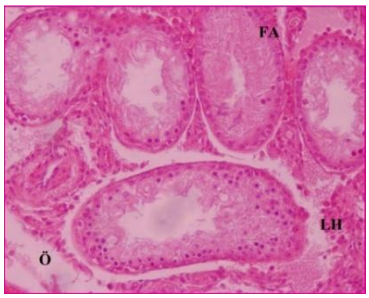
In our study, decrease in body weight (**p<0.01**), decrease in appetite, skin and body posture changes during 7 days of exposure to boric acid support the findings of Weir and Fisher [14]. In the presented study; the control group showed the expected body weight gain (**p<0.001**) at the end of the 7th day. Parallel to the decrease in appetite in the experimental group, the weight loss observed as a result of the food intake, which started from the 3rd day of the boric acid intake and gradually decreased on the 5-6th days and ended completely on the 7th day, was consistent with many studies [7,15]. Although the water and food intake of the experimental group increased in the first 2 days of the study compared to the controls, the decrease in the body weight of the subjects from the 3rd day suggested that the organism was faced with a widespread toxicity.

Since the third day of the experiment, the decrease in food and especially water intake, the fact that the subjects who could not drink water from their normal drinkers could not even drink the water dripped into the cage, but could drink the water dripped into their mouths showed that it was difficult to find the place of the water, that is, disorientation occurred. This revealed the presence of toxicity that also affects the central nervous system. In the presented study, the changes that occurred especially after the 3rd day were compatible with the literature and supported our opinion [1,16]. For example, physical activity, which increased in the first 2 days, started to decrease from the 3rd day, and this decrease and the change in body postures became very evident on the 5th day. In addition, water intake started to decrease from the 3rd day and almost completely disappeared by the 4th and 5th days. In addition to decreased food intake, a number of central nervous system findings have emerged, ranging from disorientation to confusion and ataxic movements.

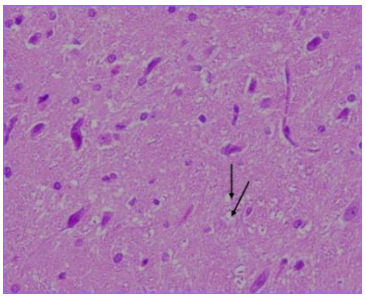
The half-life of boric acid in the organism is about 21 hours. When given orally, 95% is excreted through the excretory system. In case of acute boric acid 56 administration; boron, which rapidly settles in bone, brain, liver, kidney and testis tissues, is eliminated through a homeostatic mechanism that is not yet known today [17]. As a result of acute oral administration, although the boron concentration in the liver tissue reaches 6 times values compared to the controls, it has been shown by various researchers that this rate is up to 10-15 in the kidney tissue [4,12]. As can be seen, boron tends to disperse with all body fluids as a borate anion [4]. Therefore, the dimensions of boron toxicity include all tissues. As a matter of fact, in our study, testis, liver, kidney and brain tissues were affected by boric



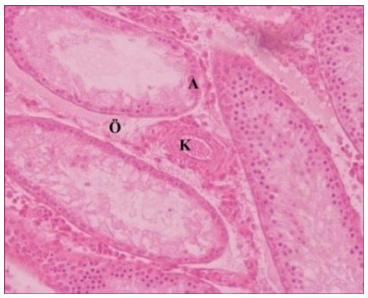
Pause in spermatonese in testis (arrows)



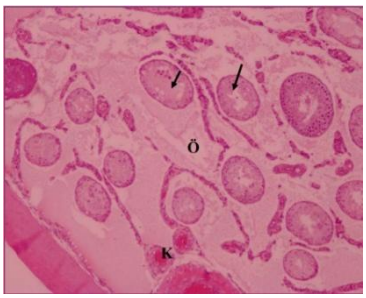
Focal atrophy (FA), Edema (Ö), Leydig Cell increase (LH) in testis



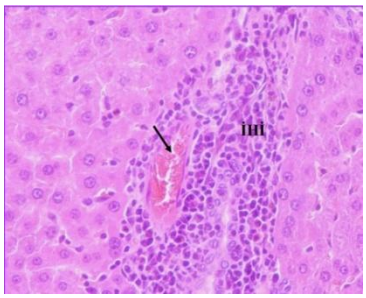
Edema (arrows) in the brain neurons



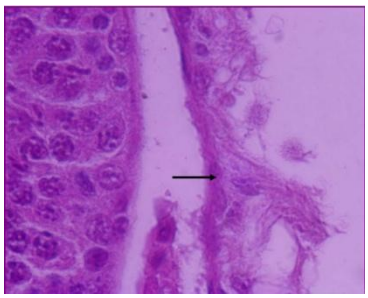
Atrophy (A), Edema (Ö), Congestion (K) in testis



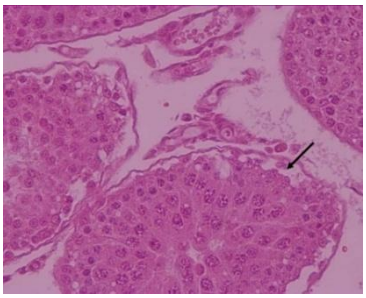
Vessel sections and congestion (K), pause in spermatogenesis (arrows) and edema (O) in testis



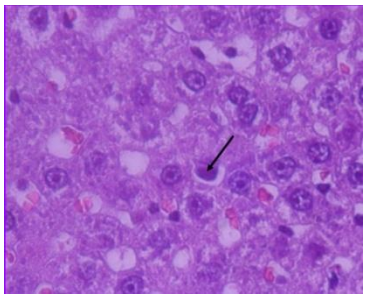
Inflammatory cell infiltration (IHI), congestion (arrow) in the liver portal area



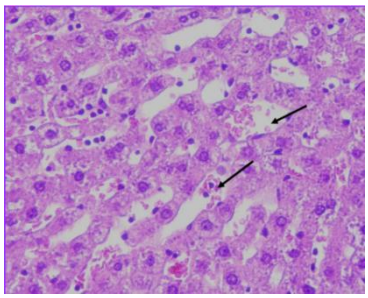
Basal membrane thickening (arrow) in testis



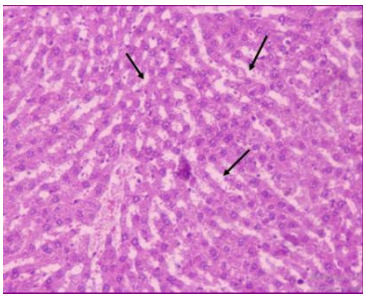
Separation of spermatogonia from basal lamina in testis (arrow)



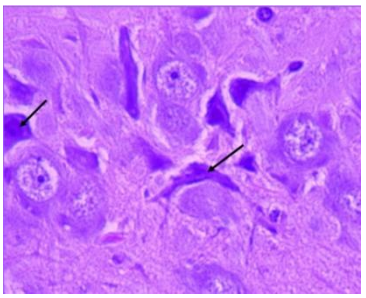
Single cell necrosis (arrow) in the liver



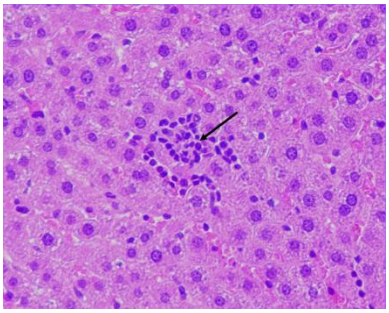
Sinusoidal dilatation and congestion in the liver (arrows)



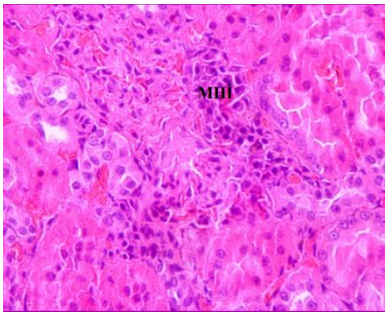
Sinusoidal dilatation (arrows) in the liver



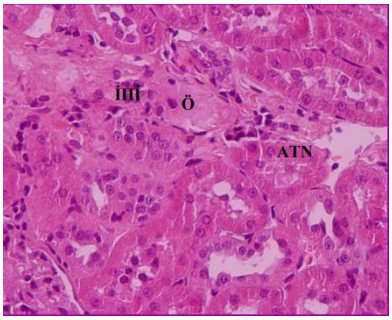
Degeneration of brain neurons (arrows)



Inflammatory cells and focal necrosis (arrow) in the liver.



Mononuclear cell infiltration (lymphoplasmocytic: inflammation containing lymphocytes & plasma cells. MHI) in the kidney



In acute tubular necrosis (ATN), inflammatory cell infiltration (IHI), edema (O) in the kidney.

Fig. 1. Histopathological changes observed in rat testis, liver, kidney and brain tissue in the application group

acid at varying rates. It is thought that the age of the experimental animal, food and water intake also have a share in this effect rate. In a similar study conducted with 8-week-old rats in the form of oral administration of boric acid at a dose of 1 g/kg per day, no remarkable feature was observed in terms of Sertoli and Leydig cells, but unusual mast cell accumulation in the testicular tissue and congestion in general were found to be very advanced [12,18]. In the histopathology of testicular tissue examined in our study, it was observed that spermatogenesis showed significant degeneration. Again, in accordance with the literature data, congestion was observed and a minimal increase in Leydig cells was also noted.

Studies carried out in recent years; As a result, it has been suggested that boric acid is a reproductive system toxicant. Acute oral toxicity of boric acid in rats develops at the level of 3.5-4g/kg. Acute oral toxicity occurs in the presence of 3.45g/kg boric acid in male Sprague-Dawley rats and 4.08g/kg in female rats. Borax and boric acid cause an acute toxicity picture with depression, ataxia, convulsions and death [1,14]. The fatal results of boric acid toxicity, which was first described in a group of infants in 1881-1887, were proven by autopsy findings. Accordingly, edema and congestion involving the brain and meninges in the central nervous system, diffuse perivascular hemorrhage in the medulla, midbrain, cerebellum, corpus striatum, tuber sinereum and lateral hypothalamus have been intensely noted [19]. As can be seen, acute boric acid toxicity spreads widely in the central nervous system and can eventually lead to fatal outcome with disorientation, confusion, ataxic movements and finally coma [3,12]. In the histopathological examinations of the brain tissues taken as a sample in our study, edema was observed in the neurons in accordance with the literature, and degeneration and congestion were observed in the neurons in a very small part of the subjects.

As a result of the histopathological examination of the tissues exposed to boric acid at toxic doses, the first effect is inhibition of spermiogenesis, followed by the loss of germ cells, followed by the loss of Sertoli cells and in a short period of 10-14 days, results up to testicular atrophy [4,20]. Our study results were in agreement with this literature information. As the prelethal symptom of acute boric acid toxicity, many investigators accept the red-violet discoloration that develops in the mucous membranes. With the transition of the acute stage to the subchronic stage, discoloration of the mucous membranes of the mouth and nose, yellowing and hunching of the nape hair, as well as inflammation of the eyes, edema of the paws and peeling of the skin of the tail were detected [1,14,21]. In our study, in the experimental group, on the fifth and sixth days, congestion in the oral and nasal mucosa, yellowish color change in the nape hair and a change in body posture in the form of hunching were observed, and these findings increased significantly on the seventh day. These signs and symptoms were interpreted by Massie [22] as premature aging.

The results of studies on rats showed that testicular lesions began to appear from the seventh day of boric acid administration and inhibition of spermiogenesis developed [6,9]. In the light microscopy examination of our study, when the experimental group and the control group were compared, significant degeneration was observed in many areas at different stages of spermatogenesis. These findings were found to be compatible with the literature data.

In the present study, focal atrophy was observed in most of the testicular tissues examined, and diffuse atrophy was observed in a very small part. In the treatment group rats, spermatogenesis was observed to continue close to normal in most of the seminiferous tubules, but in some of them, it was determined that there was a pause in spermatogenesis at varying rates. In addition, it was observed that spermatogonia were separated from the basal lamina below in the seminiferous tubules. Basal membrane thickening was detected in some of the seminiferous tubules, and excessive degeneration was noted in the tubules. These results were consistent with the studies of other reserchers: Treinen and Chapin [23] suggested that they found a decrease in serum testosterone levels in rats exposed to boric acid 59. It can be thought that the administration of boric acid may cause the formation of tubulobulbar complex by decreasing the serum testosterone level and, therefore, the arrest of spermiogenesis.

More than 95% of the absorbed boron is excreted by the kidneys [9,24]. Therefore, kidneys are also exposed to boron and its compounds. In histological studies, increased renal tubular dilation was detected in the kidneys of the mother rat as a result of boron administration to pregnant rats [9,20]. As a result of our study, acute tubular necrosis, characterized by an increase in acidophilic (pink) staining in the tubular cytoplasm and a decrease in the number of nuclei, was observed in a very few samples in the kidney tissues of the rats in the administration group, and inflammatory cell infiltration and edema were observed in the interstitium.

It has been shown that boric acid administration can impair nucleic acid synthesis in rat liver cells [1,25]. In our study, when compared with the control group, focal necrosis, sinusoidal dilatation and single cell necrosis were observed in some of the liver sections of the application group. In addition, inflammatory cell infiltration in the portal area has also attracted attention in very few samples. Congestion was detected in the livers of rats in the administration group.

5. CONCLUSION

In the presented study, it was determined that acute boric acid administration caused toxic effects on metabolism, central nervous system and reproductive system in experimental animals and it was seen that the findings were compatible with the literature. The results obtained from the experimental study presented in our country, which has rich boron deposits, are among the first examples of experimental studies conducted in our country on this subject. It has been requested to draw the attention of scientific circles to the potential toxic effects of boron and boron compounds on the health of large populations working in boron deposits and living in that region. The conclusions reached at the end of the study and the interpretation of the results by discussing have been realized within the purposes stated at the beginning. It is considered appropriate to carry out many more and advanced studies in order to examine the subject in terms of human health.

6. LIMITATIONS OF THE STUDY

We are well aware of the limitations of the present study. Firstly, it was performed in a single district, and in only one laboratuary, therefore the sample may not be enough representative about histopathological effects of boron. Thus, in order to definitively answer this question, a large sample containing a lot of animals requires.

7. AVAILABILITY OF DATA AND MATERIALS

The datasets used and/or analyzed during the current study which is ADFE's doctorate thesis are available from the Journal Editorial Office on reasonable request.

ETHICAL APPROVAL

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All

experiments have been examined and approved by the appropriate ethics committee.

CONSENT

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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