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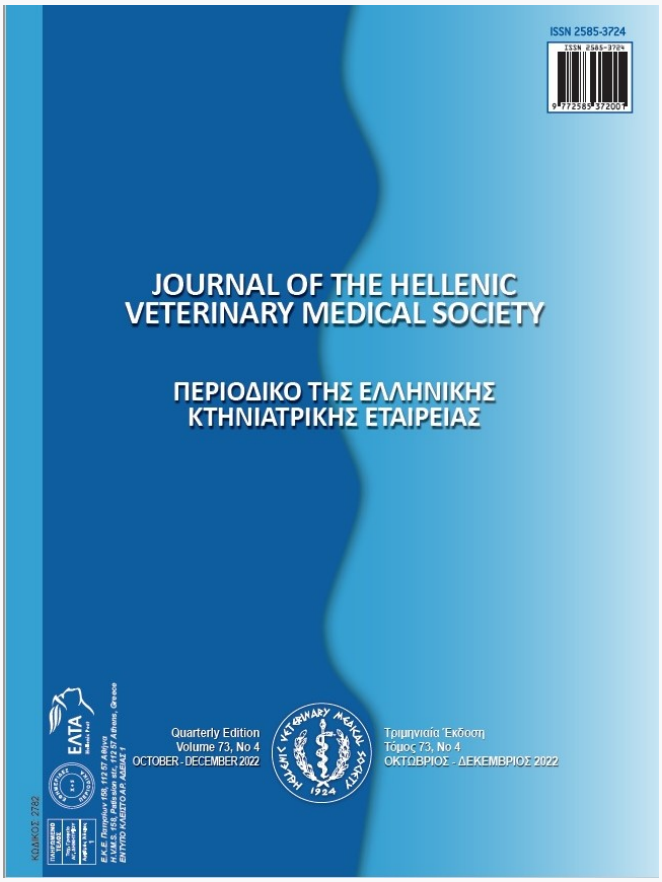

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Research article
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The effects of boron on oxidative stress, reproductive parameters and DNA damage in testicular tissue in wistar rats on a fatty diet

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ABSTRACT: This study aimed to investigate the protective effect of boron (B) against fat diet (FD)-induced spermatological, histopathological, biochemical and DNA damage in the testes of Wistar Albino Rats. Male rats were divided into five groups; the control group was fed a normal diet, the FD group was fed a normal diet with 40% beef tallow, the FD+B5 group was fed a fat diet containing 5 mg/kg/daily boron, the FD+B10 group was fed a fat diet containing 10 mg/kg/daily boron, and the FD+B20 group was fed a fat diet containing 20 mg/kg/daily boron. The spermatozoon motility, membrane integrity and glutathione (GSH) levels increased in the rats' testicular tissue in the groups given B. Conversely, the abnormal spermatozoon rate, malondialdehyde (MDA) levelsand deoxyribonucleic acid (DNA) damage were high in the FD group. Histopathologically, a decreased spermatozoon concentration in the tubulus seminiferus contortus lumen was observed in the FD and FD+B5 groups.FD+B10 administration reversed FD-induced spermatological parameters, MDA levels and DNA damage in the rats' testes. These results indicated that boron protects spermatological parameters and repairs DNA damage, reduces FD-induced high MDA levels, enhances the antioxidant defense system and eliminates tissue injury in rats' testes.

Keywords: Boron, DNA Damage, Fat Diet, Rat, Sperm.

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INTRODUCTION

Reactive oxygen species (ROS) can produce oxidative stress indirectly by reducing the enzymatic defense of spermatozoa. On the other hand, oxidative mechanisms play a key role in controlling the function of mammalian spermatozoa. Small amounts of ROS are required for the fertilization capabilities of spermatozoa. The addition of antioxidants increases semen quality by reducing oxidative stress during the storage of spermatozoa. It has been known for a long time that diets high in fat content increase oxidative stress and ROS amounts in various tissues and cells, and they consequently reduce the antioxidant capacity (Sreekumar et al. 2002). The imbalance between fat intake in the diet and energy expenditure is a factor that affects the prevalence of obesity (Küçükkurt et al. 2016). Fat diets (FD) have long been known to increase oxidative stress in fat tissue and ROS levels in various tissues and reduce the antioxidant capacity (Sreekumar et al. 2002). It may be possible to reduce oxidative damage by increasing the level of antioxidants in the body. Data obtained from livestock and laboratory animals have revealed the protective effect of antioxidants on the reproductive system (Feillet-Coudray, 2009).

Boron (B), which a natural mineral, is used extensively in the health sector, industry, agriculture and animal husbandry, especially in the cosmetics sector. B is rapidly absorbed at the point where it is given by diffusion and immediately spreads throughout the body (Murray, 1998). There have been many views regarding both the chemical and physiological functions of B until today. B can function in many different systems as a metabolic regulator. It also inhibits ROS by increasing glutathione levels and minimizes oxidative stress. A recent study reported that boron prevents lipid peroxidation and increases antioxidant activity, thus creating an antioxidant effect (İnce et al., 2010). At the same time, B demonstrates hepatoprotective and deoxyribonucleic acid (DNA) damage preventing effects (İnce et al., 2012).

Some studies have been carried outon FD in rats. Fonseca et al. (2016) reported that feeding rats with a fatty diet reduced sperm counts and motility. Fernandez et al. (2011) revealed that a fatty diet affects sperm quality by reducing motility in rats. However, there is a deficiency about the change caused by a fatty diet in the spermatozoon DNA and the effect of B on it. In this study we are presenting, the effectsof B on changes in the sperm quality, spermatozoon DNA integrity and testicular histopathology of male rats fed with a fatty diet were evaluated.

MATERIALS AND METHODS

Chemicals

The boric acid used in the present study was purchased from Sigma-Aldrich (Interlab, Turkey). All other chemicals were purchased from commercial sources. Ketamine (10-ml vial, Ketamine HCl 50 mg/ml) was purchased from Pfizer (Turkey), andxylazine (20 mg xylazineincluded in each mL of solution) was purchased from Bayer (Turkey).

Animals and Experimental Design

Forty healthy adult male Wistar Albino rats (all 8-week-old, with a mean weight of 386.5 gr) were procured from the AfyonKocatepe University Experimental Research Center in Afyonkarahisar, Turkey. The rats were allowed to acclimatize to the animal facility for at least 7 days before the start of the experiments. During the experiments, 6 animals were housed in each polypropylene cage with laboratory-grade pine sawdust as bedding and under a 12h light/12h dark cycle (light on from 08:00 to 20:00) at 20±3°C. A commercial pellet diet and fresh drinking water were available *ad libitum* to all animals. The animal use protocol of this study was approved by the Local Ethics Committee on Animal Research, Afyon Kocatepe University (approval number 2017-49533702/28). The animals were divided into five groups (n = 8). The rats in group I (control group) were fed a normal diet, group II received a normal diet with 40% beef tallow (FD), group III received a fat diet containing 5 mg/kg/day boron (FD+B5), group IV received a fat diet containing 10 mg/kg/day boron (FD+B10), and group V received a fat diet containing 20 mg/kg/day boron (FD+B20). The dose of boron was determined according to the report by Acaroz et al. (2019).The administrations of the substances were performed with a gastric gavage once a day for 56 days. An eight-week implementation is needed to determine the effect of a fatty diet on the male rat reproductive system. The reason for this is that the spermatological cycle takes 45-55 days in rats.At the end of the experimental periods, after an overnight fast, the rats were euthanized with an overdose of ketamine and xylazine (87 mg/kg ketamine and 13 mg/kg xylazine, intramuscularly (i.m))

Sample Collection

The epididymides, testes, prostate, bulbourethral

vesicles and seminal vesicles were removed from the reproductive organs of the rats, and following the clearance of fat tissues, these parts were weighed. One of the testicles was placed into 10% formalin for histopathological examinations. The other testis was stored at −20 °C for biochemical analysis. The left epididymis was used for sperm collection and determination of sperm motility, DNA integrity, membrane integrity and abnormal spermatozoon rate.

Evaluation of Semen Parameters

The percentage of forward progressive sperm motility was evaluated using a phase contrast microscope with a heated stage as described by Sönmez et al. (2005). For this process, a slide was placed on the phase contrast microscope with a heated stage warmed up to 37 °C, and then, several droplets of a Tris buffer solution [0.3 m Tris (hydroxymethyl) aminomethane, 0.027 m glucose, 0.1 m citric acid] were dripped onto the slide, a very small droplet of fluid obtained from the left caudal epididymis with a pipette was added to the Tris buffer solution and mixed with a cover slide. The percentage of forward progressive sperm motility was evaluated visually at 200x and 400x magnification. Motility estimations were performed on three different zones in each sample. The average of the three successive estimations was used as the final motility score. The proportion of morphologically abnormal sperm cells was estimated by a wet-mount slide using two to three drops of semen diluted in Hancock's solution. A drop of this mixture was placed on a slide and covered with a cover slide. The percentage of total spermatozoon abnormality (abnormal heads, abnormal mid-pieces, abnormal tail and total abnormal spermatozoa) was determined by counting a total of 400 spermatozoa under the phase contrast microscope (magnification 1000z, oil immersion)(Schafer and Holzmann, 2000).

The Hypo-osmotic Eosin stain test (HE test), was used to analyze the semen samples in which the ratios of dead-live spermatozoa were determined, and hypo-osmotic swelling tests were applied together, 1 ml of 100 mOsm HOST solution at 37°C was added to Eppendorf tubes in a water bath, and 10 µl from the semen sample was added to each tube. Then, eosin stain was added, and the mixture was kept in a 37°C water bath for 30 min. After incubation, a drop of this mixture was taken onto the slide, and smears were taken and dried in a very short time. In the prepared smears, 400 spermatozoa were classified into four types according to whether the whole or part of the head part of the spermatozoon was stained or not, and whether there was swelling in the tail. The results were classified into four types (Type I: tail swollen and head white, H+/E-; Type II: tail non-swollen and head white, H-/E-; Type III: tail swollen and head red, H+/E+; Type IV: tail non-swollen and head red, H-/E+) according to the staining status of the sperm head and curling of the sperm tail in the smears(Avdatek et al. 2018).

Homogenate preparation

Ice-cold 0.9% NaCl was utilized to wash the testicular tissues acquired from the animals. The tissues were trimmed free of extraneous tissue, rinsed in cooled 0.15 M Tris–HCl buffer (pH 7.4), and their homogenization in the buffer was performed to obtain a 10% (w/v) homogenate. Afterward, centrifugation was performed at 2100 g for 10 min, and the homogenates were kept in a deep freezer until use (Kucukkurt et al., 2008).

Biochemical evaluation

The method reported by Ohkawa et al. (1979) was used to determine malondialdehyde (MDA) levels, and the method described by Beutler et al. (1963) was used to evaluate reduced glutathione (GSH) levels. To determine the superoxide dismutase (SOD) levels in the tissue samples, the method described by Sun et al. (1988) was utilized. The method reported by Aebi (1984) was used to determine catalase (CAT) levels. The protein concentration in the tissue was evaluated by the colorimetric method according to the technique reported by Lowry et al. (1951).

DNA integrity

The single-cell gel electrophoresis (SCGE) method was used to determine DNA damage in the spermatozoa. In this method that is also called the COMETassay, alkaline pH is based on the migration of DNA molecules with different molecular weights and electric charges in the electrical field. The sperm were diluted at the ratio of 1:1 with a Ca^{+2}- and Mg^{+2}-free phosphate buffer solution (PBS) and washed by centrifugation at 800 g for 10 minutes at a temperature of +4 °C. At the end of centrifugation, the supernatant was discarded, the semen was diluted again, and the centrifugation process was repeated. The supernatant was discarded again, and the sperm was diluted again with PBS to reach a concentration of $20x10^6$/ml. 120 µl of 0.75% low-melting-point agarose (low-melting agar, LMA) gel prepared in PBS was taken, it was

dripped on to specially frosted microscope slides, and then, it was spread in the form of a smear and allowed to dry at room temperature. 5 µl of the diluted sperm suspension was taken, mixed with 85 µl of 1% LMA gel at 37 °C, layered onto the first agarose layer, covered with a 24 x 60 mm cover slideand kept for solidification at +4 °C. After solidification, the slides were prepared by carefully lifting the cover slides. A lysis solution was used to ensure that the DNA strands were released in the agarose gel by lysing the cell and nuclear membrane, and after the spermatozoa were embedded in the agarose gel, the slides were incubated in the lysis solution in a CometAssay™ Reagent Kit for Single Cell Gel Electrophoresis containing high concentrations of salt and detergent for approximately 1 hour at +4 °C. At the end of one hour, 40 mM of dithioerythritol (DTT) was added to the lysis solution, and the slides were incubated for 1 hour at +4 °C. Then, 100 µg/ml of proteinase K was added to the lysis solution, and the slides were incubated overnight at 37 °C. The slides were allowed to incubate for 20 minutes in freshly prepared and cooled electrophoresis buffer for separation of DNA chains before running on electrophoresis. After the incubation of the agarose-embedded spermatozoa in the electrophoresis buffer (300 mMNaOH and 1 mM EDTA, pH 12.5) was completed, the DNAs were subjected to processing in this buffer solution in a 300mA and 20-volt electrical field for 20 minutes. Neutralization was ensured by washing the slides in freshly prepared Tris buffer (40 mMTrisHCl, pH 7.4) 3 times. The slides stained with ethidium bromide (5 µg/ml) were covered with a cover slide, and 100 DNA images were evaluated with a fluorescence microscope (Olympus CX-31) at 400x magnification. Since the length of migration varied depending on the number of fragments, DNA chain breaks and the level of alkaline-labile regions, scoring was performed by classifying DNA images into 5 categories according to the degree of damage. Those with complete DNA integrity were scored as0, and those with disrupted integrity were scored between 1 and 4 visually. The results were evaluated in arbitrary units (AU) (Avdatek et al., 2018).

Histological Evaluation

Testicular tissues were collected from all animals, fixed in Bouin's solution, then embedded in paraffin blocks, and sections with a thickness of 5 µm were obtained. Mayer's hematoxylin and eosin (H&E) were used for the staining process. Tissue samples were evaluated under a light microscope (Olympus Bx51 model) with a camera (Olympus DP20).The evaluations were made using an image analysis program ((Nikon DS FI3, microscopic digital camera systems, Tokyo, Japan) by measuring at least 10 tubulus seminiferus contortus(TSC) diameters from each sample.

Statistical Analysis

The normality of the distribution of the data for the evaluated parameters was confirmed using Shapiro-Wilk test. Afterward, analysis of variance (ANOVA) was applied to detect the differences among the groups, and Duncan's test was used to identify the sources of the differences. Besides, Kruskal-Wallis test was used as the nonparametric test. All analyses were performed in SPSS® 20.0 for Windows, and $p<0.05$ was considered statistically significant. The data are reported as arithmetic mean ($\bar{X}$) ±standard error of the mean (SEM).

RESULTS

The findings on epididymal spermatozoon motility and abnormal spermatozoon ratio are given in Table 1, while the results regarding membrane integrity are presented in Table 2. A significant increase in spermatozoon motility ($p<0.05$) was determined in all groups given B compared in to the group given HF. The decrease in the abnormal spermatozoon rate in all groups given B and the control group compared to the HF group was statistically significant ($p<0.05$). When we looked at the plasma membrane integrity (HE test) results, a significant increase ($p<0.05$) was observed in the control group and the groups given B in comparison to the FD group. The MDA, GSH, SOD and CAT levels obtained from the testicular tissues of the rats are given in Table 3. In terms of the MDA results, a significant decrease was observed in the FD + B5 and FD + B10 groups in comparison to the FD group ($p<0.05$). There was a numerical decrease in the GSH level in the FD group compared to the control and other groups, but it was not statistically significant. The CAT activity in the testicular tissues of the control and FD + B5 groups was significantly lower ($p<0.05$) in comparison to the FD group. On the other hand, the changes in the MDA, GSH and CAT levels caused by FD were reversed in the boron-treated groups. The DNA damage results of the epididymal spermatozoa of the rats are given in Figure 1. High levels of DNA damage (56.37±0.90 AU, 53.75±1.86 AU) were detected in the FD+B20 and FD groups in comparison to the control group (39.1±1.93 AU) ($p<0.05$). In the FD+B5 and FD+B10

Table 1. Mean (± SEM) spermatological parameters in epididymal rat semen

Groups	Motility %	Head %	Mid-piece %	Tail %	Total %
Control	73.75±1.82^{bc}	0.31±0.13	1.00±0.13^{ab}	3.56±0.23^{cd}	4.75±0.34^{c}
FD	61.25±2.26^{d}	0.50±0.16	1.31±0.16^{a}	6.31±0.33^{a}	8.12±0.33^{a}
FD+ B5	77.50±1.63^{b}	0.18±0.09	1.25±0.09^{a}	4.00±0.13^{bc}	5.43±0.19^{bc}
FD+ B10	71.25±1.25^{a}	0.25±0.09	0.75±0.09^{b}	2.93±0.14^{d}	3.87±0.12^{d}
FD+ B20	74.00±1.51^{c}	0.29±0.05	1.25±0.16^{a}	4.62±0.18^{b}	6.12±0.20^{b}

Values (Mean ± S.E.M.) with different superscripts (a-d) within the same column showed significant differences (P < 0.05)
B: Boron, FD: Fatdiet, Head (%):Percentage of abnormal spermatozoa attached to the head, Mid-piece (%):Percentage of abnormal spermatozoa attached to the mid-piece, Tail (%):Percentage of abnormal spermatozoa attached to the tail, Total (%):Percentage of abnormal spermatozoa attached to the total abnormality
FD + B5: a fat diet containing 5 mg/kg/daily boron, **FD + B10:** a fat diet containing 10 mg/kg/daily boron, **FD + B20:** a fat diet containing 20 mg/kg/daily boron

Table 2. Mean (± SEM) HE test parameters in epididymalrat semen

Groups	H+/E- %	H-/E- %	H+/E+ %	H-/E+ %
Control	52.62±2.73^{bc}	39.12±2.56^{a}	4.12±0.98^{b}	4.12±1.15^{d}
FD	35.37±3.41^{d}	39.00±1.76^{a}	10.12±1.36^{a}	15.50±2.25^{a}
FD+ B5	55.62±1.58^{b}	30.50±2.20^{b}	5.25±0.52^{b}	8.62±0.94^{bc}
FD+ B10	66.00±1.65^{a}	21.75±1.69^{c}	5.50±1.08^{b}	6.87±0.66^{cd}
FD+ B20	48.12±1.44^{c}	34.50±0.90^{ab}	5.87±0.98^{b}	11.75±1.09^{ab}

Values (Mean ± S.E.M.) with different superscripts (a-d) within the same column showed significant differences (P < 0.05).
H+/E-: tail swollen and head white; H-/E-: tail non swollen and head white; H+/E+: tail swollen and head red; H-/E+: tail non swollen and head red.
B: Boron, FD: Fat diet **FD + B5:** a fat diet containing 5 mg/kg/daily boron, **FD + B10:** a fat diet containing 10 mg/kg/daily boron, **FD + B20:** a fat diet containing 20 mg/kg/daily boron

Table 3. Mean (± SEM) Oxidativestressparameters in epididymalrat semen

Groups	MDA(nmol/ml)	GSH (nmol/g doku)	SOD (U/µg protein)	CAT (k/µg protein)
Control	4,77±0,46^{b}	22,9±2,28^{a}	6,33±1,08	0,04±0,01^{b}
FD	7,28±1,02^{a}	19,89±0,65^{b}	5,78±1,48	0,06±0,02^{a}
FD+ B5	5,5±1,01^{b}	21,45±3,02^{ab}	5,23±0,98	0,04±0,01^{b}
FD+ B10	5,69±1,18^{b}	21,53±1,54^{ab}	5,94±0,98	0,04±0,02^{ab}
FD+ B20	7,18±1,25^{a}	21,03±1,41^{ab}	5,28±0,48	0,04±0,02^{ab}

Values (Mean ± S.E.M.) with different superscripts (a-b) within the same column showed significant differences (P < 0.05).
FD + B5: a fat diet containing 5 mg/kg/daily boron, **FD + B10:** a fat diet containing 10 mg/kg/daily boron, **FD + B20:** a fat diet containing 20 mg/kg/daily boron
MDA: Malondialdehyde, GSH: Glutathione, SOD: Superoxide Dismutase, CAT: Catalase

Table 4. Mean (± SEM) Diameter of ST and GCLT inrattestis.

Groups	Diameter of ST (µm)	GCLT (µm)
Control	311,83±3,04^{a}	130,33±2,18^{a}
FD	238,82±3,53^{b}	102,17±1,42^{b}
FD+ B5	245,81±5,36^{b}	104,00±1,26^{b}
FD+ B10	314,17±4,43^{a}	127,67±2,62^{a}
FD+ B20	302,45±1,21^{a}	128,17±3,53^{a}

Values (Mean ± S.E.M.) with different superscripts (a-b) within the same column showed significant differences (P < 0.05).
B: Boron, FD: Fat diet, **FD + B5:** a fat diet containing 5 mg/kg/daily boron, **FD + B10:** a fat diet containing 10 mg/kg/daily boron, **FD + B20:** a fat diet containing 20 mg/kg/daily boronST: Seminiferous tubules, GCLT: Germinal cell layer thickness

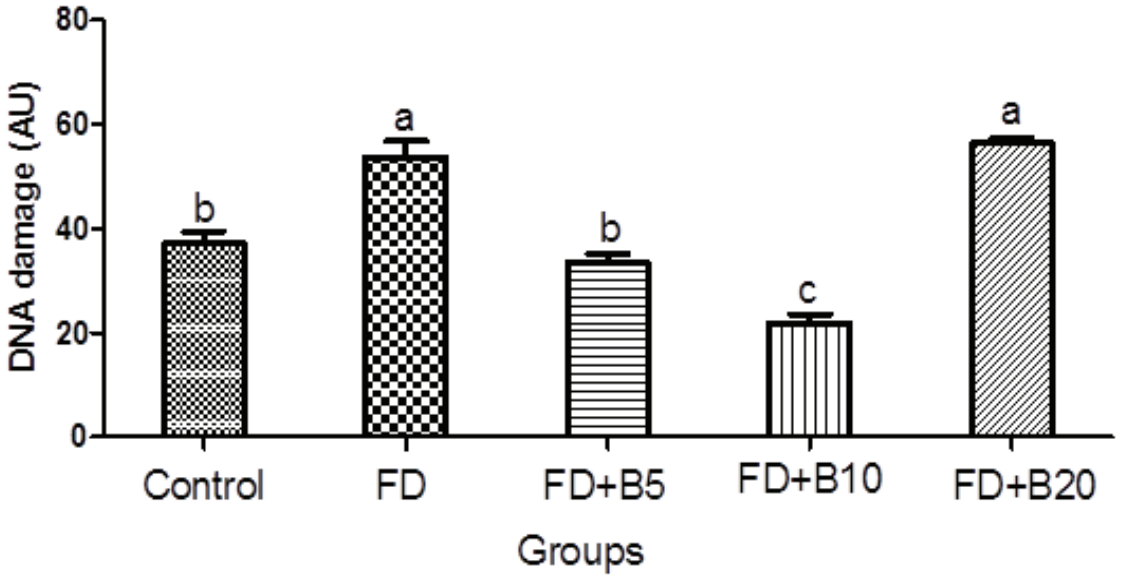

Fig. 1. The effect of FD alone and treated with B on DNA damage in rats. FD, FD+B5, FD+B10 and FD+B20 were administered at 40 % beef tallow, 40 % beef tallow + 5 mg/kg, 40 % beef tallow + 10 mg/kg and 40 % beef tallow + 20 mg/kg, respectively by oral gavage. Results are expressed as mean + *SEM* of eight rats. a,b,c,: different letters show statistically significant differences ($p < .05$)

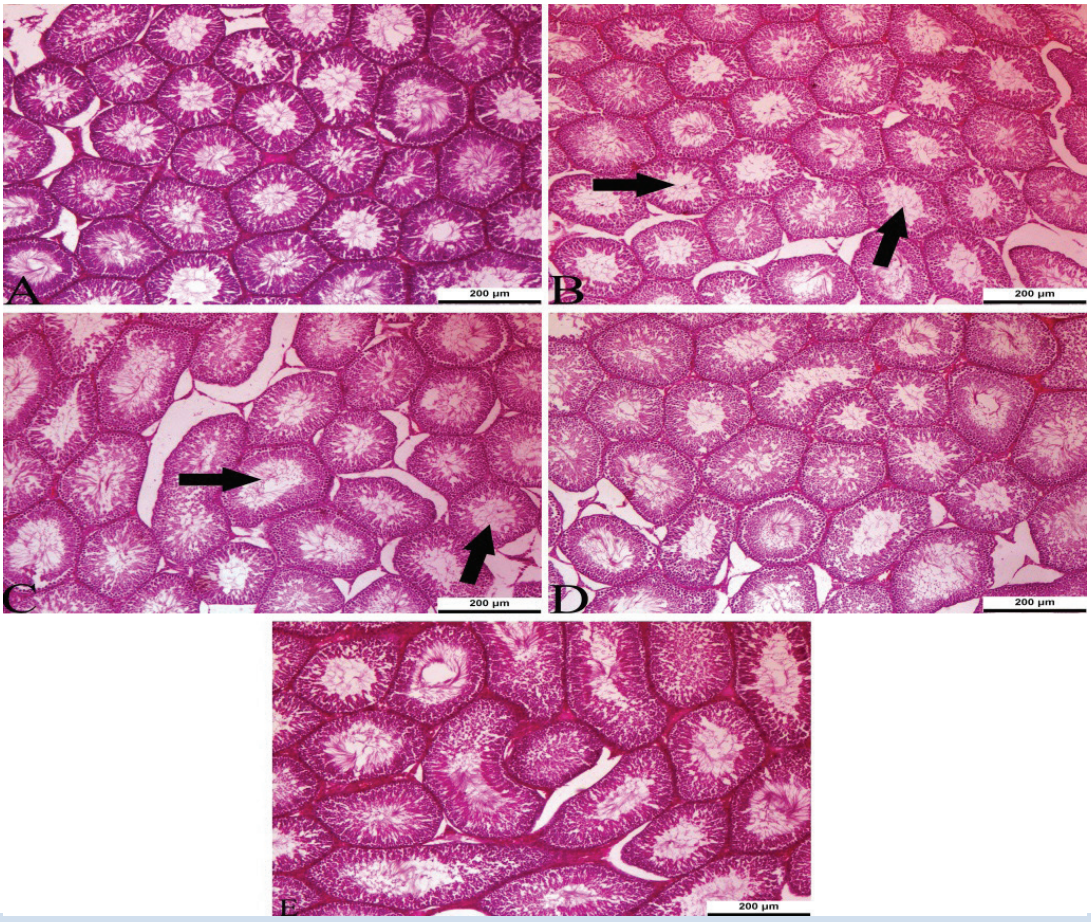

Fig 2. Theeffect of Boron (B) on FD-induced damage in testis of rats. Representative figures were stained with H&E.

Arrows and arrowheads indicate decreases sperm concentration in tubulus seminiferus contortus lumen in FD and FD+B5 groups. (Fig. 2 B and C) Control group (A), animals treated with FD (B) animals treated with FD+B5 (C), animals treated with FD+B10 (D), animals treated with FD+B20 (E).

groups, the DNA damage levels were determined to be 33.62±1.61 and 21.87±1.79 AU, respectively. The aforementioned findings indicated that administering FD+B5 and FD+B10 inhibited the DNA damage caused by the fat diet in the sperm cells ($p<0.05$). The findings on diameter seminiferous tubules (ST),: germinal cell layer thickness (GCLT) are given in Table 4. The histopathological changes in the testicular tissues of the groups are given in detail in Figure 2. Histopathologically, a decreased spermatozoon concentration in the tubulus seminiferus contortus lumen was observed in the FD and FD+B5 groups (Fig. 2 B and C). However, no significant difference was observed in terms of the testicular histopathology of the other groups including control, FD + B10 and FD + B20 (Fig. 2 A, D, E).

DISCUSSION

In our study, while the addition of B in the diet of the rats fed via FD preserved a significant degree of semen quality in comparison to those fed with only FD, there were also increases in some groups. A significant increase in spermatozoon motility ($p<0.05$) was determined in all groups given B compared in to the group given HF. The decrease in the abnormal spermatozoon rate in all groups given B and the control group compared to the HF group was statistically significant ($p<0.05$).The addition of B to the diet of the rats contributed positively to the rats' reproductive performance by increasing spermatozoon motility and vitality, as well as reducing DNA damage and the abnormal spermatozoon ratio. As a result of feeding the rats with a fatty diet, the enzymes that make up the antioxidant system decreased, and the levels of reactive oxygen species increased, resulting in a significant decrease in their spermatological parameters. In light of these data, the cause of the negative effects on the spermatological parameters after FD feeding may have been due to insufficient spermatozoon mitochondrial activity (Ferramosca et al., 2012). As a possible effect of obesity that develops with a fatty diet, an increase in temperature may occur around the male genital system. Fatty tissue is formed in the tissues around the testicles of obese mice; thus, the scrotum temperature rises, resulting in a decrease in spermatozoon quality. It has been determined that heat forming in the genital system has a negative effect on spermatological parameters and DNA integrity in rats and mice. It was also suggested that heat may be the source of the increase in the amount of ROS in spermatozoa (Perez-Crespo et al., 2008). Bakos et al. (2010) reported that obesity in mice reduces spermatozoon capacity and motility and causes significant damage to the reproductive system by increasing oxidative stress and DNA damage. Sallmen et al. (2006) asserted that obesity reduces spermatozoon DNA integrity in men and causes severe motility reductions. Nguyen et al. (2007) argued that excess weight in men might directly or indirectly affect physiological events that cause a reduction in fertilization ability. Ferramosca et al. (2016) reported in their studies where they applied a high-fat diet in rats that the animals had an increase in body weight and fat in tissues, and eventually, there was a decrease in spermatozoon concentration and motility. Fernandez et al. (2011) observed that a fatty diet affects sperm quality only by reducing motility among spermatological parameters and does not affect other spermatological parameters. In males, FD causes nutritional deficiency, resulting in a decrease in semen quality and eventually decreased fertilization potential.

With an increase in ROS levels in spermatozoon cells, the level of lipid peroxidation is also increased, while the amount of intracellular ATP decreases. Ferramosca et al. (2013) found that mitochondrial activity was negatively affected by oxidative stress, and there was a negative relationship between ROS and motility. Oxidative stress occurs when the quantity of antioxidants in the cell decreases. For this reason, GSH, CAT and SOD activities, which form the antioxidant defense system, are essential in cell defense against ROS (Scandalios, 2005).ROS significantly damage the lipids in the spermatozoon cell membrane and the integrity of the DNA of spermatozoa. It is known that oxidative damage occurs in the DNA of aerobic cells with enriched mitochondria (Chen et al., 2013). MDA, an indirect marker of LPO resulting from ROS damage, is used as an indicator of oxidative stress. In our study, FD caused an increase in the quantity of MDA in the testicular tissues, leading to LPO formation in the spermatozoa of the rats. These data showed that FD reduced the antioxidant levels by increasing the quantities of ROS. So, there was a remarkable increase in the MDA levels. A statistically significant decrease in the MDA levels was found in the testicular tissues of the groups given B. It was determined that significantly less lipid peroxidation occurred in the groups given B, depending on the dose compared to the rats given only a fatty diet. The mitochondrial activities of the spermatozoa of the rats fed with a fatty diet with increased LPO levels may be significantly damaged (Ferramosca et al., 2016).While the fatty

diet decreased the GSH level in the testicular tissue, boron application increased this level. All these data showed that the addition of boron to the diet increased the GSH levels against oxidative stress caused by a fatty diet and directly inhibited LPO. In comparison to the FD group, the CAT levels in the testicular tissue of the rats in the B groups were high, and this event indicated that these enzymes could regenerate themselves and have a dose-dependent protective effect. Antioxidants can prevent the formation of free radicals. Both SOD and CAT can degrade O^2 and decompose H_2O_2, resulting in reduced oxidative stress.

Since increased DNA damage is associated with infertility, imperfect embryonal development, implantation failure and recurrent abortions, detection of different levels of DNA damage between the spermatozoa of fertile and infertile men highlights the utility of spermatozoon DNA damage as a marker for evaluating male fertility potential (Agarwal et al., 2003). Three hypotheses have been proposed as the source of DNA damage in spermatozoa, the first of which is improper packing and binding during the development of spermatozoa, the second is the damage caused by oxidative stress, and the third is the DNA damage caused by apoptosis. Among these, oxidative stress is associated with the single- and double-strand breaks of DNA (Agarval and Saleh, 2002; Sakkas et al., 2002). In our study, the DNA damage was found to be higher in the FD group compared to the control and other groups. Moreover, the addition of B to the diet showed a protective effect on DNA integrity due to the antioxidant and cytoprotective properties of B. High levels of DNA damage in spermatozoon cells adversely affect fertilization. Antioxidants may be used for the treatment of male infertility caused by DNA damage (Greco et al., 2005). Chavarro et al. (2010) reported high spermatozoon DNA damage levels in their studies on obese men.

In the histopathological examination of the testicular tissues of the rats, a decrease in the number of spermatozoa, tubular diameter and epithelium height was observed due to feeding them with a fatty diet (Figure 2). As a result of feeding with FD, the increase in the temperature in the testicles due to the fat in the tissues around the genital organs may have been the reason for the increase in the abnormal spermatozoon rate. Mortazavi et al. (2014) reported that the seminiferous tubules of mice on a high-fat diet deteriorated, and the number of spermatozoa and maturing spermatids decreased. Zhang et al. (2012) in the detailed histopathological analysis they conducted on the testicular tissues of mice fed a fatty diet, determined that the seminiferous tubules' basement membranes were partially thickened, and there were significant changes such as rippling.B application allows preventing testicular toxicity caused by a fatty diet and preserving the normal testicular histopathology.

CONCLUSION

The results of this study demonstrated that B, in a dose-dependent manner, effectively reduced fat diet-induced oxidative stress, prevented DNA damage and preserved spermatological parameters in the rats. The protective effects of B are due to both an increased activity of the antioxidant defense system, as well as an inhibition of LPO.

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