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Protective Effects of Wheat Sprout on Testicular Toxicity in Male Rats Exposed to Lead

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Abstract

Objective: Negative effects of lead on the male reproductive system and sperm fertility parameters have been shown broadly. In recent years, use of medicinal herbs in reducing heavy metal toxicities has increased worldwide. One of these herbals, wheat sprout, contains high amount of vitamins (especially vitamin E), antioxidants and phytoestrogen compounds. This study investigated the effects of wheat sprout extract (WSE) and vitamin E on testicular oxidative stress in rats exposed to lead acetate.

Methods: Thirty-five rats were divided randomly into seven groups: G1 (control group) received 1 ml/kg/day of normal saline, G2 received 20 mg/kg/day of lead acetate, G3 and G4 received 100 mg/kg/day and 200 mg/kg/day of WSE respectively, G5 and G6 received 100 mg/kg/day and 200 mg/kg/day of WSE respectively with 20 mg/kg/day of lead acetate, and G7 received 100 mg/kg/day of vitamin E with 20 mg/kg/day of lead acetate. After 35 days, rats were sacrificed and blood, sperm, liver and testicle tissue samples were collected for histomorphological and histochemical studies.

Results: Results showed that count, motility and viability of sperms decreased following the administration of lead acetate ($P<0.01$). Histomorphological studies showed a significant decrease in tubular differentiation index (TDI), spermiogenesis index (SI), repopulation index (RI), number of Leydig and Sertoli cells, and epithelium height and diameter of seminiferous tubules in groups receiving lead acetate ($P<0.05$).

Conclusion: Summary, results of the current study show that dose dependent WSE significantly prevents testicular toxicity and oxidative stress effects of lead acetate.

Keywords: Wheat sprout extract; Lead; Testis; Oxidative stress; Rat

Introduction

Reports on reduction in male fertility reveal the role environmental contaminants in etiology of infertility in humans [1]. Lead is an important environmental contaminant which affects many body organs including male genital system; specifically in workers of lead mines and metal melting and battery factories [2,3]. Other resources of lead include storage of drinking water in tanks, packing food stuffs in newspapers and canned foods [4]. Effects of lead on male genital system and sperm fertility parameters have been shown [5-7]. Kakkar *et al.* (2005) have shown that disorders in structure and function of male genital system and quality of sperms are principal symptoms of exposure to lead [8]. Lead can be absorbed through respiration, digestion and skin and accumulated in body [9]. Heavy metals such as lead can show their toxic effects through the production of reactive oxygen species (ROS) or inhibition of antioxidant enzyme activity [10,11]. Upasani *et al.* (2001) has reported that lead toxicity is associated to increased lipid peroxidation [12]. Therefore, it can be concluded that antioxidant compounds are possibly effective against lead toxicity [13,14].

Wheat sprout includes a long background in Iranian culture. Wheat sprout is a healthy food which contains high nutritional values; mostly produced by *Triticum aestivum* [15]. Sprouting or germination causes extensive changes in seeds; in which, synthesis of many useful compounds such as vitamins, phenols and antioxidants occur. Wheat sprout includes redox enzymes such as catalase and peroxidase and is rich in antioxidants including phenolic acids, tocopherol, alkylresorcinols, aminophenol acids, vanillic acid and aminobenzoics. These compounds include free and bounded forms and show strong antioxidant activity with medicinal properties and a high absorption value [16,17]. In recent years, wheat sprout has widely been presented in markets of European countries, United States and India [17]. Tocopherols (vitamin E) are strong fat-soluble antioxidants; mainly found in wheat sprout [18]. Medical properties of wheat sprout have previously been shown, including anti-hyperglycemia, antidiabetics, anticancer and antimutagenicity *in vivo* [19-22]. It has been reported that extracted antioxidants from wheat sprout prevent DNA oxidative damage *in vitro* [23]. The aims of this study included studying protective roles of hydro alcoholic extract of wheat sprout and vitamin E against tissue damages and oxidative stress induced by lead in the testis of rats.

Materials and Methods

In this study, lead acetate (Merck, Germany), vitamin E in α-tocopherol form (Zahravi Pharmaceutical Co, Iran) and testosterone assessment kit (DRG Instruments, Germany) were used. The hydro alcoholic extract of wheat sprout was prepared using maceration (volumetric) method in Center of Pharmacological Researches of the Faculty of Veterinary Medicine, Chamran University, Ahvaz, Iran. Other chemicals were purchased from Merck, Germany.

Preparation of animals and study design

Thirty-five healthy adult male Wistar rats (220 ± 20 g) were purchased from the Laboratory Animal Breeding Center of the Faculty

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of Veterinary Medicine, University of Tehran, Tehran, and equally divided into seven groups, including control (1 ml/kg of normal saline), wheat sprout extract (WSE) (100 mg/kg/day); WSE (200 mg/kg/day); lead (20 mg/kg/day); lead (20 mg/kg/day) and WSE (100/200 mg/kg/day); and lead (20 mg/kg/day) and vitamin E (100 mg/kg/day) groups. Rats were housed under a 12-h day/night illuminating cycle at 23–25°C and *ad libitum* access to food and water. Animals were acclimatized to environmental conditions for one week before the intervention. The study was adopted according to the regulations by the Iranian Veterinary Organization and manuals published by the Ethical Committee of the Faculty of Veterinary Medicine, University of Tehran. Control group received 1 ml/kg of normal saline as daily intraperitoneal injection. Lead group received 20 mg/kg of lead acetate as daily intraperitoneal injection [7,24,25]. WSE group received 100 mg/kg of wheat sprout extract orally (via gavage) daily. WSE group received 200 mg/kg of wheat sprout extract orally. Lead and WSE group received 20 mg/kg/day lead acetate as daily intraperitoneal injections with 100 mg/kg/day of WSE orally (via gavage) daily. Lead and WSE group received 20 mg/kg/day of lead acetate as daily intraperitoneal injection with 200 mg/kg/day of wheat sprout extract orally (via gavage) daily. Lead and vitamin E group received 20 mg/kg/day of lead acetate as daily intraperitoneal injection with 100 mg/kg/day of vitamin E orally (via gavage) daily [26]. The study continued for 35 days. To modify drug doses, animals were weighed weekly. On Day 36, rats were sacrificed using chloroform. Blood and liver samples were collected for the assessment of testosterone and lead, respectively. Samples were stored at -20°C until use. To analyse number, motility and live/dead ratio of sperms, epididymis was carefully separated from the testis. Right testes were stored at -20°C for the assessment of thiobarbituric acid reactive substances (TBARS). After recording weight and volume, testes were fixed in 10% of buffered formalin for histochemical and histomorphometrical studies.

Lead assessment in liver

Lead was quantified in homogenized liver specimens to ppm levels using Optima 7300 DV ion conductivity plasma instrument (Perkin Elmer, USA) in Center of Toxicological Research of the Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran.

Evaluation of sperms

This was carried out using hemocytometer slides and light microscope and included sperm count in volumetric unit and stabilized dilution, and assessment of sperm motility. Furthermore, eosin-nigrosin staining was used for the assessment of live/dead ratio of sperms. Five rats from each group and 10 slides from each sperm specimen were used for the study. Three parts of epididymis including head, body and tail were discrete. Tail was placed in a 3 cm Petri dish containing 1 ml of human tubal fluid (HTF) culture media. After cutting epididymis tail into small pieces, sperms were removed at 37°C and 5% concentration of CO_2 and then studied [27].

Assessment of testicular TBARS

Malonyldialdehyde, a substance showing reaction with tiobarbituric acid, was assessed in homogenized testis specimens using a method originally described by Lukaszewicz-Hussain *et al.* in 2007 [28]. Ten percent of the testis tissue were homogenized in potassium chloride (0.15 M) and centrifuged at 10,000g for 30 min. Half a milliliter of 50% trichloroacetic acid was added to 0.5 ml of the supernatant and centrifuged for 5 min at 5,000g. Then, tubes containing 0.5 ml of supernatant and 0.5 ml of tiobarbituric acid were covered with aluminum foil and incubated at 90°C for 1 h. Absorption was read at 540 nm at room temperature using spectrophotometer and standard calibration curve.

Histomorphometrical and histochemical studies

For macroscopic study, testes were weighed using digital scale (with a minimum accuracy of 0.001g). Volume of testes was calculated using water displacement method [29]. For microscopic study, Dino-Lite digital lens and Dino Capture 2 Software were used. Furthermore, histometrical structures of testes were analyzed, including thickness of capsule, height of germinal epithelium of seminiferous tubules, number of Sertoli cells, size of Leydig cells, number of Leydig cells in a marked scale and diameter of seminiferous tubules. Spermatogenesis and spermiogenesis were studied using analysis of TDI, RI and SI indices [30]. To analyze TDI index, ratio of seminiferous tubules with four lines or more of differentiated cells from the Type A spermatogonia was calculated. To analyze SI index, ratio of seminiferous tubules containing spermatid cells was reported as SI positive and tubules lacking spermatids as SI negative. To analyze RI index, the ratio of Type B to Type A spermatogonia was calculated. Formalin fixed samples were processed using standard histological method. Paraffin blocks were sectioned at 5-6 μm and stained with Hematoxylin and Eosin (H&E), Periodic Acid Schiff (PAS), Alkaline Phosphatase (ALP) and Sudan black. For lipid staining, samples were embedded with optimal cutting temperature compound (OCT gel) and sections of testicular tissues were prepared to 15-20 μm levels at -40°C using cryostat (SLEE, Germany).

Assessment of serum testosterone

Blood samples were collected from rat heart and incubated at 37°C. Blood serum was separated by centrifugation at 3,000g for 10 min and stored at -20°C until use. Serum testosterone was assessed using Enzyme Linked Immunosorbent Assay (ELISA).

Statistical data analysis

Data were analyzed statistically using SPSS V.18 Software. Data were present as mean ±SD (standard deviation). One-way ANOVA and Tukey's post hoc test were used for data analysis. Differences were considered as significant when $P<0.05$.

Results

Macroscopic studies

Rats were weighed a day after the last injection. As shown in Table 1, lead group (average weight of 214 ± 14.7 g) and combined lead and WSE (200 mg/kg/day) group (average weight of 207.4 ± 26.9 g) had the least rate of weight within the groups. The other groups receiving WSE showed an insignificant increase in weight, compared to that of control group. Furthermore, the body weight of rats in combined lead and WSE (200 mg/kg/day) group showed a significant decrease, compared to that in WSE (200 mg/kg/day), combined lead and vitamin E, and WSE (100 mg/kg/day) groups ($P<0.01$). Body weight of rats in combined lead and vitamin E, and WSE (200 mg/kg/day) groups showed a significant increase, compared to that of lead group ($P<0.05$). WSE (200 mg/kg/day) group included the heaviest testis net weight within the groups, compared to combined lead and WSE (100 mg/kg/day) group ($P<0.05$). However, net weight of the testes in groups receiving lead showed an insignificant decrease, compared to that in other groups. Combined lead and WSE (100 mg/kg/day) group (average weight of 5.4 ±0.6 g) included the least rate of relative testis weight compared to other groups ($P<0.05$) (Table 1). Quantitative results of the testis volume revealed that the lead group included the least average volume, compared to

Testis volume (ml)	Testis/body weight ×10^{-3}	Net weight (g)	Body weight (g)	Group
1.2 ± 0.2	6.4 ± 0.1^{ab}	1.5 ± 0^{ab}	231.2 ± 4.2^{ab}	Control
1 ± 0	6.4 ± 0.4^{ab}	1.4 ± 0.1^{ab}	214 ± 14.7^{bcd}	Lead
1.2 ± 0.3	6.5 ± 0.6^{ab}	1.5 ± 0.2^{ab}	237.4 ± 10.5^{ab}	WSE (100*)
1.2 ± 0.2	6.3 ± 0.2^{ab}	1.6 ± 0.1^{a}	251.8 ± 8^{a}	WSE (200*)
1 ± 0.2	5.4 ± 0.6^{c}	1.3 ± 0.1^{b}	244.6 ± 12.8^{ac}	Lead + WSE (100*)
1.1 ± 0.1	6.5 ± 0.7^{b}	1.4 ± 0.1^{ab}	207.4 ± 26.9^{bd}	Lead + WSE (200*)
1.3 ± 0.2	5.6 ± 0.2^{ac}	1.4 ± 0.1^{ab}	250 ± 20.5^{a}	Lead + vit E

*mg/kg/day, Different letters represent significant differences between groups (*P*<0.05).

Table 1: Rat body weight, net weight, testis/body weight and testis volume.

other groups. Groups receiving WSE demonstrated an increase in volume of testes, compared to control group. No significant difference was seen in volume of testes within the groups (Table 1).

Histochemical and microscopic studies

In the current study, exposure of testis tissues to lead acetate caused disorders in spermatogenesis, reduction of seminiferous germinal epithelium height, degenerative changes in seminiferous tubules, increased edema in interstitial tissue, and increased lumen diameter of seminiferous tubules. Furthermore, disorders in unification and adherence of spermatogenic cells were seen. Vacuolar degeneration in germinal epithelium of seminiferous tubules was observed. Termination of spermatozoa maturation and absence of spermatozoa in lumen of a majority of seminiferous tubules were significant. In tissue sections from groups received lead acetate, combined lead and vitamin E, combined lead and WSE (200 mg/kg/day) and combined lead and WSE (100 mg/kg/day), degenerating seminiferous tubules were seen. This was not observed in other groups. Groups receiving dose dependent WSE and vitamin E showed improvement and unity in testicular tissue structure and increased active seminiferous tubules. Therefore, groups receiving WSE, especially with doses of 200 mg/kg/day, included no degenerating seminiferous tubules, vacuolar degeneration in germinal epithelium and cellular disruption in seminiferous tubules. It is worth to mention that increased spermatogenesis due to WSE was more obvious in peripheral tubules close to the capsule of testis (Figure 1). As shown in Figure 2, SI index in groups that received lead and combined lead and WSE (100 mg/kg/day) showed an average of 81% ± 4.6 and 76.2% ± 3.8 respectively with a significant decrease, compared to that in control group (92.3% ± 4.2) (P<0.05). A significant increase was seen in SI index in dose dependent WSE groups, compared to that in lead group (P<0.01). As shown in Figure 3, TDI index showed a significant increase in groups receiving dose dependent WSE and vitamin E, compared to that in lead group (P<0.001). TDI index showed a significant increase in WSE (200 mg/kg/day) group with average of 85.4% ± 0.6, compared to that in control group (76% ± 2.7) (P<0.001). RI index revealed a significant increase in WSE (200 mg/kg/day) group (80.6% ± 1.2), compared to that in other groups (P<0.001) (Figure 4). The least average value of RI index was seen in groups receiving lead acetate. RI index in lead group (55.7% ± 4) and combined lead and vitamin E group (56.1% ± 5.26) showed an insignificant decrease, compared to that in control group (62% ± 5.2). Furthermore, WSE (100 mg/kg/day) and combined lead and WSE (200 mg/kg/day) groups showed a significant increase, compared to control group (P<0.01), (Figure 4).

Germinal epithelium height of seminiferous tubules showed a significant decrease in lead group, compared to that in control group (P<0.001). On the contrary, this showed the highest rate in WSE (200 mg/kg/day) group (89.19 ± 4.30 μm), compared to that in other groups (P<0.001). Results have shown that dose dependent WSE and lead can increase germinal epithelium height of seminiferous tubules. Germinal epithelium height in combined lead and WSE (200 mg/kg/day) group showed a significant increase, compared to that in lead group (P<0.001)

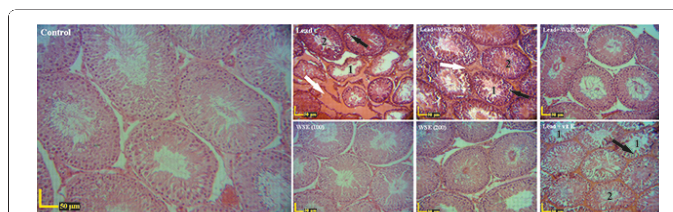

Figure 1: Histological structure of rat testis using H&E staining. Inactive and degenerative seminiferous tubules (1), edema (white arrows), discontinuity and vacuolation changes (black arrows) were seen in seminiferous tubule epithelium in groups which received lead, compared to those in control group. Active and inactive seminiferous tubules and edema were seen in combined lead and WSE (100 mg/kg/day), and combined lead and vitamin E groups. Combined lead and WSE (200 mg/kg/day), WSE (100 mg/kg/day) and WSE (200 mg/kg/day) groups showed normal activity. Number 2 shows active seminiferous tubules. Values in parentheses are shown in mg/kg/day.

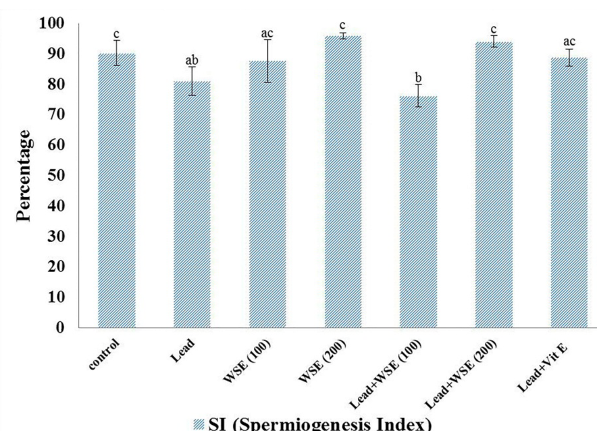

Figure 2: Mean ± SD and changes in SI index of the study groups. Different letters represent significant differences within the study groups (*P*<0.05). Values in parentheses are shown in mg/kg/day.

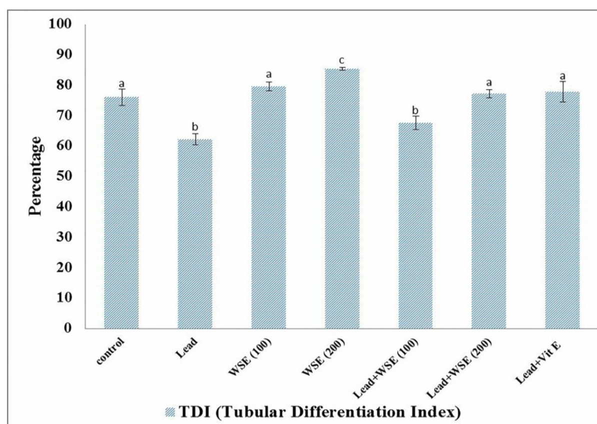

Figure 3: Mean ± SD and changes in TDI index of the study groups. Different letters represent significant differences within the study groups (*P*<0.05). Values in parentheses are shown in mg/kg/day.

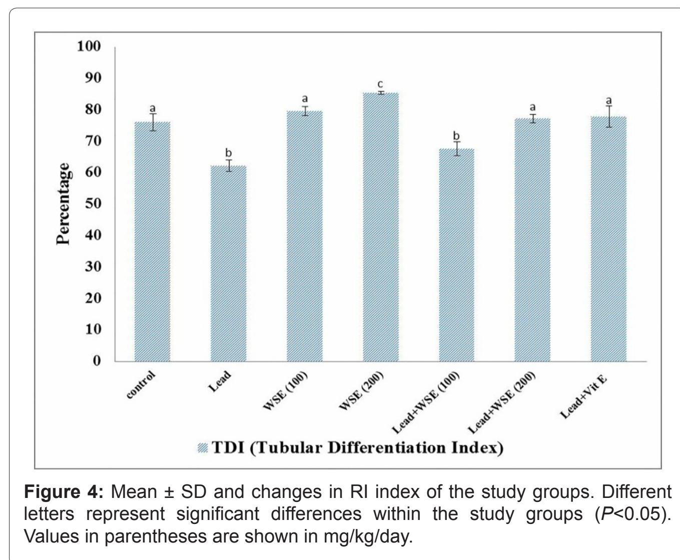

Figure 4: Mean ± SD and changes in RI index of the study groups. Different letters represent significant differences within the study groups (*P*<0.05). Values in parentheses are shown in mg/kg/day.

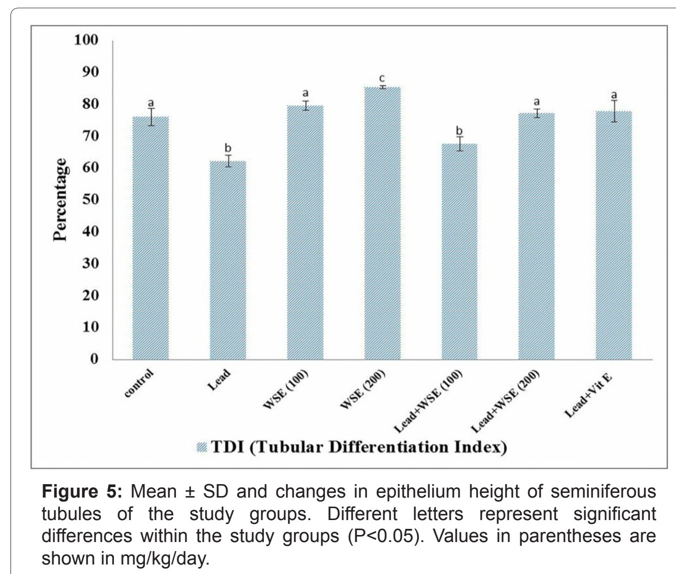

Figure 5: Mean ± SD and changes in epithelium height of seminiferous tubules of the study groups. Different letters represent significant differences within the study groups (P<0.05). Values in parentheses are shown in mg/kg/day.

(Figure 5). Diameter of seminiferous tubules in lead group showed a significant decrease, compared to that in control group (P<0.001). Diameter of seminiferous tubules in WSE (200 mg/kg/day) group revealed a significant increase, compared to that in groups received lead, combined lead and WSE (100 mg/kg/day), combined lead and vitamin E, and WSE (100 mg/kg/day) (P<0.01). Diameter of seminiferous tubules in combined lead and WSE (100 mg/kg/day) group (186.5 ± 10 µm) showed a significant decrease, compared to that in control group and groups received WSE (100 mg/kg/day), WSE (200 mg/kg/day), combined lead and WSE (200 mg/kg/day), and combined lead and vitamin E (P<0.001). Moreover, results have shown that WSE (100 mg/kg/day) cannot improve the diameter of seminiferous tubules against toxic effect of lead (Table 2). As shown in Table 2, minimum number of Leydig cells was seen due to exposure to lead acetate, compared to exposure to other compounds. An insignificant decrease was observed in Leydig cells in lead group, compared to that in control group. The majority of Leydig cells were seen in WSE receiving groups. The average number of Leydig cells showed a significant increase in WSE (200 mg/kg/day) group (13.5 ± 1.7), compared to that in control group and groups received lead, combined lead and vitamin E, and combined lead and WSE (200 mg/kg/day) (P<0.05). No significant difference was observed in size of Leydig cells in other groups.

Histometrical results of testicular tissue structure showed that the majority of Sertoli cells were observed in WSE (200 mg/kg/day) group, compared to that in other groups except combined WSE (200 mg/kg/day) and lead group (P<0.001). Lead group included the smallest number of Sertoli cells in seminiferous tubules (16.4 ± 0.8), compared to that in other groups except control group (P<0.001). It is worth to mention that number of Sertoli cells in seminiferous tubules in all treated groups with combined WSE and vitamin E significantly increased, compared to that in control group (P<0.01) (Figure 6). Statistical analysis of data showed that no significant difference was seen in capsule thickness of testes within the groups (Table 2). A significant finding in histometrical study included increased characteristics of testicular tissue structure in group receiving WSE (200 mg/kg/day) and lead, compared to that in combined lead and vitamin E group. Furthermore, significant increase was observed in Sertoli cells, RI index, height of germinal epithelium, and diameter of seminiferous tubules.

Results of testicular tissue section staining using ALP, PAS and Sudan black have revealed positive effects of prescription of dose dependent WSE on lead induced toxicity. In the current study, cytoplasm of Leydig cells, peritubular interstitial tissue and basement membrane reacted with PAS. As shown in Figure 7, PAS positive particles were seen in cytoplasm of upper series cells of germinal epithelium close to lumen of seminiferous tubules. Receive of combined lead acetate (20 mg/kg/day) resulted in no reaction in cells which were closed to basement membrane to PAS, especially in Sertoli cells. On the contrary, WSE (200 mg/kg/day) with or without lead resulted in a significant decrease in PAS positive particles in cytoplasm of upper series cells, compared to that in other groups. PAS positive particles were detected lesser in cytoplasm of upper series cells of germinal epithelium in combined lead and vitamin E group than that in lead group (Table 3). In seminiferous tubules, brown to black particles which contain lipid were barely seen inside the cytoplasm of cells close to lumen of seminiferous tubules, especially in Leydig cells. Prescription of 20 mg/kg/day of lead induced black particles containing lipid inside the cytoplasm of cells of lower series of germinal epithelium close to the basement membrane. A decrease was seen in volume of lipid deposit in Leydig cells exposed to lead. Furthermore, lipid particles were observed in cytoplasm of cells close to basement membrane. Dose dependent WSE caused increased reaction to Sudan black in Leydig cells and cells adjacent to lumen of seminiferous tubules. A majority of Sertoli cells included dense bodies and Sudan black positive bodies, especially in lead receiving groups. These dense bodies were mostly seen in cytoplasm of Sertoli cells and series cells close to the basement membrane of degenerated seminiferous tubules (Figure 8). ALP staining showed small brown to black particles in cytoplasm of damaged cells in testicular tissue sections. As shown in Figure 9, minimum rate of reaction in ALP staining was shown in control, WSE (100 mg/kg/day), WSE (200 mg/kg/day), and combined lead and WSE (200 mg/kg/day) groups. Maximum rate of reaction in ALP staining was shown in groups receiving lead acetate other than combined lead and WSE (200 mg/kg/day) group. ALP staining results showed small brown to black particles in cytoplasm of damaged cells in combined lead and WSE (100 mg/kg/day) group (Figure 9).

Sperm evaluation

Injection of lead acetate caused a significant difference in sperm count in groups, compared to that in control group (P<0.01). Sperm count in control group was calculated with an average of 69.6 ± 7.9 million ml^{-1}. Sperm count in lead group showed an average of 48.9 ± 9.1

Number of Leydig cells	Size of Leydig cells (µm)	Diameter of seminiferous tubules (µm)	Capsule thickness (µm)	Group
10.3 ± 0.8^{bc}	7.1 ± 1.1	252.4 ± 13.3^{ac}	31.8 ± 2.4	Control
9.5 ± 0.9^{b}	7.1 ± 0.2	206.6 ± 8.9^{b}	32.5 ± 6	Lead
12 ± 1.4^{ac}	8 ± 0.5	235.7 ± 11.2^{c}	28.9 ± 4.5	WSE (100*)
13.5 ± 1.7^{a}	8.2 ± 0.3	261.5 ± 18.2^{a}	33.2 ± 3	WSE (200*)
13.6 ± 1.3^{a}	7.1 ± 0.7	186.5 ± 10^{b}	35.6 ± 5.2	Lead + WSE (100*)
10.9 ± 1.2^{bc}	7.7 ± 0.7	268.4 ± 8.6^{a}	33.6 ± 3.1	Lead + WSE (200*)
10.6 ± 0.7^{bc}	7.3 ± 0.8	235.6 ± 12.4^{c}	32.3 ± 2.3	Lead + vit E
*mg/kg/day. Different letters represent significant differences between groups (*P*<0.05)				

Table 2: Characteristic changes in rat testes (mean ± SD).

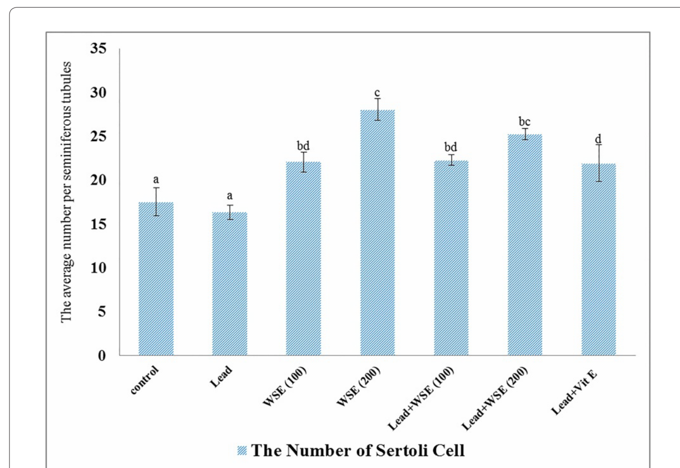

Figure 6: Mean ± SD and changes in number of Sertoli cells in the study groups. Different letters represent significant differences within the study groups (*P*<0.05). Values in parentheses are shown in mg/kg/day.

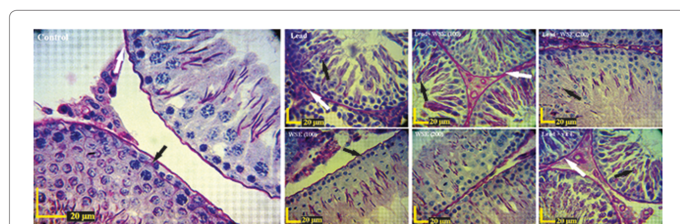

Figure 7: Histological structure of rat testis using PAS staining. Positive PAS particles were seen inside cytoplasm of spermatogenesis cells closed to basement membrane in control, combined lead and WSE (200 mg/kg/day), WSE (100 mg/kg/day) and WSE (200 mg/kg/day) groups. Furthermore, positive PAS particles were shown inside cytoplasm of cells closed to lumen of seminiferous tubule, combined lead and WSE (100 mg/kg/day), and combined lead and vitamin E groups. Black arrows show positive PAS particles. PAS positive reactions in basement membrane are shown by white arrows. Values in parentheses are shown in mg/kg/day.

Group	PAS intensity	ALP intensity
Control	+1	+1
Lead	+5	+5
WSE (100*)	+1	+1
WSE (200*)	+1	+1
Lead + WSE (100*)	+5	+3
Lead + WSE (200*)	+2	+2
Lead + vit E	+4	+3
*mg/kg/day		

Table 3: Qualitative evaluation of PAS and ALP staining of testicular tissue among the study groups. Nos. 1-5 were used to show minimum and maximum intensity levels of PAS and ALP staining.

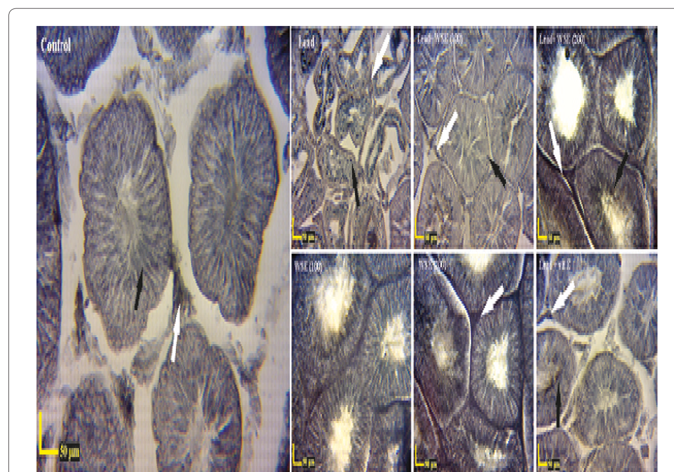

Figure 8: Histological structure of rat testis using Sudan black staining. Sufficient amounts of lipids were seen in Leydig cells (white arrows) and spermatogenesis cells (black arrows) in control group. Combined Lead and WSE (200 mg/kg/day), WSE (100 mg/kg/day) and WSE (200 mg/kg/day) groups showed an increase in storage amount of lipids in all cells. On the contrary, lead, combined lead and WSE (100 mg/kg/day), and combined lead and vitamin E groups showed a decrease in storage amount of lipids in all cells. Values in parentheses are shown in mg/kg/day.

million ml^{-1}. Sperm count showed an increase due to dose dependent WSE. Sperms in combined lead and WSE (200) group were calculated as 54 ± 7.34 million ml^{-1}. Most number of sperms (average number of 78.1 ± 7.5 million ml^{-1}) was reported in WSE (200 mg/kg/day) group. No significant difference was seen in sperm count in WSE (200 mg/kg/day) group, compared to that in control group. Whereas, sperm count in WSE (200 mg/kg/day) group showed a significant increase, compared to that in other groups (P<0.001) (Figure 10). As shown in Figure 11, sperm motility showed a significant decrease in lead group (average percentage of 36.2% ± 4.3) compared to that in other groups (P<0.001). In general, 76% ± 5.1 and 64.2% ± 6.4 of all sperms were motile in WSE (100 mg/kg/day) and WSE (200 mg/kg/day) groups, respectively. Sperm motility in WSE (200 mg/kg/day) group showed a significant increase, compared to that in control group (60% ± 4.3) (P<0.01). A significant difference was seen in sperm motility in groups receiving WSE (200 mg/kg/day) and WSE (100 mg/kg/day) (P<0.05). Sperm viability showed a significant increase in groups receiving dose dependent WSE, compared to that in other groups except control group (P<0.001). Furthermore, sperm viability showed a significant decrease in lead group (61.4% ± 6.1), compared to that in control group (87.6% ± 4) (P<0.001). Sperm viability showed a significant increase in combined lead and WSE (200 or 100 mg/kg/day) group, compared to that in control group (P<0.001) (Figures 12 and 13).

Testicular TBARS assessment

Testicular TBARS assessment showed a significant increase due

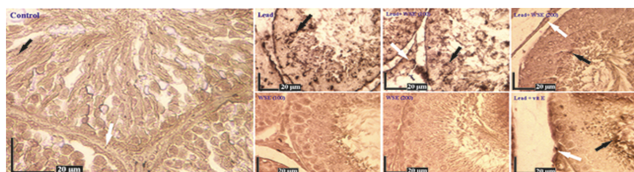

Figure 9: Histological structure of rat testis using ALP staining. Reaction to ALP staining (black arrows) was seen rarely in control group. Lead, combined lead and WSE (100 mg/kg/day), and combined lead and vitamin E groups showed progressively more severe reactivity to ALP staining. On the contrary, combined lead and WSE (200 mg/kg/day), WSE (100 mg/kg/day) and WSE (200 mg/kg/day) groups showed decreased ALP reactivity. White arrows show ALP reactivity inside cytoplasm of Leydig cells. Values in parentheses are shown in mg/kg/day.

to exposure to lead acetate (1.61 ± 0.15 µmol), compared to that in control group (1.1 ± 0.1 µmol) (P<0.05). Testicular TBARS showed an insignificant decrease in combined lead and vitamin E (1.53 ± 0.28 µmol), and combined lead and WSE (100 mg/kg/day) (1.54 ± 0.37 µmol) groups, compared to that in lead group. Testicular TBARS showed an insignificant increase in combined lead and vitamin E, and combined lead and WSE (100 mg/kg/day) groups, compared to that in control group. WSE (200 mg/kg/day) group included the smallest value of TBARS, compared to that in lead, combined lead and WSE (100 mg/kg/day), and combined lead and vitamin E groups (P<0.05) (Figure 14).

Liver lead assessment

Findings of lead assessment verified the presence of lead in liver and body of rats exposed to 20 mg/kg/day of lead acetate. Liver lead assessment showed a significant increase in lead group, compared to that in control group and groups receiving WSE (P<0.01). Furthermore, liver lead showed an insignificant decrease in combined lead and WSE (200 or 100 mg/kg/day), and combined lead and vitamin E, compared to that in lead group. Whereas, liver lead showed an insignificant increase in combined lead and WSE (200 or 100 mg/kg/day) and combined lead and vitamin E, compared to that in lead group (Figure 15).

Serum Testosterone Assessment

Serum testosterone showed a significant decrease in lead group, compared to that in control group (P<0.001). Serum testosterone revealed an insignificant decrease in groups that received lead acetate, compared to that in other groups. The highest rate of testosterone was seen in WSE (200 mg/kg/day) group. Serum testosterone results showed a significant increase in WSE (200 mg/kg/day) group, compared to that in lead, combined lead and WSE (100 mg/kg/day), and combined lead and vitamin E groups (P<0.01) (Figure 16).

Discussion

Humans are exposed to a variety of environmental contaminations. In recent decades, environmental contamination by heavy metals has increased extensively as modern industries rapidly develop. One of these heavy metals is lead. Negative effects of environmental chemical factors (e.g. lead) on male genital system have been considered intensively [7,31]. Exposing to lead threatens human and animal health [32]. Occupational exposure to lead in men, especially in professional workers, results in disorders including infertility [33]. Many studies have shown that genital toxicity is a dominant characteristic of lead toxicity, including destruction of spermatogenesis and germinal epithelium apoptosis in testis [34-36]. Rubio *et al.* (2006) have stated that severe effects of lead on function of male genital system occur through changes in spermatogenesis and sperm function [37]. Various

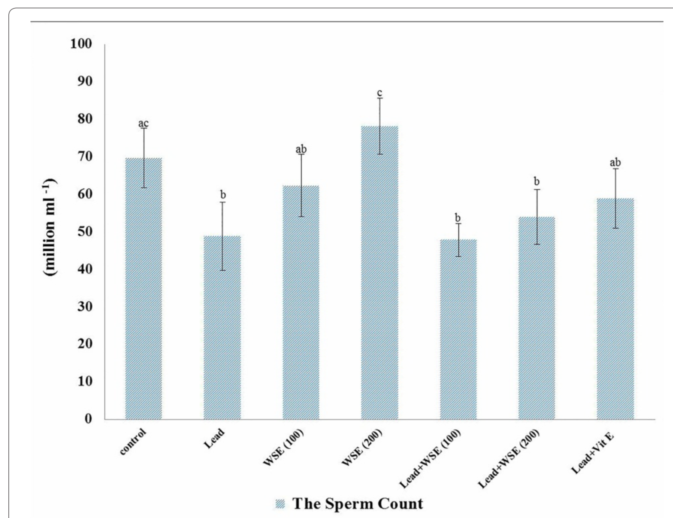

Figure 10: Mean ± SD and changes in sperm count of the study groups. Different letters represent significant differences within the study groups (*P*<0.05). Values in parentheses are shown in mg/kg/day.

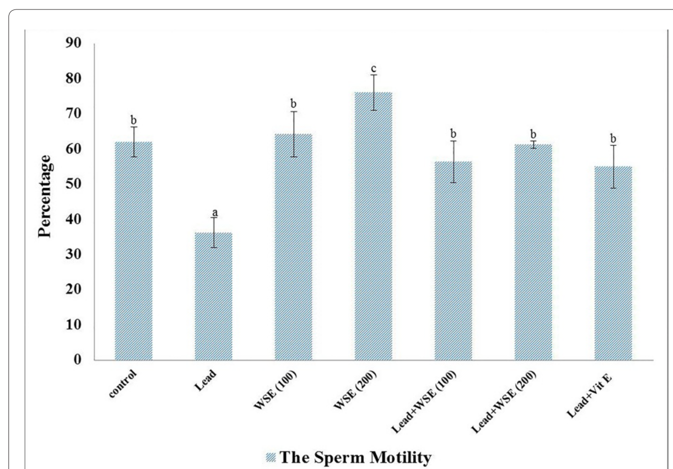

Figure 11: Mean ± SD and changes in sperm motility of the study groups. Different letters represent significant differences within the study groups (*P*<0.05). Values in parentheses are shown in mg/kg/day.

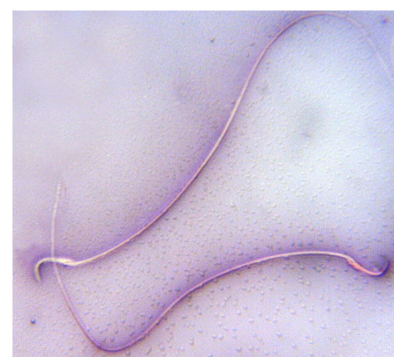

Figure 12: Eosin–nigrosin staining: Live sperms are unstained (1); dead sperms are stained pink or red (2)

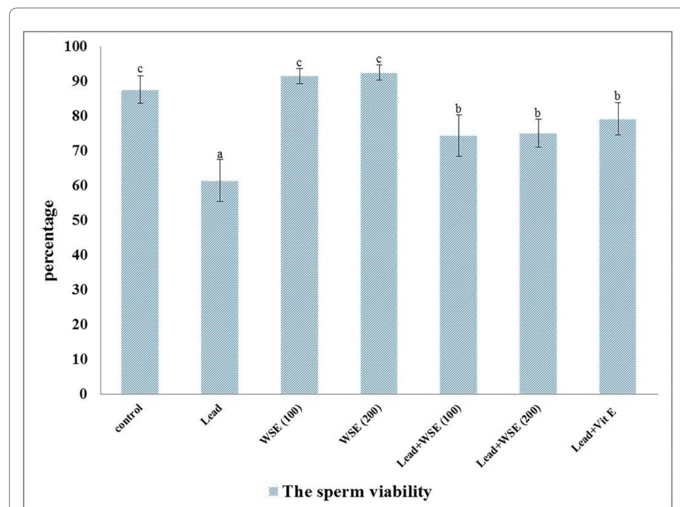

Figure 13: Mean ± SD and changes in sperm viability of the study groups. Different letters represent significant differences within the study groups (P<0.05). Values in parentheses are shown in mg/kg/day.

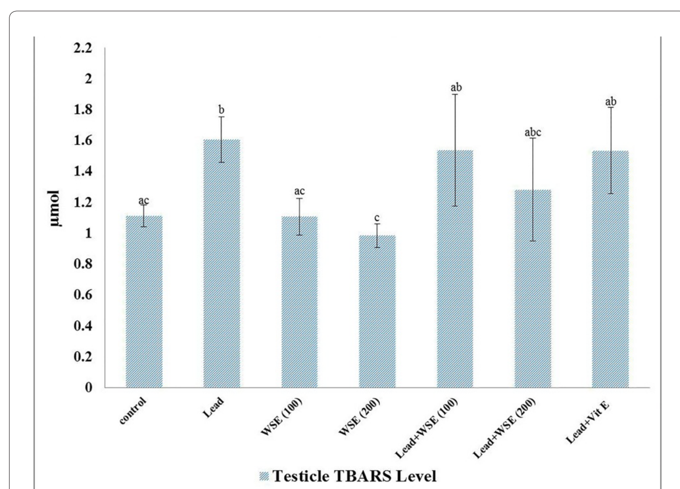

Figure 14: Mean ± SD and changes in testicle TBARS level of the study groups. Different letters represent significant differences within the study groups (P<0.05). Values in parentheses are shown in mg/kg/day.

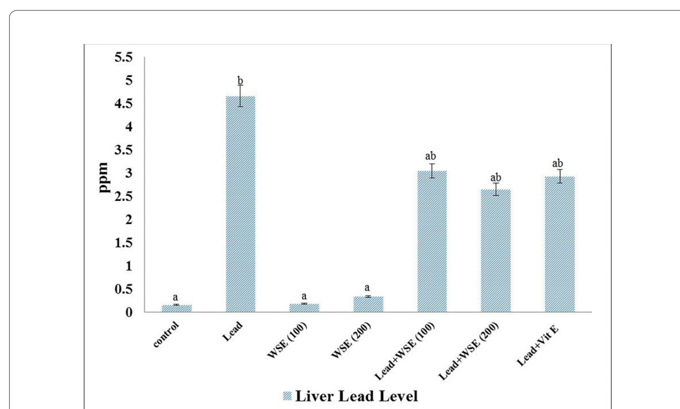

Figure 15: Mean ± SD and changes in liver lead level of the study groups. Different letters represent significant differences within the study groups (P<0.05). Values in parentheses are shown in mg/kg/day.

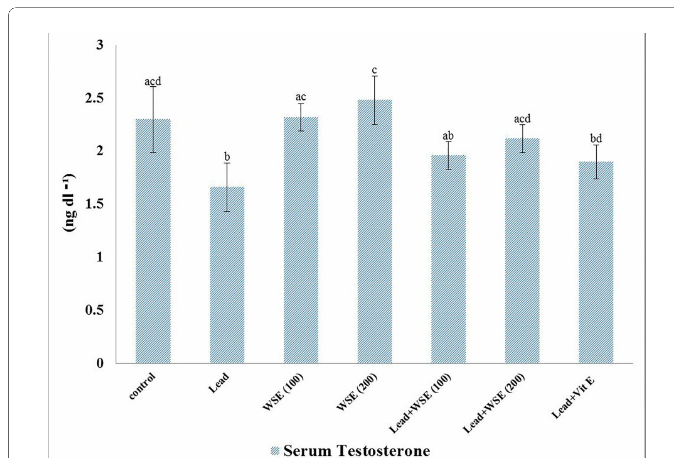

Figure 16: Mean ± SD and changes in serum testosterone level of the study groups. Different letters represent significant differences within the study groups (P<0.05). Values in parentheses are shown in mg/kg/day.

theories have been published on effect mechanisms of lead. Results of the current study have shown that exposure to lead results in disorders in male genital system including extensive degenerative changes, inactivation of spermatogenesis, decreased diameter of seminiferous tubules, and severe edema in connective tissue and decreased germinal epithelium height in rat testes. Furthermore, intraperitoneal injection of 20 mg/kg/day of lead acetate for 35 days cause a significant decrease in count, motility and viability of sperms in rats, compared to control group.

Overall, results of the lead effects on sperms in the current study are similar to those from other recent investigations [5,7,9,38]. Significant decreases in Sertoli and Leydig cells, height of germinal epithelium and diameter of seminiferous tubules in rats have been reported [5,9,38]. Epithelium height and seminiferous tubule diameter decreased following reduced metabolic activity of germinal cells and cell number. This caused increased interstitial space and consequently tissue edema [30]. Damaged DNA or increased apoptosis in germinal cells of seminiferous tubules are thought to be one of the effect mechanisms of lead [34,39]. Another mechanism is suppression of hypothalamic-pituitary-testicular axis [9]. Reactive oxygen species (ROS) is another mechanism for tissue damages induced by heavy metals [40]. ROS causes detachment and fluidity of cell membrane structure through oxidation of lipids [5]. ROS was first investigated in sperms [41]. In the current study, significant increase in TBARS level suggests that oxidative stress is a major mechanism of lead effect in testicular tissues. However, results of TBARS assessment showed a significant decrease in groups receiving dose dependent WSE. Therefore, it can be concluded that WSE plays a protective role through its antioxidant activity. It has been reported that one of the main reasons for infertility in individuals is excessive production of ROS or reduction of antioxidant activity in semen which causes oxidative stress and consequently increased death and decreased number and motility of sperms [42]. In this study, spermatogenesis and spermatogenesis indices, including TDI, SI and RI, showed a significant decrease due to lead induced oxidative stress, compared to control group. Induction of oxidative stress causes significant decrease in TDI, RI and SI indices in seminiferous tubules of testes in mice; as seen in the current study [30,43]. In the current study, prescribing lead acetate resulted in insignificantly decreased body weight, volume and weight of rat testes, compared to control group. Weight of genital organs, especially testes, decreases in oxidative stress;

therefore, weighing testes is one of the most important parameters in toxicological studies. In similar studies, it has been reported that oxidative stress induced by lead causes decreased body weight, and net and relative weight and volume of testes in rats [7,9,30,38]. However, Asadpour *et al*. (2013) reported that lead included no effects on body and testis weight in rats [5]. After 35 days in the present study, WSE (200 mg/kg/day) and combined lead and vitamin E groups showed the heaviest body weight, compared to that of other groups. Antioxidant compounds can increase body weight and volume and weight of genital organs [7,9,29,30].

The current study is the first study on wheat sprout extract (WSE) use for protection and prevention of lead toxicity in spermatogenesis, sperm parameters and tissue structure of testes. Results of this study showed that dose dependent prescription of WSE for 35 days included protective effects on sperm parameters (count, motility and viability) and tissue structure of testes in rats exposed to oxidative stress induced by lead, compared to other groups. Sperm count in epididymis can reflex meiosis and spermatogenesis in testes. This is one of the most sensitive tests for evaluation of spermatogenesis [44]. In the current study, capsule thickness of testes showed an insignificant change. Indices of RI, TDI and SI were calculated for spermiogenesis and spermatogenesis. Results have shown that dose dependent prescription of WSE causes an increase in TDI, RI and SI indices, compared to those in other groups. WSE (200 mg/kg/day) can completely inhibit toxic effects of oxidative stress induced by lead in tissue structure of testes. WSE is rich in vitamins, minerals and phytoestrogen and antioxidant compounds. Moreover, WSE contains magnesium, zinc, calcium, vitamin E, vitamin C, folic acid, thiamin, riboflavin, iron, niacin and vitamin B_{12} [45,46]. Direct effects of WSE have not been reported on disorders in male reproductive system and sperm parameters induced by lead. However, effects of elements, minerals and vitamins have been shown on disordered male reproductive system and sperm parameters due to lead. In the current study, prescription of combined vitamin E and lead resulted a significant increase in parameters including seminiferous tubules diameter, number of Sertoli cells and motility and viability of sperms. Positive effects of vitamins E, C, folic acid, thiamin, niacin and B_{12} have been reported in genital system and sperm fertility parameters in males exposed to lead [5-7,47,48]. Another determinant of cell damage by lead is ALP enzyme. Damages to cell membrane increase release of ALP into the cell and serum. Thus, assessment of ALP enzyme is used as indicator for damages in testicular tissues [30]. In the current study, findings of ALP staining in WSE groups revealed a significant decrease in color depth using dose dependent WSE, compared to that in lead group. Normally, spermatogenic cells closed to basement membrane of seminiferous tubules contain carbohydrate resources; whereas, cells closed to luminal space of seminiferous tubules use lipids for their metabolism. In disorders in metabolic cycle, cell metabolism changes consequently. In such a condition, cell uses other nutritional sources for its metabolism [30].

Results of the current study showed an increase in PAS positive particles (carbohydrates) in cells closed to lumen of seminiferous tubules in groups that received lead. Moreover, a decrease was seen in Sudan black positive particles (lipids) in Leydig cells and spermatogenic cells closed to basement membrane. These results indicate disorder in metabolism of testicular cells due to lead exposure. WSE with doses of 200 mg/kg/day can play a complete protective role against toxic effects of lead. Therefore, WSE can induce a normal metabolism in testicular cells exposed to lead. Sudan black staining showed that a majority of Sertoli cells included Sudan black positive bodies in groups that received lead. These dense bodies were mostly observed in cells closed to basement membrane of degenerating seminiferous tubules. Some of important roles of Sertoli cells include phagocytosis of degenerating spermatogenic cells and removing residues of spermatid cells. These residues are combined with lysosomal enzymes and hence called lipofuscin; distinguished by yellowish-brown pigments in Sudan-black staining [49]. In the present study, these dense bodies were seen in Sertoli cell cytoplasm possibly due to excessive phagocytic activity of degenerating spermatogenic cells induced by lead oxidative stress. Assessment of serum testosterone showed a significant decrease in lead group, compared to that in control group. Furthermore, reduction in number and chromophily of Leydig cells in Sudan black staining indicated a decrease in activity of these cells due to oxidative stress. Dorostghol *et al*. (2013) have reported that oxidative stress causes a decrease in serum testosterone in rats exposed to lead; as shown in the current study [9]. In the current study, serum testosterone increased in groups receiving dose dependent WSE or vitamin E. Increased serum testosterone due to medicinal herbs, antioxidant and mineral substances such as vitamins C and E, and zinc has been reported in various studies [6,9,26,29]. Moreover, Leydig cells showed an increase in groups receiving vitamin E or WSE. Ultrastructure study of Sertoli and Leydig cells by electron microscopy can be used to better understand the action mechanisms of WSE, vitamin E and lead acetate.

Conclusions

In summary, it can be concluded that 20 mg/kg/day of lead for 35 days can induce oxidative stress in testicular tissues in rats. On the contrary, dose dependent WSE can inhibit toxic effects of lead in testicular tissues. Results of the current study shows that oral consumption of WSE include a higher inhibitory effect on oxidative stress in testicular tissues in rats exposed to lead than that of vitamin E with doses of 100 mg/kg/day.

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