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Assessment of Fish Oil Effects on the Testicular Structure of Male Rats

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Abstract: The aim of this study was to investigate the protective effects of fish oil on lead acetate induced testis toxicity in rats. Forty male rats were randomly divided into 4 groups, 10 rats in each. Control rats received normal saline, while treated rats received lead acetate (150 mg/kg body weight) three times weekly, lead acetate and fish oil (650 mg/kg body weight/day) and fish oil (650 mg/kg body weight/day) for six weeks by gavage tube. The results showed an insignificant increase in mean testis weight with highest and lowest testis weight gained was of 3.09±0.31g and 2.82±0.76 g respectively. Testicular histology of rats treated with fish oil revealed slight changes in the uniformity of arrangements of seminiferous tubules. Data from present study suggests that fish oil have been initiating the positive effects on testicular normal structure in lead acetate treated rats.

Key words: Fish oil · Lead acetate · Testis histology structure

INTRODUCTION

The various deleterious effects on our health today are due to heavy metal exposure distributed in the environment and it is considered as one of important issues concern around the world. Lead is one of the important abundant toxic metals and industrial pollutants in the earth. The widespread of lead through the Earth's crust occurs naturally in the environment [1]. However, most of the high concentration found in the environment comes from human- caused processes. Some processes could include volcanic emissions, forest fires and various erosion processes. Similarly, human activity, particularly mining of metallic ores, leads to concentration and dispersion [2]. Gonads structures and functions are main target for environmental toxins. One of these toxicants is Pb, it is concedered one of the very harmful materials to testicular function [3].

Testis is composed of seminiferous tubules and interstitial tissues.

Seminiferous tubules are the site for spermatogenesis and they contain three types of cells: male germ cells, Sertoli cells and peritubular myoid cells, while Leydig cell are located between neighboring seminiferous tubules [4]. According to developmental progression at the base of the seminiferous tubules have spermatogonia, spermatocytes in the middle, and spermatids near the apex of the seminiferous epithelium [5]. Pb is considered as one of the toxic heavy metals and the most dangerous environmental pollutants. Therefore, it has been a big concern for human health. Recently there is increase in the levels of Pb in the environment compared to that recommended by the World Health Organization due to human activities [6].

Lead may inhibit spermatogenesis and reduce young spermatids, spermatocytes, and mature spermatids [7]. Gonadal dysfunction and congenital malformation are the main alterations caused by these substances in the male reproductive system [8].

Similar results were also found in studies of Pb as heavy metals harmful effects to the body when the organism is exposed to high levels of these metals [9].

Fish oil contains long-chain poly unsaturated fatty acids (n-3 PUFA) most important are the omega-3 fatty acid Docosahexaenoic acid, or (DHA), it is believed to play a main role in the development of human body systems, Eicosapentaenoic acid, or (EPA) and Alpha-linoleic acid (ALA) [10]. Fish oils are considered one of the most public dietary supplements in the world. In the United States, more than a third of the 17.7% of dults who use dietary supplements take fish oil [11,12].

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The Consumers take fish oil supplements for many reasons, the major kind is because it has shown promising effects of lowering inflammation [13]. Numerous studies in mice have supported the beneficial roles of fish oil consumption. Succinctly, in mice, fish oil may have beneficial effects on arthritis, cancer, cardiac arrhythmias and on bone mass during aging [14].

It might be no data available on the protective effect of fish oil against the toxicity of heavy metals on male testicular tissue structure. Administration of fish oil before exposure to lead could reduce many of its side effects. Therefore, the present study was carried out to investigate the protective role of fish oil against the effect of lead acetate on testicular male albino rats.

MATERIALS AND METHODS

Ethical Approval: This experimental study was approved by the Ethical Committee of the Animal Care and King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia.

Animals and Housing: A total number of 40 male albino rats of the Wistar strain (*Rattus norvegicus*), were used in the present study and their weight ranged between 200 and 250g. The experimental animals were obtained from the Experimental Animal Unit of King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia. Food and water were supplied *ad libitum* on normal commercial chow. Rats were housed at a controlled temperature of (20±1°C), 65% humidity and under a 12 h light: 12 h dark schedule.

Experimental Design and Treatment: After the acclimatization period the animals were divided into 4 groups. The first one (n = 10) were used as control and received only normal saline (0.9 ml) every day during the experiment period. The second one (n = 10) were administrated lead acetate at concentration of 150 mg/kg body weight of 1% solution by gavage tube, 3 times weekly. The third one (n = 10) were administrated lead acetate at the same dose given to group 2 of 1% solution and fish oil (650 mg/kg body weight/day) by gavage tube for six weeks. The last group (4) was orally supplemented with fish oil at a dose of 650 mg/kg body weight/ day.

Organ Weight Measurement and Histological Analysis: At the end of the study period (seven weeks), rats were euthanized and organs were dissected. Testes glands are removed and the weight was measured for each rat in treated and control groups.

Histological Analysis: The testis tissues were preserved in 10% formalin immediately after rats dissected and removal from the animals. Dehydrated the testis tissues, through ascending grades of isopropyl alcohol by immersed in 80% isopropanol overnight and 100% isopropyl alcohol for one hour. The dehydrated tissues were cleared in two changes of xylene, one hour each. Embedded the impregnated wax tissues in paraffin blocks, used the same grade of wax. Then embedded the paraffin blocks and cut with rotary microtome at 4 micron thickness. The testis tissues were floated on a tissue floatation bath at 40°C and taken on glass slides and smeared with equal parts of egg albumin and glycerol. Then the samples were melted in an incubator at 60°C and allowed to cool after 5 minutes. The tissues through immersing it in xylene for 10 minutes in horizontal staining jar. After that the samples were washed in 100% isopropyl alcohol and stained in Ehrlich's hematoxylin for 8 minutes in horizontal staining jar. The staining samples were washed in tap water and dipped in acid alcohol (8.3% HCl in 70% alcohol). Then the tissues were placed in running tap water for 10 minutes for bluing (slow alkalization). Then counter stained in 1% aqueous eosin for one minute, the excess stain was washed in tap water. After that the samples were placed for 5 minutes in the incubator at 60°C. The samples were cooled and mounted in DPX mount having to optical index of glass, then wetted it in xylene and inverted on to the mount and placed on the coverslip [15]. In the microscope unit at King Fahd Medical Research Center the specimens were examined using light microscope (Olympus BX61- USA) with motorized controller unit (Olympus BX-UCB- USA) and photographed by a camera (Olympus DP72- USA).

Statistical Analysis: Statistical Package of Social Science (SPP version 21.0) was used for data management and analyses. Obtained data were statistically analyzed by one way ANOVA to compare the mean of studied parameters in all subject groups. The value of $p<0.05$ is considered as significant.

RESULTS

Body Weight Gain: Following seven weeks treatment, obtained data of SD rats from experimental groups indicated an increasing pattern in mean daily body weight (Fig. 1). Initially they were weighing between 200-250 g, but it was found that there were slight enhancements in the body weight measures towards the end of the experiment, with the highest increment was observed in

Table 1: Average testis weight in all treatment groups

Groups (Treatments)	Weight (gr)	p-Value
G1 Control (mean)	2.94±0.36	-
G2 Lead (mean)	3.09±0.31	0.17
G3 Fish Oil (mean)	3.08±0.26	0.21
G4 Lead+Fish Oil (mean)	2.82±0.76	0.35

Each group data compared with G1 control group. Data are expressed as Mean±SD, (n=10) and p-value is from comparison to control, $p<0.05$ = significant.

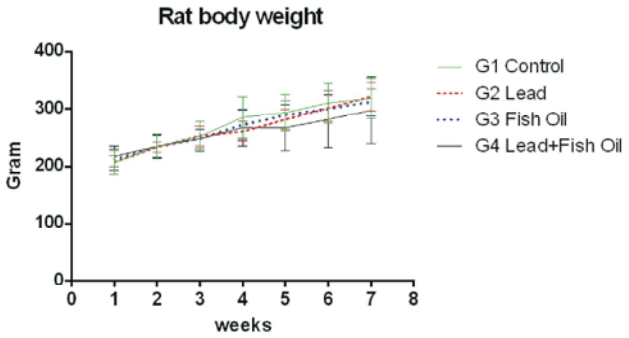

Fig. 1: Trend of body weight (g) gain of experimental groups. Net body weight increment in each experimental group is as follows; control (G1) (103.8±13.88 g), lead acetate group (G2) (94.1±11.57 g), lead acetate and fish oil group (G3) (88.7±6.03 g) and fish oil group (G4) (64.06±32.41 g).

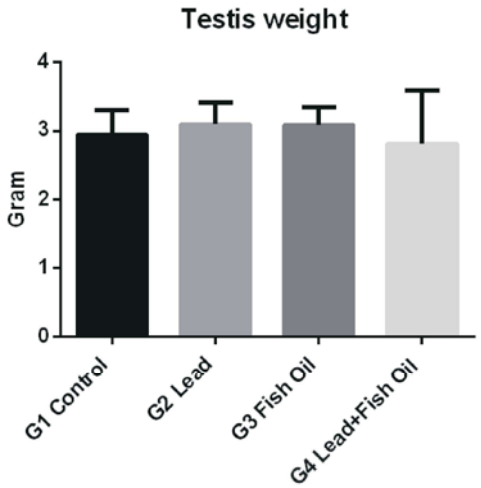

Fig. 2: Slight deterioration in testicular weight in all treatment groups compared to control group.

control group with addition of 103.8 g and the least body weight gained was from fish oil group with increment of only 64.6 g.

Histological Analysis of Testes: Result of testes histology in control group showed normal and undisturbed pattern in the arrangement and shape of tubules (Fig. 3). The connective tissue septa arrange the testicular histological structure of lobules. Each of these lobules has seminiferous tubules. Control group showed normal arrangement of seminiferous tubules. The layers of tubules ordered from outer to inner side with germinal epithelium including spermatogonia with sertoli cells, primary spermatocytes, secondary spermatocytes and spermatids respectively. All of these cells faced to lumen of seminiferous tubules.

Histological of testicular rats result from the control compared to experimental groups showed a range of differences in the shape and uniformity in tubular arrangement.

As seen from the obtained results, histological changes in treated tissues group with Pb revealed changes appeared in non-intact arrangements of seminiferous tubules, this lead to a wide range space between the tubules. Moreover, Pb treated rats showed several abnormalities involving the structure of the tubules change to oval-like shape, lipid vacuolation, interstitial edema and absence of primary, secondary spermatocytes and spermatids spermatogonia (Fig. 4). Beside this conformation, changes in the tubular structure results in an elongation of the tubules thus increase the diameter of seminiferous tubules with shrinkage in tubular size resulting in reduction of spermatogonial cells available for development (Fig. 5). Furthermore, increment of space between germ cells was detected (Fig. 6).

Reduction in the diameter of seminiferous tubules and detachment of the basement membrane was showed in testis of group three (Pb plus fish oil) treated rats. In addition, same histological shape and abnormalities with slight alterations in comparison with Pb treated group. Moreover, lipid vacuolation and the absence of the germinal epithelium was detected (Fig. 7 & Fig. 8).

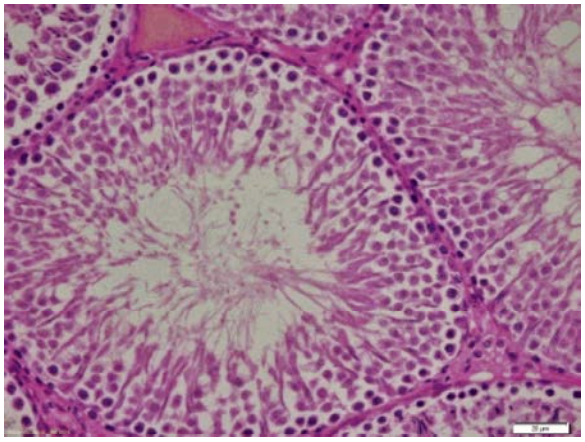

Fig. 3: Histological photomicrograph of testis tissue of control group showing normal histological structure of active mature functioning seminiferous tubules with complete spermatogenic series: Spermatogonia, sertoli cells, primary spermatocyte, secondary spermatocyte, spermatids and normal spermatozoa (H&E, X 400).

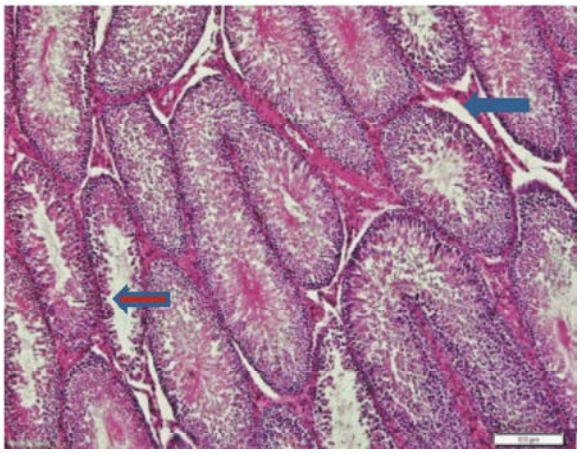

Fig. 4: Histological photomicrograph of testis tissue of Pb treated rats showing absence of spermatogonia, sertoli cells, primary and secondary spermatocyte and spermatids (red arrow), more obvious congestion of blood vessel of testis, elongation of the tubules and lipid vacuolation (blue arrow), (H&E, X 200).

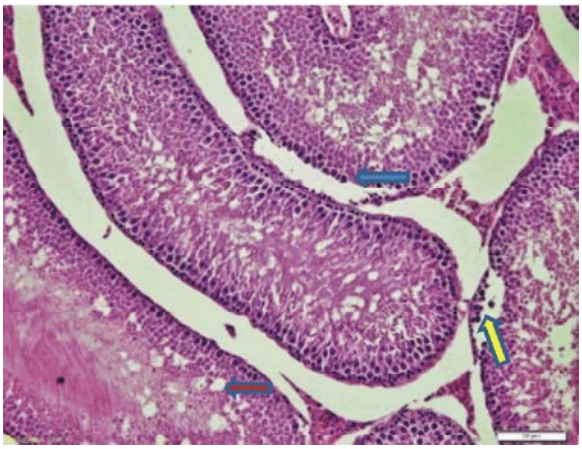

Fig. 5: Histological photomicrograph of testis tissue of Pb treated rats showing more obvious lipid vacuolation, reduced diameter of seminiferous tubule (red arrow) and detachment of the basement membrane (blue arrow), dilated and damage in seminiferous tubule (yellow arrow) (H&E, X 100).

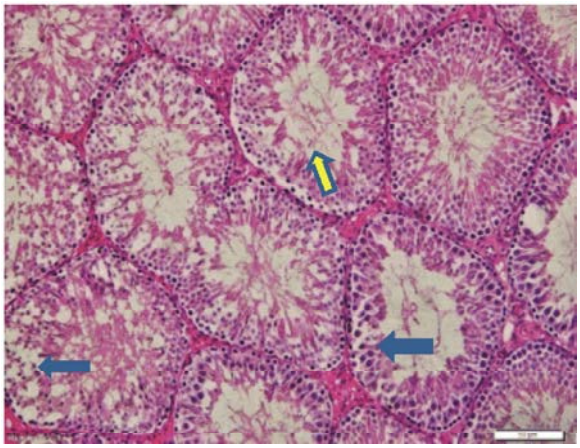

Fig. 6: Histological photomicrograph of testis tissue of Pb treated rats showing empty space between germ cells, absence of tail of spermatozoa in the lumen of seminiferous tubule (yellow arrow) (H&E, X 200).

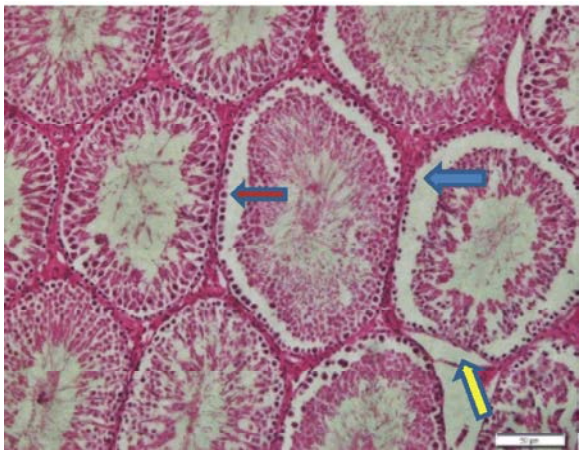

Fig. 7: Histological photomicrograph of testis tissue of Pb plus fish oil treated rats showing absence of the germinal epithelium (red arrow), slight tubulointerstitial congestion, basement detachment with reduced seminiferous tubules (blue arrow) and lipid vacuolation (yellow arrow) (H&E, X 200).

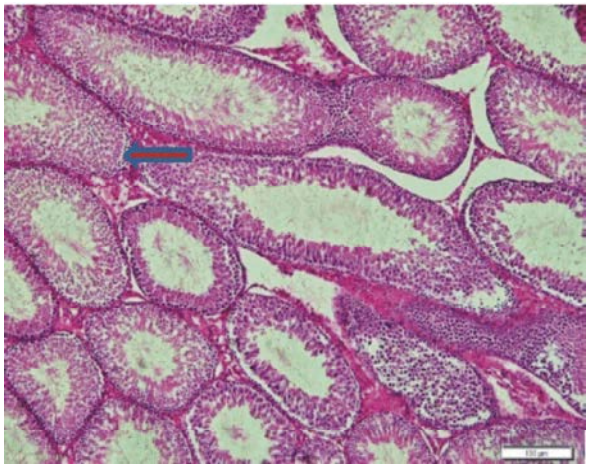

Fig. 8: Histological photomicrograph of testis tissue of Pb plus fish oil treated rats showing a slight empty space between germinal epithelium (H&E, X 400).

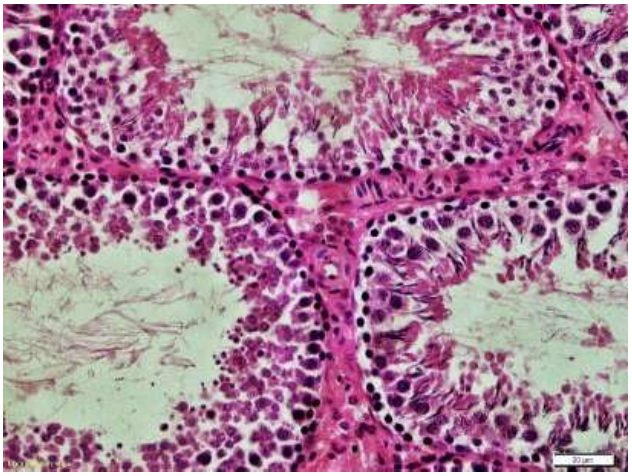

Fig. 9: Histological photomicrograph of testis tissue of fish oil rats showing normal layered germinal epithelium and normal spermatozoa (H&E, X 400).

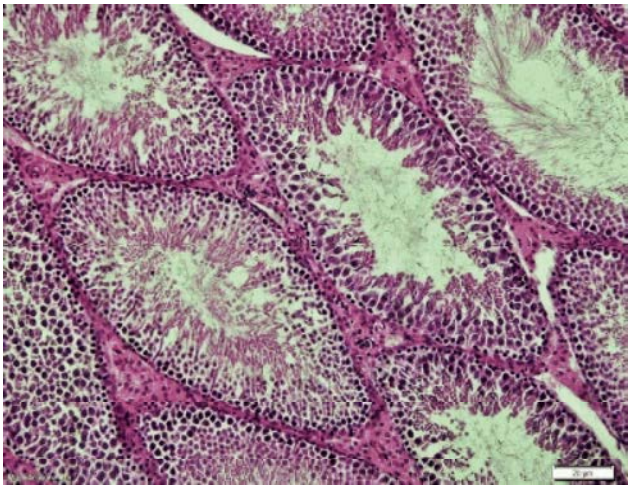

Fig. 10: Histological photomicrograph of testis tissue of fish oil treated rats showing normal appearance of layered germinal epithelium, normal spermatozoa location and creation, appearance of leydig cells and normal seminiferous tubules diameter (H&E, X 400).

The seminiferous tubules in testes from fish oil treated rats were observed to remain in circular form, just as observed in control group, but with slight elongation of the tubules. However, the arrangements of seminiferous tubules were observed to be similar in both Pb and Pb with fish oil treated groups where both exhibit the appearance of wide interstitial spaces. In fish oil treated rats the seminiferous tubules appeared in normal shape and uniformity in tubular arrangement. The layered germinal epithelium of spermatogonia, primary spermatocytes, secondary spermatocytes showed in normal structure and arrangement. The diameters of seminiferous tubules are normal diameters beside the position of leydig interstitial cells appeared same as control groups (Fig. 9 & Fig. 10).

DISCUSSION

The obtained data from present study suggest rats treated with Pb at a dose of 150 mg/kg body weight, induced some histological alterations in the testis tissue of male Wister rat. These results suggest that treated rats have begun to get affected by exposure of Pb. This is because there are signs of negative effects found in testes of experimental rats, thus providing supportive findings from this study. The acquired data from present experiment obviously depicted that Pb changes normal state of testicular histology, basically, led to several changes in the diameters of seminiferous tubules and their structure. The increment in body weight and testis weight following the exposure of Pb in all

treatment groups that would have been expected and are in general agreement with other research, as previously reported [16 , 17].

Meanwhile, histological results of testes from rats treated with Pb and Pb plus fish oil showed effects on seminiferous tubules including differences in normal diameters, degeneration of spermatogonia, primary and secondary spermatocytes and spermatids of most seminiferous tubules. Where these changes will definitely results in abnormality of normal testicular functions. However, these changes in testis structure slightly less when compared to Pb group. For instance, the cellular damage induced by Pb exposure does affect the decrement of spermatogenesis and the disturbance in appearance, arrangement and distribution of normal spermatogonial cells in Pb plus fish oil group, which were found to vary within the tubular among different treated rats groups. The contents of seminiferous tubules are the primary pointer to testicular weight and the possibility of spermatogenesis that occurs in testis [18]. Besides, the differences appeared in normal diameters, degeneration of spermatogonia in tubular structure leads to a chance whereby the placements of the testicular tubules are seen to be disaggregated and moving away from each other with lipid vacuolation. This leads to increment of spatial interstitial area between the tubules and in term of long-term probably has influence on the function of entire testicular functions.

The reproductive system is always vulnerable to damage caused by Pb toxicity and this damage appears in the form of a reduction in sperm concentrations was found with both random and fixed-effect models after Pb exposure [19]. Pb is considered one of the nephrotoxicants and can generate reactive oxygen species (ROS). Moreover, the pathogenesis of Pb nephrotoxicity that led to oxidative stress due to disrupting the delicate prooxidant/antioxidant balance within mammalian cells [20 - 23]. A reduction in the levels of antioxidant enzymes activities was found as the other causes of cellular damage in rats exposed to lead, which leads to increment the oxidative stress in testis treated tissue resulting [24]. Result in rats groups exposed to lead showed negative testicular development cells due to the increment in highly reactive cytotoxic compounds formation [25]. Beside that Russ *et al.* [26] who worked on Pb toxicity found that the antioxidant effect of grape seed oil has a protective factore against Pb toxicity on the rat's testicular tissues.

The testicular damage include degeneration with loss of spermatogenic series in the seminiferous tubules obtain when male albino rates exposed to lead acetate at a dose of 1.5 g/L for 8 weeks induced [16].

Examination by the light microscope showed that supplementing rats with fish oil enhanced the histopathological damage induced by Pb toxicity. This reflects the positive effect of fish oil in improving tissue of rats' testis and preventing it against Pb toxicity. Food supplement fish oil gives an effective effect to reduce the contusion injury in the muscles of aging. In addition when use as a supplement food it works as an anti-coagulant and pro-oxidant [27].

The histopathological alterations in seminiferous tubules that appeared in the form of decrement of diameters increase the gap between bules and lipid vacuolation. The decrement length of seminiferous tubules or a reduction density of cells within a given length including decreased density due to damage of spermatogenic tissue would affect testes weight lead to either fewer spermatogonial stem cells or a lower mitotic activity of these stem cells [28]. The present results showed that the reduction of sertoli cells, primary spermatocytes, secondary spermatocytes and spermatids appeared clearly in Pb plus fish oil treated samples and damage in Pb groups. This indicated the capability of fish oil in reducing the negative effect of Pb toxicity. Moreover, when the fish oil and lead acetate administrated to rats, the level of adverse tissue alternation was decrement compared to its level in rats treated only with lead.

Furthermore, a reduction in testicular sperm count was detected, compared with control rats as a result of Pb exposure [29]. The histological changes in this results suggested that the administration of Pb in rats induces pathological changes in the testes. Lead acetate is known to cause free radical damage in tissues by two mechanisms: Increased generation of ROS, including hydroperoxides, singlet oxygen and hydrogen peroxides, and by causing direct depletion of antioxidant reserves [30, 31]. The supplemented dietary intake of antioxidants such as herbal and animals extraction can be prevented the oxidative stress that caused by heavy metals [31, 32]. Fish oil has usually applied as a supplement factor because it contains high levels of n-3 fatty acids and can inhabit some diseases. An increasing number of people are use supplements of fish oil and its omega-3 poly-unsaturated fatty acids eicosapenatenoic acid (EPA) and

docosahexaenoic acid (DHA). These fatty acids can only be synthesized in mammals, α-linolenic acid [33] Studies suggest that consumption of fish oil or of its omega-3 fatty acids may have beneficial effects on stroke, depression, diabetes mellitus and Alzheimer's disease. Numerous studies in mice have supported the beneficial roles of fish oil consumption. Succinctly, in mice, fish oil may have beneficial effects on arthritis, cancer, cardiac arrhythmias and on bone mass during aging.

Rats fed on fish oil observed a significant reduction in body weight and weight of fatty tissue in the epididymis [34].

There is a need to enrich our diet with antioxidant compounds to prevent cellular damage that occur due to oxidative stress [35]. There for the use of fish oil may improve the cellular works to prevent the oxidative stress caused by pb toxicity.

CONCLUSIONS

In conclusion, the present study showed that fish oil has a protective effect on lead induced testicular negative changes and oxidative stress that lead to damage of tissues. Therefore, this study suggests that fish oil could be beneficial in the prevention or treatment of decrement and damages in testicular tissues against Pb toxicity.

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