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The Protective Effect of Grape (*Vitis vinifera*) Seed Oil on Testicular Structure of Male Rats Exposed to Lead

[1]N.A. Alawi, [1,2]G.A. EL Hag, [1]S.O. Rabah, [1]N.M. Al-Baqami,
[1]N.S.Fahmy, [1]A.M. Al-Attar and [1]I.M. Abu Zeid

[1]Department of Biological Sciences, Faculty of Sciences,
King Abdulaziz University, P.O. Box: 139109, Jeddah 21323, Saudi Arabia
[2]Department of Fisheries Science, Faculty of Agricultural Technology and Fisheries Science,
Al-Neelain University, P.O. Box: 12702, Khartoum, Sudan

Abstract: The present study was conducted to assess the possible protective effects of grape seed oil on testis toxicity induced by Lead (Pb) in male rats. Four groups of rats were used in this experiment. Rats of the first group were served as control. The second group of rats was exposed to Pb (100 mg/kg) three times weekly. Rats of the third group were treated with grape seed oil (600mg/kg body weight/day) plus Pb. The fourth group was supplemented with grape seed oil at the same dose given to group three. After six weeks, the histopathological alterations were estimated. The testicular structure of rats exposed to Pb revealed some histological changes. The findings of this work indicated that grape seed oil slightly attenuated the histological alterations induced by Pb. Furthermore, the result of the present study suggests that the antioxidant properties of grape seed oil could be attributed to the protective effect against toxicity induced by Pb.

Key words: Grape Seed Oil · Lead Toxicity · Testis · Histological Changes

INTRODUCTION

Environmental system is a complex system which consists of physical and chemical factors that combine to create the environment. The physical factors are considered as an important conditions for living organisms. These factors include temperature, pressure, radiations, noise, humidity, light, gravity, air, soil and land. The chemical factors include organic compounds, non-organic compounds, hydrogen ion concentration (pH) and salinity. Recently, human's activities changed the harmony of the environmental system, so a lot of pollution was created. Moreover, human activities are not the only reason that causes pollution. The other reason is the alterations that happen because of the nature's disasters [1, 2]. Unfortunately, pollution became a much more serious problem that threatens humans and animals [3].The pollutants can get in the living organism's system through food. So it is important to protect animals that positioned in the food chain from pollutants. Also they can enter in the living organism's system through skin, eyes, nose and ears [4].

Heavy metals are an example of pollutants. They are stable high density metals that available in the environment because they are one of the components of earth's crust [5]. The amount of heavy metals in air, water, soil and tissues of living organisms has increased because of the human's activities that caused a rise in the input of heavy metals to the environment [2]. Heavy metals are absolutely toxic, but the dose, degree of exposure and the chemical form of these metals lead to a difference in the extent of their toxicity, as well as the gender, age and genetics of the exposed animals [5].

Lead (Pb) is an example of a highly toxic heavy metal and not like other heavy metals, it has no importance to sustain life [3]. Lead is accumulated in soil, air and water due to pollution that happen because of human activities such as fossil fuel burning, mining and manufacturing [6].

Corresponding Author: Ghada Ahmed El Hag, Department of Biological Sciences, Faculty of Sciences, King Abdulaziz University, P.O. Box: 139109, Jeddah 21323, Saudi Arabia. Tel: +966506553617.

Through the food chain, Pb is trans-located to human and animals and the extant of Pb toxicity is depending on duration of exposure, dose, sex and species [3, 4].

The use of herbal treatment and herbal products has strongly raised over the past 30 years, because of the general belief that herbal medicines are without any side effects and cheap [7]. Vegetable oil is a widespread usable oil due to its many nutritional and therapeutic benefits [8]. The main vegetable oil's components are three fatty acids with triglycerides – esters of glycerol [9]. The antioxidant activity is the main reason to make vegetable oils considered as therapeutic substance beside it's nutritional benefits [10]. Tocopherols such as vitamin E, are an essential substance for body's health which are available in many vegetable oils. These tocopherols have the power of preventing cell membrane damage that happen by reactive oxygen species due to its antioxidant properties [11, 12]. Certain types of vegetable oils had the ability of shortening the stroke prone survival in hypertensive rats beside their ability to inhibit chronic kidney disease. Vegetable oils that are rich in polyunsaturated fatty acids, such as soybean oil, sun flower oil and corn oil can protect the body against coronary heart disease [13]. Also, the anti-inflammatory effect of canola oil was registered [14]. Moreover, palm oil which is rich in saturated fatty acids, appeared a significant effect on increasing high density lipoprotein cholesterol (HDL-C) [15]. Furthermore, an antibacterial, antimalarial, antifungal, anti-inflammatory, antiparasitic and immune modulatory properties were found in some vegetable oils like neem oil. This oil proved its ability to ameliorate toxicity that occurs in liver, kidney and testicles [16]. Moreover, many plant source oils were studied to prove their ability to treat or ameliorate the toxicity that occurred due to heavy metals exposure. Flax seed oil decreased lipid peroxidation that was induced by cadmium [17]. This oil which is rich in omega-3 fatty acids and lignans, has proved its ability to decrease or prevent renal and cardiovascular disorders progression and reduce oxidative damage induced by Pb through antioxidant defense mechanism [18].

Herbal medicines have the ability to ameliorate the toxicity of heavy metals and can prevent oxidative stress that caused by them. It was found that a blend of 17 kinds of herbs which was administrated to rats orally were useful against the heavy metal in reducing oxidative stress [19]. Many plants showed remarkable ameliorative effects against Pb poisoning such as turmeric or *Curcuma longa*, black and green tea, garlic or *Allium sativum* and ginger or *Zingiber officinale*. These plants revealed their anti-inflammatory, cytoprotective and antioxidant properties [20].

The grape plant (*Vitis vinifera)* is rich in phytochemicals, which reduce the chronic diseases risk such as cardiovascular diseases and cancer and it has a tough antioxidant activity and suppressing platelet aggregation, besides reducing cholesterol [21]. It has anti-inflammatory, antioxidant, antihistamine, antiallergenic and immune boosting properties. Moreover, it's therapeutic features protect the body against carcinogens and allergens. The grape seed extract is effective against eye strain [22].

Grape juice, is considered as a rich polyphenol, antioxidants and vitamins mixture, it has the ability to prevent lipid peroxidation, decrease hepatocellular degeneration and reactive species levels and reduce liver steatosis in rats. Furthermore, products of the grape are considered as useful products to treat hepatic and metabolic diseases [23]. Grape seed oil is rich with fatty acids, phenolic compounds and vitamins. It is known that this oil has anticancer, antimicrobial, cardioprotective and anti-inflammatory properties due to the presence of tocopherol, resveratrol, linolenic acid, procyanidins, quercetin, phytosterols and carotenoids. Therefore, it is used in pharmaceutical industry [24]. Grape seed oil contains high concentrations of antioxidants such as flavonoid, phenols and vitamin E. Therefore, grape seed oil can provide protection against oxidative stress and cellular damage caused by free radicals that usually formed after exposing to heavy metals [25]. Grape seed oil attenuates the toxicity that occurs due to heavy metal toxicity such as cadmium (Cd). The oil showed a remarkable ameliorative effect against oxidative hepatic dysfunction induced by Cd in rats [26]. The present study was carried out to evaluate the possible protective effects of grape seed oil on testis toxicity induced by Lead (Pb) in male rats.

MATERIALS AND METHODS

Test Animals: Male albino rats of the Wistar strain (*Rattus norvegicus*), were used in the present study. The experimental animals were obtained from the Experimental Animal Unit of King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia. Rats were acclimatized to the laboratory conditions for one week prior to the initiation of experimental treatments. The experimental animals were housed in standard plastic cages and maintained under controlled laboratory conditions of humidity (65%), temperature (20±1°C) and 12:12 h light: dark cycle. Rats were fed *ad libitum* on normal commercial chow and had free access to water.

The experimental treatments were conducted in accordance with ethical guidelines of the Animal Care and Use Committee of King Abdulaziz University.

Experimental Design and Treatment: After the acclimatization period, a total of forty rats were assigned into four groups of 10 rats in each cage. The experimental groups were formulated to contain variables proportions of lead and oil. Group (1) was served as control and received saline solution (0.9% NaCl) orally, daily for 6 weeks. Group (2) was given 100 mg/ kg body weight of lead acetate orally, 3 times weekly. Group (3) was orally supplemented with grape seed oil (Rafael Salgado Co. Spain) at a dose of 600 mg/ kg body weight/ day. Moreover, they received lead acetate at the same dose given to group 2. While the last group (4) was orally supplemented with grape seed oil at a dose of 600 mg/kg body weight/ day.

Histopathological Study: Rats were dissected and the testis tissues were preserved in 10% formalin immediately after removal from the animals. The testis tissues were prepared into sections and stained with hematoxylin according to method described by Dunn [27]. Testis sections were examined using light microscope (Olympus BX61- USA) connected to motorized controller unit (Olympus bx-ucb- USA) and photographed by a camera (Olympus DP72- USA) in the microscope unit at King Fahd Medical Research Center.

RESULTS

The testis sections of control rats showed normal structure (Fig. 1). The structure of testis consist of many lobules which separated by connective tissue septa. The separated lobules contain seminiferous tubules. These tubules consist of layers of germinal epithelium including spermatogonia attached with sertoli cells, primary spermatocytes, secondary spermatocytes and spermatids respectively that oriented to the lumen of seminiferous tubules. In spermatogenesis process, spermatids are converted eventually to spermatozoa which are located in the lumen of seminiferous tubules. Leydig cells are responsible for secreting testosterone. They are interstitial cells that are located between seminiferous tubules.

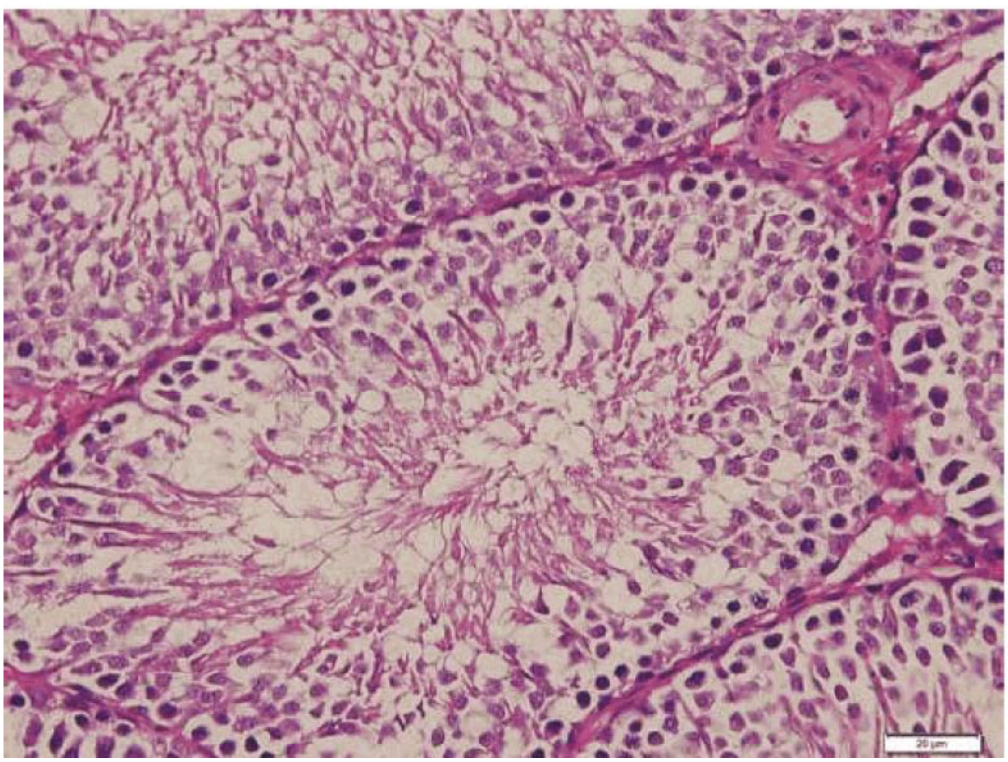

Fig. 1: Photomicrograph of testis section of control group showing normal structure of seminiferous tubule, spermatogonia, sertoli cells, primary spermatocyte, secondary spermatocyte, spermatids and normal spermatozoa (eosin and hematoxylin's staining). Original magnification X400

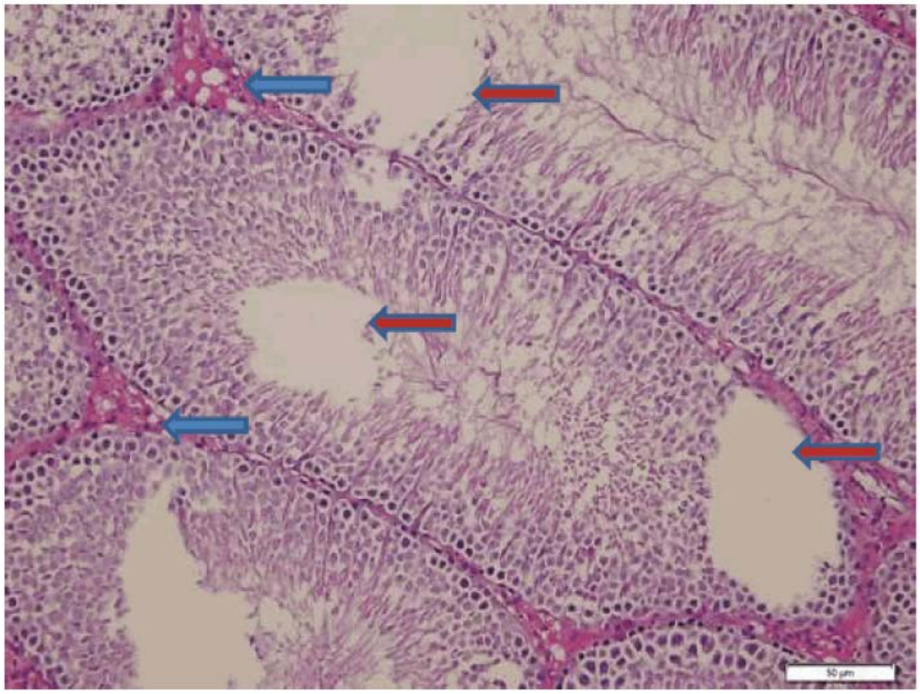

Fig. 2: Photomicrograph of testis section of Pb treated rats showing absence of spermatogonia, sertoli cells, primary and secondary spermatocyte and spermatids (red arrow), and lipid vacuolation (blue arrow) (eosin and hematoxylin's staining). Original magnification X200

Fig. 3: Photomicrograph of testis section of Pb treated rats showing reduced diameter of seminiferous tubule (red arrow), detachment of the basement membrane (blue arrow), absence of tail of spermatozoa in the lumen of seminiferous tubule (yellow arrow) and dilated blood vessel (green arrow) (eosin and hematoxylin's staining). Original magnification X200

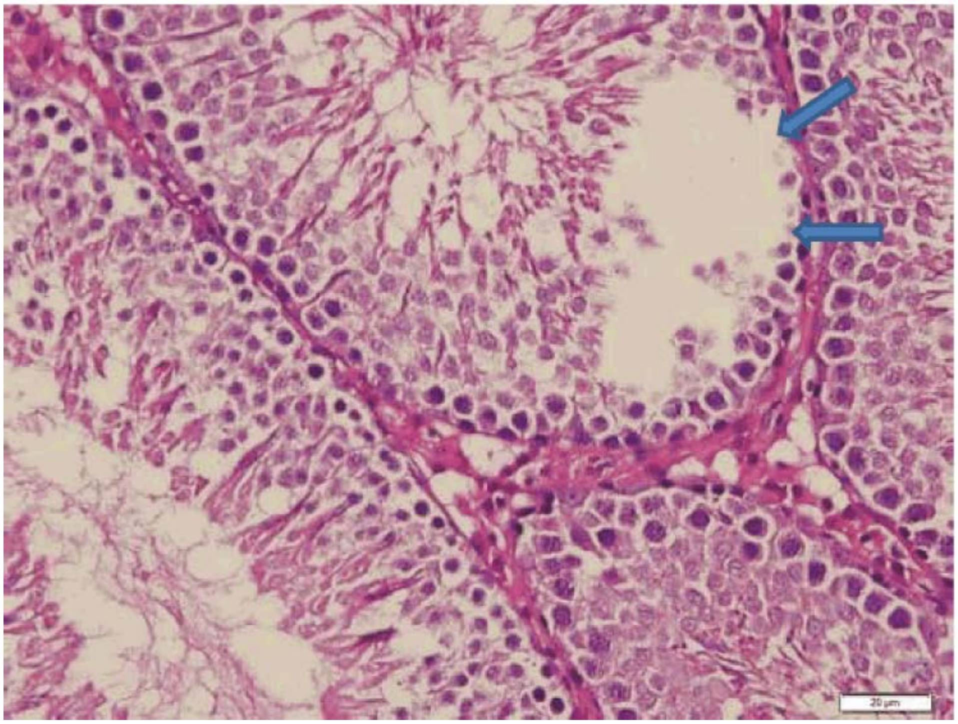

Fig. 4: Photomicrograph of testis section of Pb treated rats showing empty space between germ cells (eosin and hematoxylin's staining). Original magnification X400

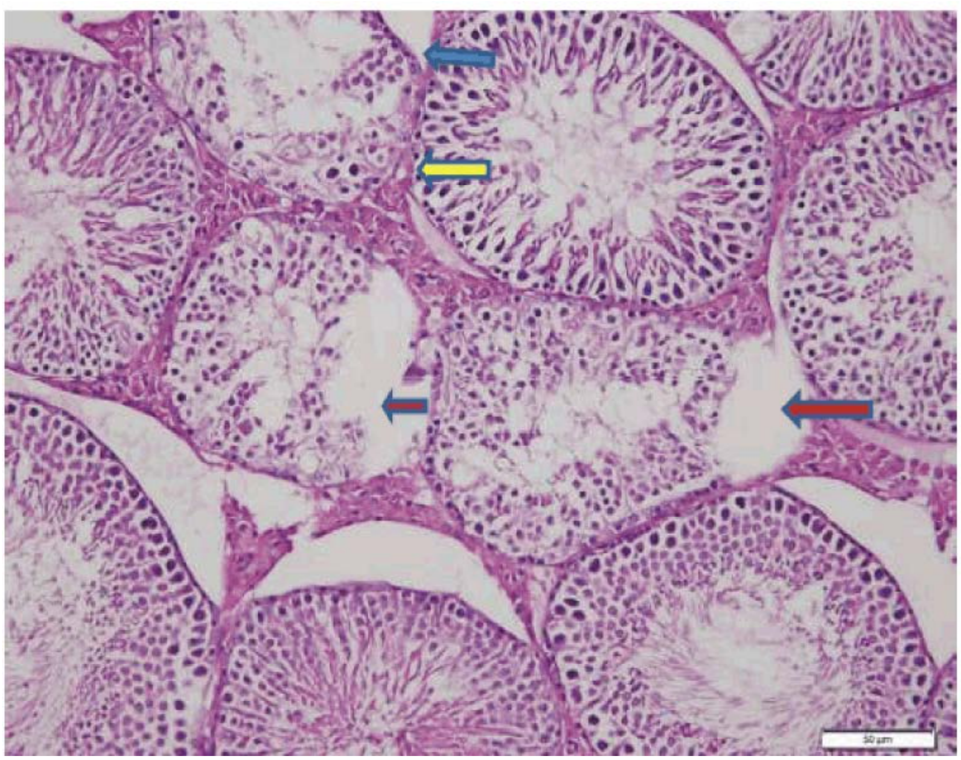

Fig. 5: Photomicrograph of testis section of Pb plus grape seed oil treated rats showing absence of the germinal epithelium (red arrow), basement detachment with reduced seminiferous tubules (blue arrow) and lipid vacuolation (yellow arrow) (eosin and hematoxylin's staining). Original magnification X200

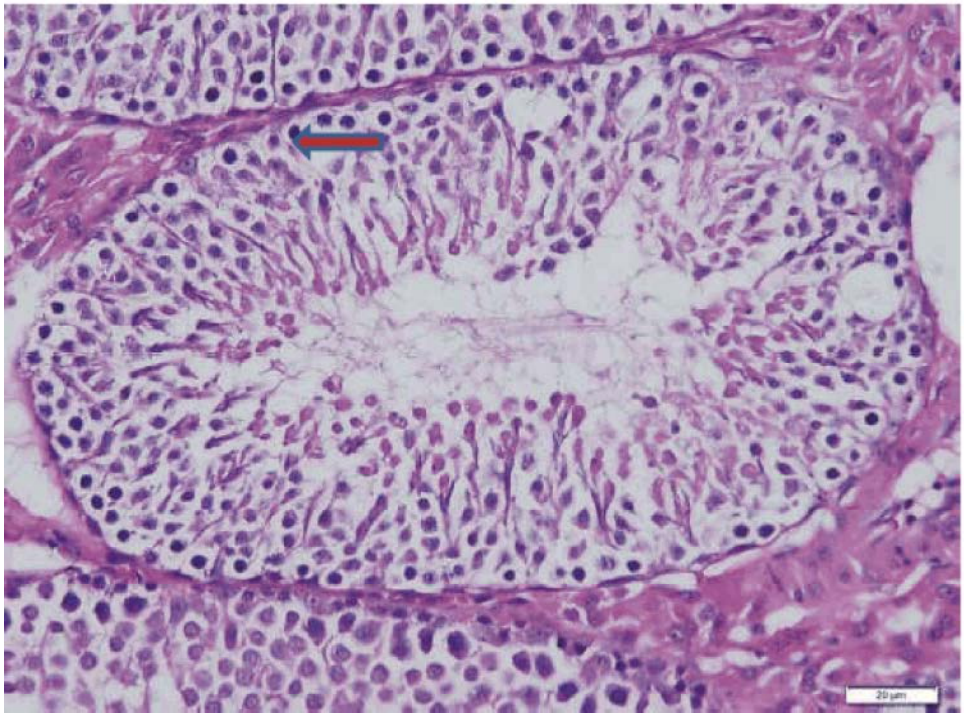

Fig. 6: Photomicrograph of testis section of Pb plus grape seed oil treated rats showing a slight empty space between germinal epithelium (Eosin and hematoxylin's staining). Original magnification X400

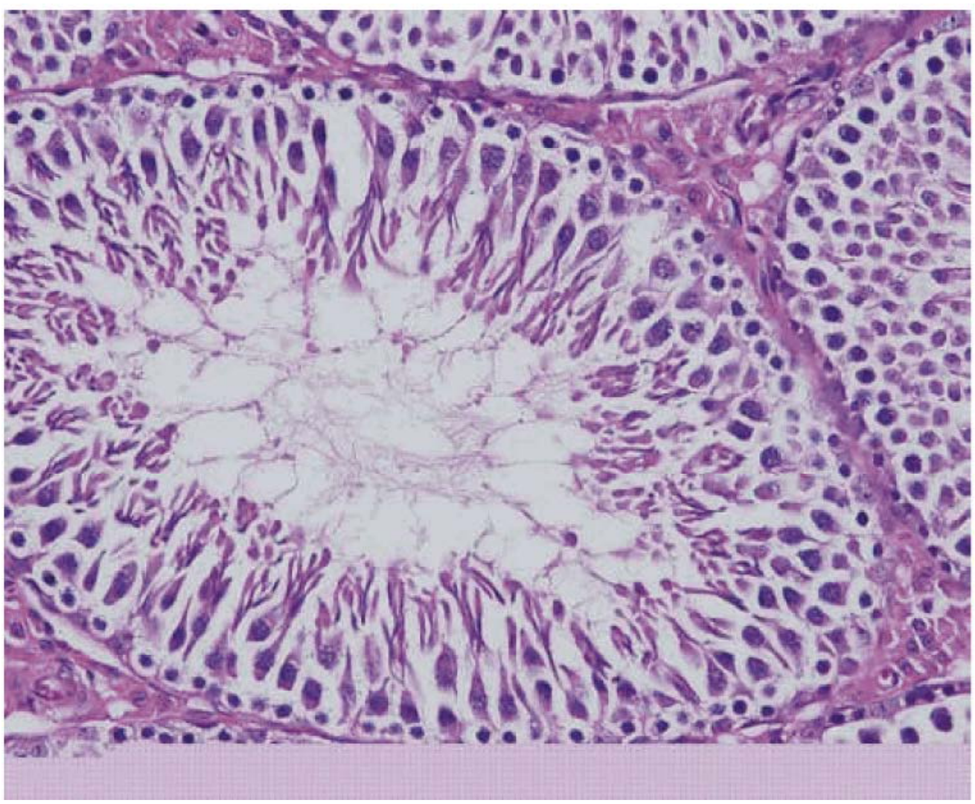

Fig. 7: Photomicrograph of testis section of grape seed oil rats showing normal layered germinal epithelium and normal spermatozoa (Eosin and hematoxylin's staining). Original magnification X400

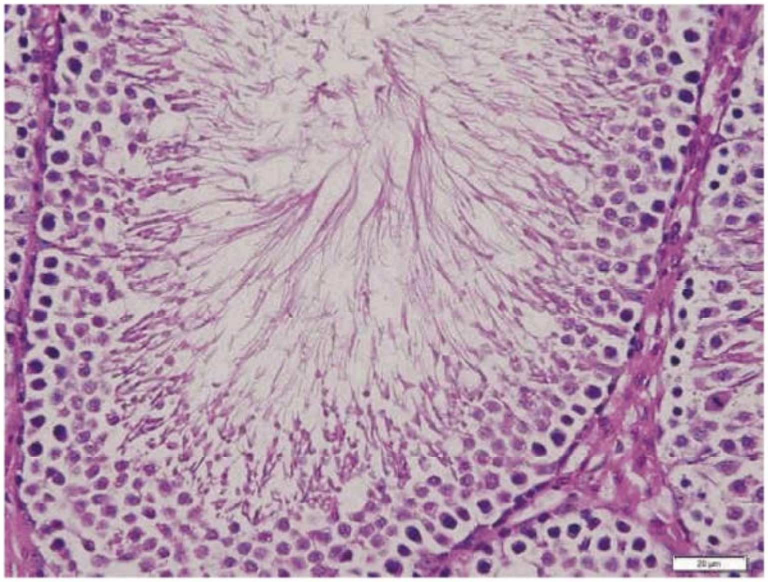

Fig. 8: Photomicrograph of testis section of grape seed oil treated rats showing normal appearance of layered germinal epithelium, normal spermatozoa location and creation, appearance of leydig cells and normal seminiferous tubules diameter (Eosin and hematoxylin's staining). Original magnification X400

The sections of testis of Pb treated rats showed several abnormalities including absence of spermatogonia, sertoli cells, primary and secondary spermatocytes and spermatids with interstitial edema and lipid vacuolation (Fig. 2). Moreover, these sections revealed reduction in the diameter of seminiferous tubules with basement membrane detachment, blood vessel dilatation and absence of leydig cells (Fig. 3). In addition, an empty space between germ cells was shown as well (Fig. 4).

The sections of testis of Pb plus grape seed oil treated rats showed the same abnormalities with slight alterations compared with testis sections of rats treated with only Pb. Abasement membrane detachment with reduction in the diameter of seminiferous tubules was detected. Moreover, the sections appeared the absence of the germinal epithelium with lipid vacuolation and edema (Fig. 5). Furthermore, slight empty space was shown between the germinal epithelium (Fig. 6).

The testis sections of grape seed oil treated rats revealed a normal testicular structure. The separated seminiferous tubules appeared the normal view of layered germinal epithelium of spermatogonia, sertoli cells, primary spermatocytes, secondary spermatocytes. The complete created spermatozoa are located in the lumen of seminiferous tubules and the leydig interstitial cells are shown in its normal position (Fig. 7). In addition, the diameters of seminiferous tubules are normal as well (Fig. 8).

DISCUSSION

Heavy metals are toxic and the bio toxic effect refers to the heavy metals harmful effects to the body when the organism is exposed to high levels of these metals [3]. Pb is widely used in the chemical industry and it is one of the most dangerous environmental pollutants. Therefore, it has been a big concern for human health. The levels of Pb in the environment are more than the levels that were recommended by the World Health Organization and that is because of human activities [28]. Pb is considered as a toxic heavy metal due to the poisonous influences which occur after being exposed to it and the nature of these influences could be toxic (Acute, sub-chronic or chronic), carcinogenic, mutagenic or neurotoxic [3]. There are many mechanisms for Pb toxicity such as involvement with neurotransmitters, oxidative stress and impact on the hematopoietic system [29].

The obtained data in the present study showed that administration of Pb at a dose of 100 mg/kg body weight, 3 times weekly for 6 weeks induced some histological alterations in the testis of male Wister rat. These observations are in general agreement with other investigations on Pb induced relative influences [30-32]. The problem of Pb toxicity comes from its poisonous effect that is usually affecting all organs and functions in the body. It was reported that Pb accumulating in organs tissues making testis a good choice to study the effect of Pb poisoning [33].

The present results about testis demonstrated that Pb intoxication basically led to several changes in the structure and diameters of seminiferous tubules. These testicular changes are mainly including, marked degeneration of most seminiferous tubules with absence of spermatogonia, sertoli cells, primary and secondary spermatocytes and spermatids in the lumen of seminiferous tubules. The absence of spermatogonia is a result of cellular damage induced by Pb exposure and this absence is the reason of other cells absence that are located inside the seminiferous tubules. The reproductive system is always vulnerable to damage caused by Pb toxicity and this damage appears in the form of reduced number and motility of sperms and infertility [34]. The free radical damage in tissues is a result of Pb exposure and this damage occurs through two mechanisms: ROS increased generation, including hydrogen peroxides, singlet oxygen and hydroperoxides and through causing direct antioxidant reserves depletion. Additionally, Pb intoxication causes a remarkable reduction in the levels of antioxidant enzymes activities: superoxide dismutase and catalase, which leads to increase in oxidative stress in testis tissue resulting in degeneration and cellular damage [35-37]. In another study, Ramah *et al*. [31] demonstrated that treating adult male albino rats with 1.5 g/L lead acetate daily in drinking water for 8 weeks induced testicular damage. Examination of light microscope showed the histopathological alterations in testis that appeared in the form of degeneration with loss of spermatogenic series in the seminiferous tubules. Moreover, a marked reduction that was associated with Pb exposure was detected in the viability of sperms, levels of testosterone, GSH and GST with abnormal morphology of sperms. Testicular toxicity was induced in male albino rats by administrating the experimental rats orally with 0, 25, 50, 75 and 100 mg/Kg body weight of lead acetate dissolved in 5 ml of distilled water for 28 days [32]. Histological examination of testis of rats exposed to Pb revealed tissues degeneration, necrosis and maturing spermatogenic cells in seminiferous tubules. Furthermore, a reduction in testicular sperm count was detected, compared with control rats as a result of Pb exposure [32].

The present results showed that supplementing rats with grape seed oil improved the histopathological alterations induced by Pb toxicity. This indicated the capability of grape seed oil in preventing against Pb toxicity. Pb is a testicular toxicant metal and that is due to its effect on changing the morphology of the tissues of testis negatively. Rats exposed to Pb reveals damaged testicular cells due to the elevation in highly reactive cytotoxic compounds formation like oxidative free radicals [38, 39]. It is possible to consider grape seed oil as a testicular protective factor against Pb toxicity and that is because of the antioxidant effect of this oil that explain the reason of its effectiveness in attenuating symptoms of Pb toxicity. Flora *et al*. [40] mentioned that exposing rats to a combination of Pb and ethanol leads to increasing oxidative stress and apoptosis possible initiation. Elgawish and Abdelrazek [30] mentioned that lead acetate exposure induces degeneration in most seminiferous tubules of testis with spermatogenic series absence and blood vessels congestion. Additionally, several studies indicated that Pb caused a significant reduction in the levels of SOD and GSH in the body [41-44].

The oxidative stress can be prevented through dietary intake of antioxidants which have the ability to prevent cellular oxidation evolution. Grape seed oil is rich in flavonoids and proanthocyanidins which are known as phenolic compounds that have antioxidant activities. The antioxidant activities of these phenolic compounds are protecting against oxidative stress, which happens due to Pb exposure. Therefore, there is a need to in rich our diet with antioxidants compounds to prevent cellular damage that occur due to oxidative stress [22, 24]. Cetin *et al*. [45] studied the effect of grape seed extract and origanum onites essential oil on hepatotoxicity induced by cisplatin (CP) in male Wistar albino rats. CP induced cellular damage in the liver and that was known through realizing the hepatocytes degeneration, Kupffer cells activation and sinusoids enlargement. Moreover, CP decreased the levels of SOD and GSH–Px. Also, it increased the levels of MDA, serum ALT and AST when compared with control rats. Treatment of rats with grape seed extract and origanum onites essential oil resulted in remarkable improvement in histopathological features and physiological parameters. They significantly increased the levels of SOD and GSH–Px. In addition, the oils decreased the levels of MDA, ALT and AST that were increased due to CP exposure. Therefore, it can be hypothesized that grape seed oil is a natural product that can protect against CP induced hepatotoxicity. Hasseeb *et al*. [46] assessed the protective effect of dietary grape seed extract against toxicity induced by aluminum chloride in male Sprague-Dawley rats. They demonstrated that the dietary grape seed extract that is rich in polyphenols is significantly effective in protecting against aluminum induced toxicity in several body organs such as liver, kidney, male genital organs and the brain. It showed a protective effect against oxidative stress that is induced by aluminum which leads to the possibility of considering grape seed extract supplementation as a therapeutic way against aluminum toxicity. In conclusion, the present study showed that grape seed oil has a protective effect on lead induced testicular injuries and oxidative stress. Therefore, this study suggests that grape seed oil could be a beneficial attenuating factor against Pb toxicity due to the features of the antioxidant activities of this oil.

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