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Effect of Vitamin E Supplementation on Testicular Tissues of Mice Exposed to Sub-chronic Lead Intoxication

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ABSTRACT

Lead is considered the most common occupational and environmental toxicants and it has serious potential health hazards to humans. Investigation of toxic effects expressed on the somniferous tubules and possible protective effects produced by vitamin E supplementation. Thirty adult male albino rats weight 100-150 g were included in the study. Rats were equally divided into three groups: Group I: Control group: Included 10 rats. They were subdivided into two subgroups: Subgroup Ia: Negative control group. Subgroup Ib: Positive control group. Group II: the intoxicated group: included 10 rats that received lead acetate orally in a dose of 60 mg kg^{-1} b.w. dissolved in distilled water by gavage every other day for 6 weeks. Group III: included ten rats that received vitamin E, diluted in soya oil 150 mg kg^{-1} body weight by gavage every other day for 6 weeks, simultaneously with lead acetate in a similar dose to that of group II. At the end of study period, rats were sacrificed; blood samples were obtained for hormonal and blood lead levels evaluation; while testicular blocks were prepared for both histological and electron microscopy evolution. Lead intoxication was associated with decreased serum testosterone levels when compared to control groups, the effect that inhibited by vitamin E supplementation. Similar results were found for LH and FSH. In addition both histological and ultrastructural findings confirmed deleterious effects of lead toxicity and protective effects of vitamin E on semineferous tubules. Lead (Pb) caused degenerative changes in the seminiferous tubules of mice testis and caused cellular abnormalities in testosterone-producing cells. In addition, vitamin E can reduce the impact of Pb toxicity in the male genital organs.

Key words: Lead, testis, vitamin E

INTRODUCTION

In the occupation fields, lead is one of the most common toxicants that have been recognized long time ago. It had been proved that, lead has serious potential threat to human health (Watson *et al*., 2004; El Shafai *et al*., 2011).

The most common source of lead is storage batteries, as it reported that, about 70% of current lead use occurs in these batteries. It also used in the production of solder for electrical devices, automobile radiators, formulation of metal alloys (e.g., manufacture of pipes, weights, cable sheathing, radiation shielding and ammunition). In addition, different lead compounds can be used as pigments, stabilizers, or binders in many industries (e.g., paints, ceramics, glass, plastic and mortar (Henretig, 2002).

Air pollution by lead can be produced by combustion of leaded gasoline in vehicles and it is the major sources of pollution in the developing countries. In addition, to contamination of air, lead also contaminates the water sources and the cultivated soils along the highways. The pollution is more on the cities with high population and heavy traffic density (Gidlow, 2004).

Other important sources of lead pollution include, storage of drinking water in tanks, wrapping the food in newspapers (ink), canned food and the use of lead glazed utensils (Henretig, 2002; Markowitz, 2000).

Lead exposure can affect reproductive system. It had been shown to change sperm shape and reduce sperm numbers in seminal fluid (Acharya *et al*., 1997) and this observation may explain some of the causes of idiopathic male infertility (Dohle *et al*., 2005).

The mechanism by which lead toxicity leads to impaired sperm morphology is not yet clear. However, it has been shown that lead is capable of inducing oxidative stress and it had been shown that testis in particular if affected greatly by oxidative stress (Tomascik-Cheeseman *et al*., 2004). Lead accumulated in the reproductive system and induced oxidative stress in testis via induction of lipid peroxidation that results in the generation of reactive oxygen species (Marchlewicz *et al*., 2004). Thus, many investigators have used a variety of antioxidants, including vitamin C (Hsu *et al*., 1998) and vitamin E (Patra *et al*., 2011) to prevent the occurrence and or reduce oxidative stress in tissues. Some investigations have reported beneficial effects of antioxidants on heavy metal- induced toxicity (Han *et al*., 2007), while others (Willems *et al*., 1982) have questioned the usefulness of these agents in the reduction of heavy metal-induced lesions in the reproductive system of laboratory animals.

Studies examine the effect of lead toxicity are contradictory, some studies showed changes observed by light microscopy (Ait *et al*., 2009; Suradkar *et al*., 2010), while ultrastructural studies showed that lead exposure causes no changes in the testis and Leydig cells (Wenda-Rozewicka *et al*., 1996). Thus, the present study was designed to investigate toxic effects expressed on the somniferous tubules and possible protective effects produced by vitamin E supplementation.

MATERIALS AND METHODS

The study was conducted in the period from March 2013 till July 2013. It was carried out on 30 adult male albino rats weighing 100-150 g. The animals were kept (in Anatomy Department, Al-Azhar Faculty of Medicine; New Damietta) in plastic cages and maintained under standard animal house conditions of illumination (illuminated for 12 h per day 0700-1900 h) and ventilation (kept in a room with a temperature of 28±2°C). They had access to standard rat chow and water ad libitum.

Rats were equally divided into three groups:

Group I: Control group: included 10 rats. They were subdivided into two subgroups:

- **Subgroup Ia:** Included 5 rats who received nothing except distilled water (negative control group)
- **Subgroup Ib:** Included 5 rats who received nothing except distilled water and soya oil (positive control group)

Group II: The intoxicated group: Included 10 rats that received lead acetate (purity>99%) (Sigma Company) orally in a dose of 60 mg kg^{-1} b.w (~1/5 of LD_{50}) dissolved in distilled water by gavage every other day for 6 weeks

Group III: Vitamin E- lead acetate group: included ten rats that received vitamin E, diluted in soya oil 150 mg kg^{-1} body weight by gavage every other day for 6 weeks, simultaneously with lead acetate in a similar dose to that of group II

At the end of the experimental period, the rats were sacrificed under ether anesthesia. Blood samples were collected from the aorta for estimation of hormonal levels (LH, FSH and total testosterone) and blood lead levels. Blood samples stood for half an hour and then centrifuged at 500x g for 15 min at 4°C to separate serum and stored at -70°C. Blood lead levels estimation were done by atomic absorption spectrophotometry.

Determination of serum hormones Serum testosterone (TT), follicle stimulating hormone (FSH) and luteinizing hormones (LH) were quantitatively measured by adopting enzyme-linked immunosorbent assay (ELISA) technique.

The testes were quickly excised suspended in cold normal saline to rinse, weighed and kept cold for homogenisation. Fresh specimens were taken from the testis of each animal and dissected into two pieces; one was immersed immediately in glutaraldehyde and the other one was fixed in Bouin's solution and paraffin-embedded.

For histological examination, the tissue was immersed in 10% formal saline for 16 h for tissue fixation. Afterwards it was rinsed with distilled water, dehydrated in graded alcohol, cleared in xylene and embedded in paraffin. Finally, they were cut into 5 μm section with a rotary microtome and stained with haematoxylin and eosin and examined under a microscope. For electron microscopy, sections were fixed in Bouin's solution and paraffin-embedded. Electron microscopic study using: transmission electron microscope.

Statistical analysis: The collected laboratory data were represented as mean and standard deviation (SD) and for comparison between groups, the one way analysis of variance (ANOVA; F) was conducted and to test significance between two groups, the post Hoc least significant difference (LSD) were calculated. P value <0.05 was considered significant.

RESULTS

Laboratory results: results of laboratory investigations were presented in Table (1) and revealed that, blood lead levels were significantly higher in group 2 when compared to groups Ia, Ib and group III, while the difference between groups Ia and Ib was non significant. In addition, there was significant decrease of total testosterone, FSH and LH in group II (lead-intoxicated) when compared to any of other groups. Vitamin E-treated group showed results near that found in control groups.

Histological results

Subgroup Ia: Sections of the testes from control rats showed the normal structure of the testis. The seminiferous tubules appeared rounded or oval in their outlines and lined by germinal epithelium showing two types of cells; germ cells and sertoli cells. Sertoli cells were detected in between spermatogenic cells as pyramidal cells with pale basal oval or triangular nuclei and prominent nucleoli. The spermatogenic cells were seen in regularly arranged rows with different stages of spermatogenesis. They were arranged from the basal compartment to the lumen of the tubules starting from spermatogonia, primary spermatocytes, rounded and elongated spermatids till mature

Table 1: Comparison between studied groups as regard to laboratory investigations

	Group Ia		Group Ib		Group II (lead)		Group III (lead-vit. E)		Statistics	
Parameters	Mean	SD	Mean	SD	Mean	SD	Mean	SD	F	P
BLL	3.98	0.19	3.96	0.36	5.26	0.11	4.66	0.14	75.51	<0.001*
TT	41.20	4.08	41.20	2.77	12.41	8.35	36.50	1.91	54.41	<0.001*
FSH	1.68	0.064	1.66	0.034	0.67	0.03	1.46	0.036	935.50	<0.001*
LH	1.06	0.045	1.08	0.021	0.45	0.04	1.10	0.056	427.40	<0.001*

NB: Running *post hoc*, LSD revealed that, group II (lead intoxicated group) had significant increase of BLL and significant decrease of TT, FSH and LH in comparison to each of other groups. In addition, no significant difference was found between groups a and b as regard any variables

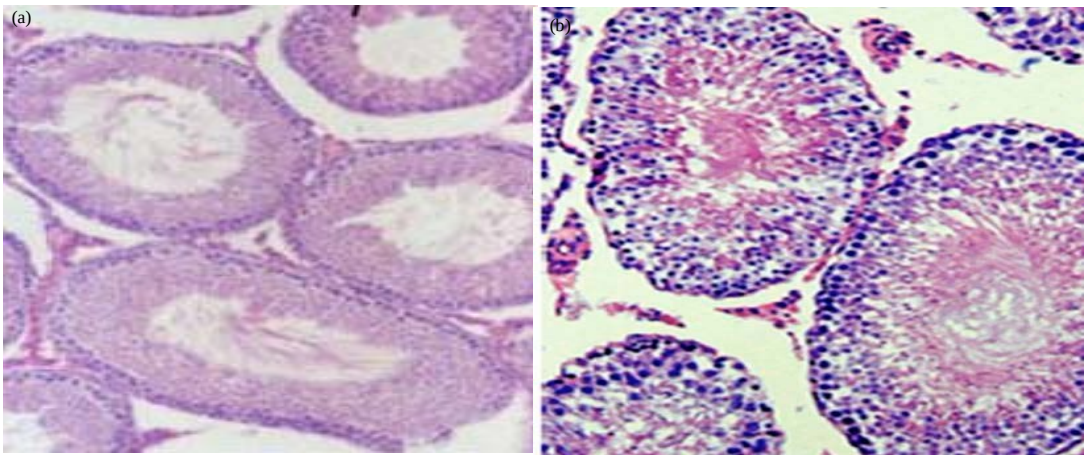

Fig. 1(a-b): Photomicrographs of rat testes (H and E, ×400) from the control group (a and b) showing the normal architecture of testicular tissue with active spermatogenesis in seminiferous tubules

spermatozoa in the lumen. The seminiferous tubules were surrounded by a thin connective tissue layer with myoid cells. The other control subgroup (Ib) showed similar histological characters (Fig. 1).

Group II (lead–intoxicated rats): Examination of sections obtained from testes of lead intoxicated rats revealed seminiferous tubules with vacuolations in the spermatogenic epithelium mostly separating primary spermatocytes from spermatogonia and surrounding nuclei of Sertoli cells. Testes of few animals showed some thin walled seminiferous tubules with wide lumen and vacuolations in the basal part of the spermatogenic epithelium with decreased number of germ cells. Apoptotic bodies were found within the basal part of the spermatogenic epithelium. Focal thickening of the connective tissue bounding the tubules was noticed in some tubules. Some spermatogonia appeared shrunken with pyknotic nuclei (Fig. 2).

Group III: (Vitamin E- protected group), animals that received lead together with vitamin E showed wide areas of testicular tissue more or less similar to the examined control sections. However, focal areas of basal vacuolations in the germinal epithelium were noticed only in limited tubules (Fig. 3).

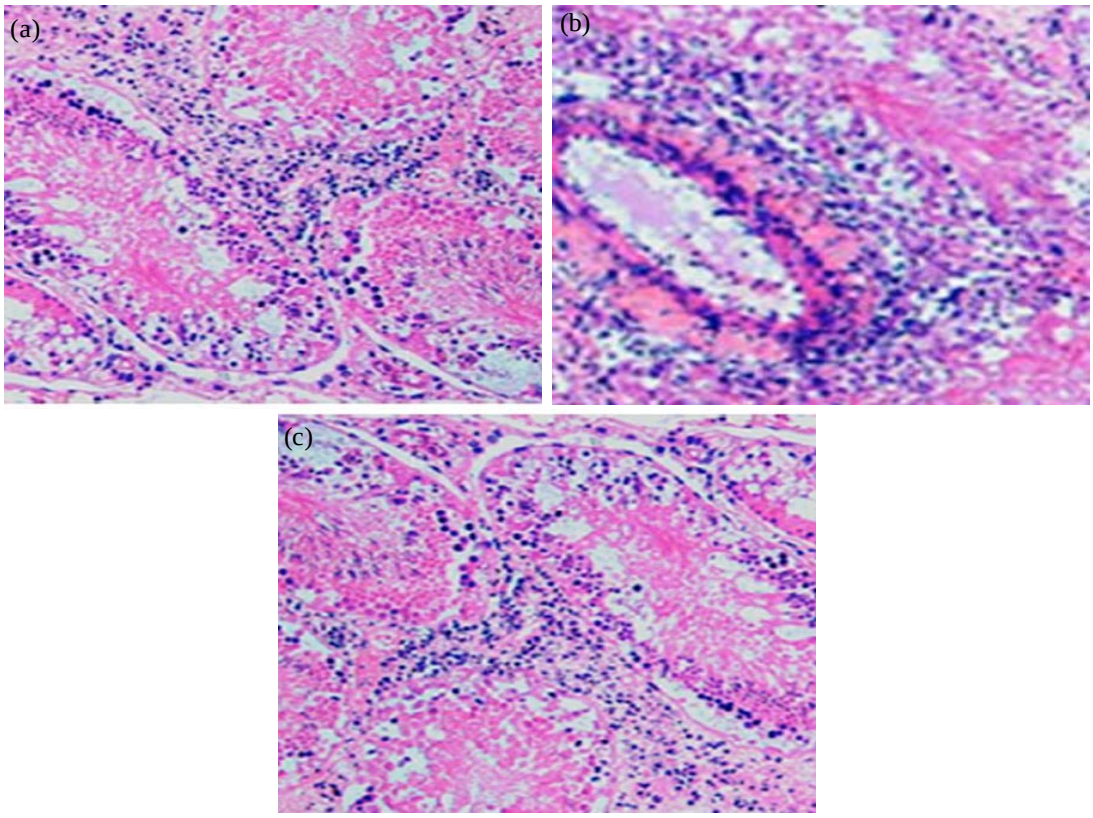

Fig. 2(a-c): Photomicrographs of group II (Lead-intoxicated) showing severe lesions in the form of diffuse necrosis affecting the germinal layer in the seminiferous tubules and interstitial tissue associated with necrotizing vasculitis, edema and nuclear debris

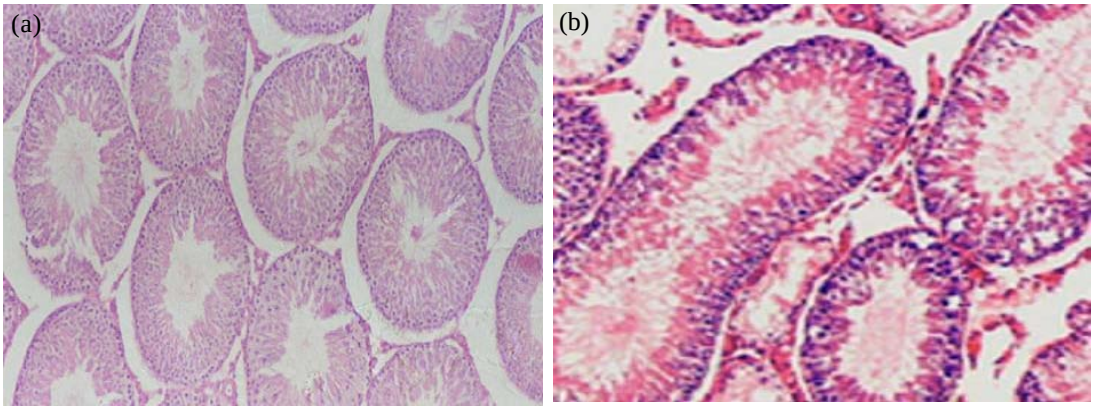

Fig. 3(a-b): Photomicrographs of group III (Lead vitamin E) showing almost normal histological picture with restoration of active spermatogenesis in most of seminiferous tubules

ELECTRON MICROSCOPIC EXAMINATION

Subgroup Ia: Electron microscopic examination of the control rat testis revealed the normal structure of the seminiferous tubules. The tubules were bounded by elongated myoid cells that encircled the basal lamina (Fig. 4). Sertoli cells were identified by their large indented nuclei with prominent nucleoli. Their cytoplasm showed numerous mitochondria, smooth endoplasmic reticulum, few lysosomes and lipid droplets. Intercellular bridges were seen between adjacent spermatogonia (Fig. 5).

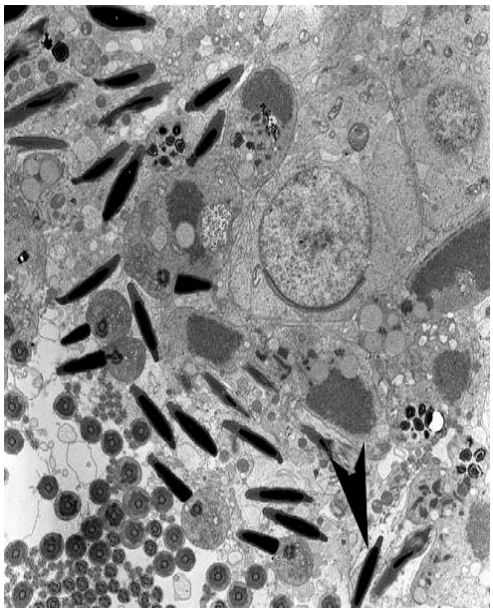

Fig. 4: Electron micrograph of luminal region of the seminiferous tubules of control group showing normal density and normal spermatids

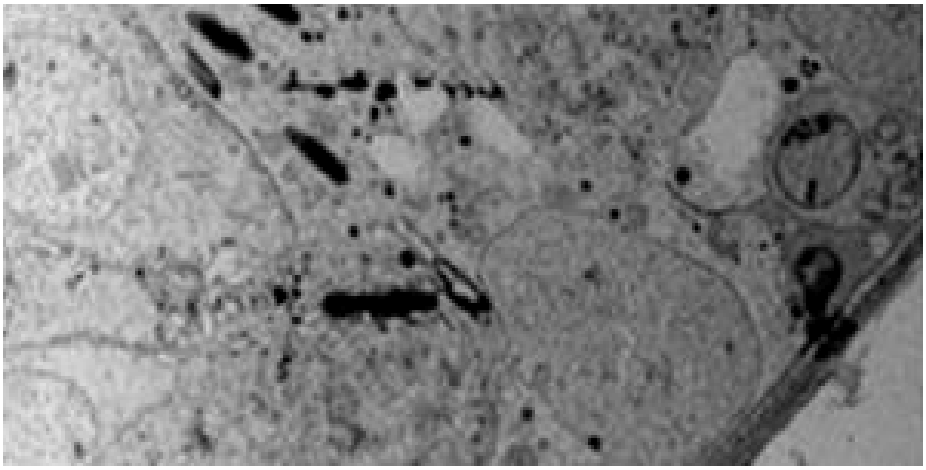

Fig. 5: Electron micrograph of rat testes from the control group showing normal basement membrane (A, black arrow) and primary spermatocytes with spermatogonia embedded in the apical region of sertoli cell (×3000)

Group II: (lead-intoxicated rats): Examination of sections obtained from testes of lead-intoxicated rats revealed many altered seminiferous tubules. They showed large vacuoles within Sertoli cell cytoplasm (Fig. 6). Some seminiferous tubules revealed many apoptotic cells with heterochromatic nuclei and dense cytoplasm with aggregated organelles. Some tubules showed irregularities in the basal lamina with increased collagen deposition and shrunken myoid cells with dense nuclei. Some spermatogonia appeared shrunken with dense heterochromatic nuclei. Few primary spermatocytes adjacent to the affected Sertoli cells showed swelling of the nuclear envelope (Fig. 7).

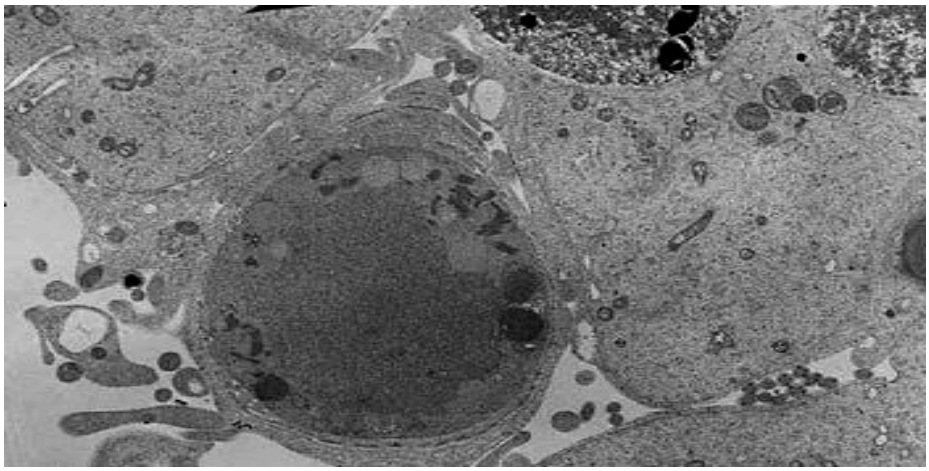

Fig. 6: Electron micrograph of luminal region of the seminiferous tubules of lead treated group showing significant decrease in the density of spermatozoa in the lumen with degenerative changes in spermatids

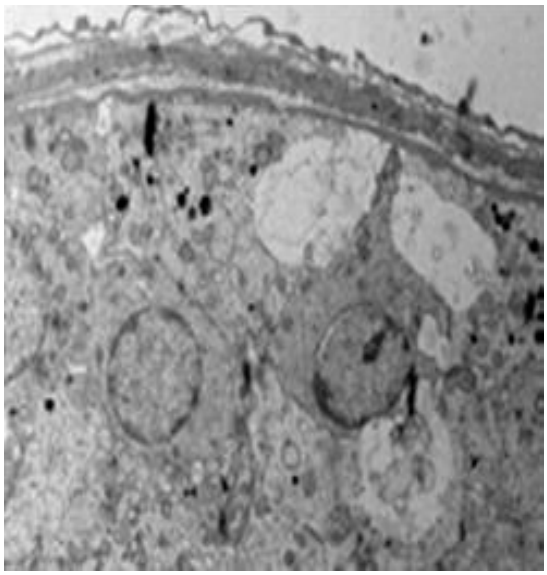

Fig. 7: Electron micrograph of rat testes from the lead-treated group showing signs of inflammatory damage of testicular tissue in the form of irregular and thickened basal lamina, blebbing of the sertoli cell membrane, cytoplasmic vacuolations in the sertoli cells and spermatocytes and dense clumped marginal chromatin in primary spermatocytes

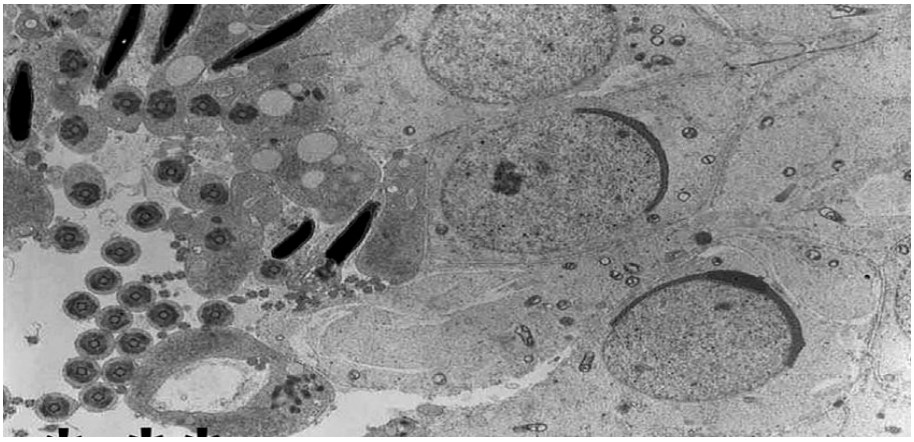

Fig. 8: Electron photograph of group III showing restoration of different structures as in the control group

Group III: (Vitamin E- protected group): Animals that received lead together with vitamin E showed preserved histological structure of most of the seminiferous tubules compared to the control group. Some tubules showed small vacuolations within the cytoplasm of sertoli cells as well as in primary spermatocytes. Other germ cells appeared normal (Fig. 8).

DISCUSSION

Lead toxicity is an environmental hazard that reported early in literature (Flora *et al.*, 2008). Lead is present in batteries, leaded gasoline, paints, water pipes, insecticides and some cosmetics. Air, water, soil, food and consumer products are the major routes of human exposure to lead (Chowdhury, 2009).

The accumulation of lead in various tissues and its interference with biological fluids and elements accounts for its pathophysiology (Berrahal *et al.*, 2011). Reproductive toxicity of lead was reported and reported to be due to oxidative stress (Abdel Moniem *et al.*, 2010).

Studies have shown that antioxidants have a far protecting effect in andrology (Kartikeya *et al.*, 2009).

The present study was designed to examine the toxic effects of lead on testicular functions and histology and extended to study protective effects of vitamin E.

In the present study, serum FSH and TT levels in the animals were altered in the lead exposed animals and these results are in agreement with those reported by Ait *et al.* (2009). They explained these effects by the fact that, increased blood lead content has been associated with disruption of hypothalamic secretion of hormones and spermatogenesis. In addition, Thoreux-Manlay *et al.* (1995) reported that, lead's direct toxicity on testicular histology or its action on the hypothalamic-pituitary axis or a combined defect involving the gonad and hypothalamic pituitary sites could inhibit spermatogenesis and this is consistent with decreased sperm densities in the present study. On the other hand, LH results showed significant alteration in lead exposed animals when compared to the control group. This is in disagreement with El-Tohamy (2003) who observed that lead suppressed TT production with no significant alteration to LH content. In addition, Salawu *et al.* (2009) showed no significant decrease in serum TT level in lead treated rats. These results are in contradiction to that of the present study.

Results of the present study also showed that, vitamin E treatment on lead intoxicated animals showed a significant increase in reproductive hormones and an ameliorative effect on the semen quality assessed. These results are in line with Ayinde *et al.* (2012).

Histological findings of the present study are in agreement with Abdel Moniem *et al.* (2010) whose work showed that lead exposure caused progressive vascular, tubular and interstitial testicular damage. One possible mechanism for this impairment could be attributed to zinc reduction, as zinc deficiency in rats have been suggested to result in atrophy of seminiferous tubules. Other possible mechanism is oxidative damage to cellular materials (Tuncer *et al.*, 2011). Hong *et al.* (2009) reported a similar increase in the density of semineferous cells and luminal spermatozoa in Boer goats supplemented with vitamin E. In a similar study, cells of the seminiferous tubules show signs of degeneration (heterochromatic nuclei, irregular basal lamina, vacuolization) in albino rats given 25 mg kg^{-1} body weight of lead acetate (El Shafai *et al.*, 2011). The results of the present study showed that in lead intoxication, spermatids underwent degenerative changes, which may lead to a reduced number of spermatozoa. The infertility observed in Pb-intoxication is probably due to the morphological changes seen in the seminiferous tubules. Pb treatment also caused vacuolization and a decrease in the number of cytoplasmic organelles of testicular cells, the cells responsible for the production of testosterone. These results are in line with that reported by Fahim *et al.* (2013).

The cause of these degenerative changes can be attributed to oxidative stress. It has been shown that Pb can cause oxidative stress (Ercal *et al.*, 1996; Acharya *et al.*, 2003), resulting in increased lipid peroxidation. Lead exposure has also been shown to reduce the tissue levels of testicular levels of endogenous antioxidants such as glutathione, catalase and superoxide dismutase in rats (Abdel Moniem *et al.*, 2010) and mice (Sharma *et al.*, 2010). These antioxidants are important markers of oxidative stress.

Different studies have examined the role of antioxidants on Pb induced oxidative stress. For example it has been shown that flaxseed oil improved the antioxidant pool in the testis of albino rats subjected to Pb intoxication (Abdel Moniem *et al.*, 2010).

The results of the present study showed that vitamin E increased sperm count in the testicular lumen and reduced the occurrence of morphological changes in the test. The observation of this study has shown that vitamin E as an antioxidant is capable of reducing the deleterious impact of Pb-induced oxidative stress in mice male reproductive organs, in accordance with literature reports. Mishra and Acharya (2004) showed that vitamin E reduced the impact of Pb-induced reduction in sperm count in Swiss mice. The fact that Pb can reduce the level of vitamin E in wild animals residing in Pb mining regions (Rodriguez-Estival *et al.*, 2011) showed a strong association of both Pb and vitamin E.

Vitamin E was found to exhibit a protective effect on the testis of rats. It is a major chain-breaking antioxidant in the sperm membranes. It acts as a free radical scavenger, scavenging superoxide, hydrogen peroxide and hydroxyl radicals (Kartikeya *et al.*, 2009). Adding vitamin E in diet increased the activity of some antioxidant enzymes, decreased nitric oxide content and lipid peroxidation products in the testis of Boer goat (Hong *et al.*, 2009). Vitamin E has been implicated in protecting sperm DNA from oxidative stress of free radicals and improving fertility (Tarin *et al.*, 1998).

CONCLUSION

Pb in its acetate form caused degenerative changes in the seminiferous tubules of mice testis and caused cellular abnormalities in testosterone-producing cells. In addition, vitamin E can reduce the impact of Pb toxicity in the male genital organs.

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