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THE PROTECTIVE EFFECT OF VITAMIN E AGAINST GENOTOXICITY OF LEAD ACETATE INTRAPERITONEAL ADMINISTRATION IN MALE RAT

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Abstract - Lead is an industrial pollutant that may exert specific toxic effects on male mammals. The aim of this study was to investigate further the protective effects of vitamin E on lead acetate (Pb) induced reproductive toxicities and genotoxic effects on male rats. Sexually mature male Wistar rats (weighing 120-150 g) were given Pb (20 mg/Kg) and vitamin E (600 mg/kg/rat) orally for 20 days. The sperm count, sperm motility, sperm morphology, chromosomal aberrations, FSH, LH and testosterone levels, and histopathological changes in the testes of the rats were investigated after 20 days. Results revealed a statistically significant ($p<0.01$) increase in the number of abnormal sperm in treated animals. Lead acetate increased the percentage of chromosomal abnormalities. A significant decrease in LH, FSH and testosterone were observed in the treated group compared to the control. Pathological examination of testicular tissues showed degenerative changes of spermatogonia and spermatocytes to advanced degeneration and vacuolation. Lead acetate can be considered to have an environmental genotoxic and cytotoxic effect in the male rat and may contribute to a reduction of fertility. Vitamin E administration could reduce the genotoxic effects of lead in somatic and germ cells.

Key words: Lead; genotoxicity, chromosome aberration, vitamin E

INTRODUCTION

Humans are exposed to various types of environmental contaminants at different stages of their life span, the majority of them harmful. In recent years, there has been growing concern about the deleterious effects of chemicals on the developing male reproductive system. Exposure to heavy metals during pregnancy has been associated with adverse effects during the development of gonads. Heavy metals can act as testicular toxicants and correspond to different compounds related to social habits, life conditions, work hazards or the use of drugs and medicines (Johnson, et al. 1970; Pomerol and Arrondo, 1994; Bustos-Obregón, 2001). Although many studies have reported the toxic and carcinogenic effects of metals in humans and animals, it is also well known that these metals form a crucial part in the normal biological functioning of cells. Many heavy metals are classical testicular toxicant, though the mechanism of their action may differ. Lead is a male reproductive toxicant (Winder, 1989); the primary mechanism of the toxic action of lead appears to be through disruption of the hypothalamic control of pituitary hormone secretion and in turn, spermatogenesis (Sokol, 1987). Since males do not possess accessory reproductive organs, male reproductive potential is related to three factors -

sperm availability, quality and quantity (Tsuji and Karagatzides, 2001).

Vitamins are essential to maintain normal metabolic processes and homeostasis in the body. Vitamins C (Vit C) and E (Vit E) are low molecular mass antioxidants that scavenge or quench free radicals (Janisch et al., 2005). Reactive oxygen species (ROS) related lead toxicity in the rat sperm was prevented by Vit C or Vit E (Hsu et al., 1998). These findings suggest the potential role of antioxidants to ameliorate lead toxicity. Natural antioxidants may be helpful in preventing or reducing the harmful effects of ROS on the testes and semen quality (Yousef, 2010). Vitamin E is the main component of the antioxidant system of the spermatozoa and is one of the major membrane protectants against ROS and lipid peroxidation (Akiyama, 1999). Supplemental vitamin E can increase total sperm output and sperm concentration in rabbits (Yousef, 2010) and rams (Yue et al., 2010). Conversely, a deficiency of vitamin E can have detrimental effects on the reproductive organs, such as degenerative spermatogonia, testicular damage and degeneration of the seminiferous tubules (Wilson et al. 2003). The influence of dietary vitamin E on semen quality has been described in the mouse (Sánchez-Gutiérrez et al., 2008), rat (Wu et al., 1979), rabbit (Cesare et al. 2002) and goat (Shi et al., 2010). Consequently, this study aimed to evaluate (1) the influence of lead acetate on the reproductive organs and fertility of male albino rats, and (2) the protective role of vitamin E in alleviating the detrimental effect of lead on male fertility.

MATERIALS AND METHODS

Thirty adult male Wistar rats (120-160 g) were used for this study. Rats were housed in temperature-controlled rooms (25°C) with constant humidity (40-70%) and a 12 h light/dark cycle. All animals were treated in accordance with the principles of laboratory animal care.

Grouping of animals and treatment

The rats were divided into 3 groups (groups A, B, and C; n = 10). The animals in group A served as the control group and drank distilled water. The animals in groups B and C received 20 mg/kg by intraperitoneal injections of lead acetate. Group C animals were treated with 600 mg/kg rat body weight (b.w.) orally of vitamin E for 20 days. Each rat was weighed every week.

Reproductive organ weights

All rats were euthanized at the end of the experiment. The testes, epididymides and accessory sex organs (seminal vesicles and prostate glands) were removed, examined and weighed. The index weight (I.W.) of each organ was calculated according to Matousek (1969): I.W. = organ weight (g)/100 × b. w. (g).

Sperm motility

Progressive motility of sperm was evaluated microscopically within 2-4 min of their isolation from the cauda epididymis as described by Sönmez et al. (2005). Fluid was obtained from the cauda epididymis with a pipette and diluted to 2 ml with Tris buffer solution. The percentage of motility was evaluated at ×400 magnification.

Sperm abnormalities

A total of 300 sperm was counted on each slide under the light microscope at ×400 magnification and the percentages of morphologically abnormal spermatozoa (detached head and coiled tail) were recorded according to Evans and Maxwell (1987). Smears for sperm morphology were prepared and stained with eosin according to Mukherjee et al. (1988). One thousand sperms were counted for each animal and abnormal shape involving the head was recorded.

Determination of serum testosterone, LH and FSH levels

Blood was collected from the abdominal vein of all anesthetized rats before scarification. Serum was separated for assessment of the total serum using electrochemiluminescence immunoassay (ECLIA).

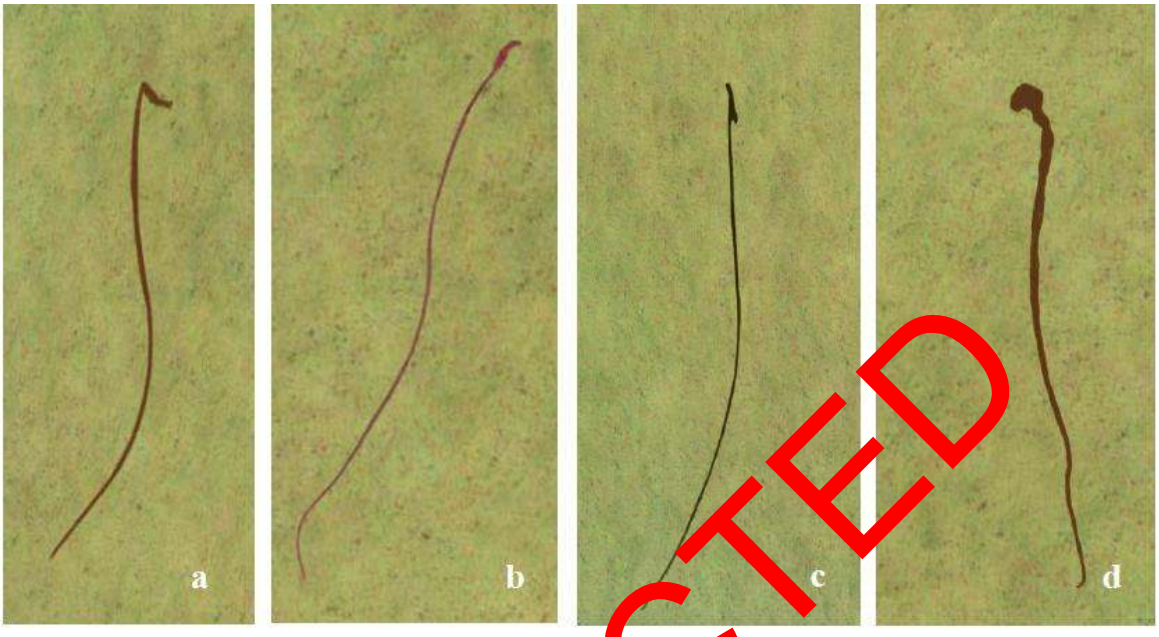

Fig. 1. Types of sperm-head abnormalities in rats treated with lead; a – hammer shaped sperm; b – banana-shaped; c – hooked; d – amorphous;

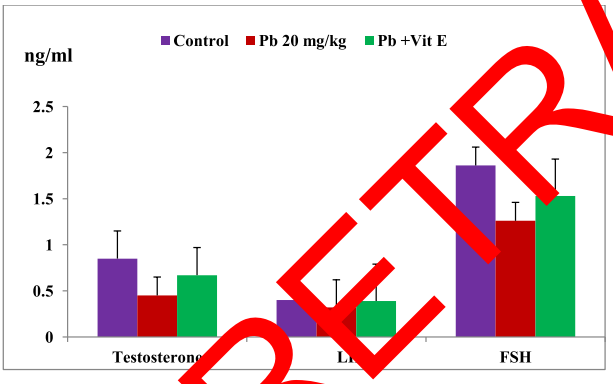

Fig 2. Serum levels of testosterone, LH and FSH after intraperitoneal lead acetate. The values expressed as mean ± SEM (n=10). * *p0.05*; ** *p<0.01*.

Chromosomal abnormalities in rat bone marrow cells

Twenty-four hours after the last treatment, the rats were injected with 0.6 mg/kg b.w. colcemid 2 h prior to scarification. Bone marrow preparations were prepared according to the method of Preston et al. (1987). Four rats were used for each dosage, where the structural alterations of chromosomes were evaluated in 75 metaphases per animal.

Histological slides preparation

Specimens from testicular tissues were fixed in 10% neutral buffer formalin, dehydrated in ascending grades of ethanol alcohols, cleared in xylol, casted, blocked, cut at 2-5 μm thickness and stained with hematoxylin-eosin for microscopic examination (Bancroft, 1975).

Statistical analysis

All data obtained from control and lead-poisoned animals were compared using the Student's t-test for unpaired means. A *p* value *<0,05* was considered significant.

RESULTS

Body weight changes

Table 1 shows that rats in the control group had a significant (P<0.05) increase in weight. Both lead acetate-treated groups lost weight when compared with their initial weights.

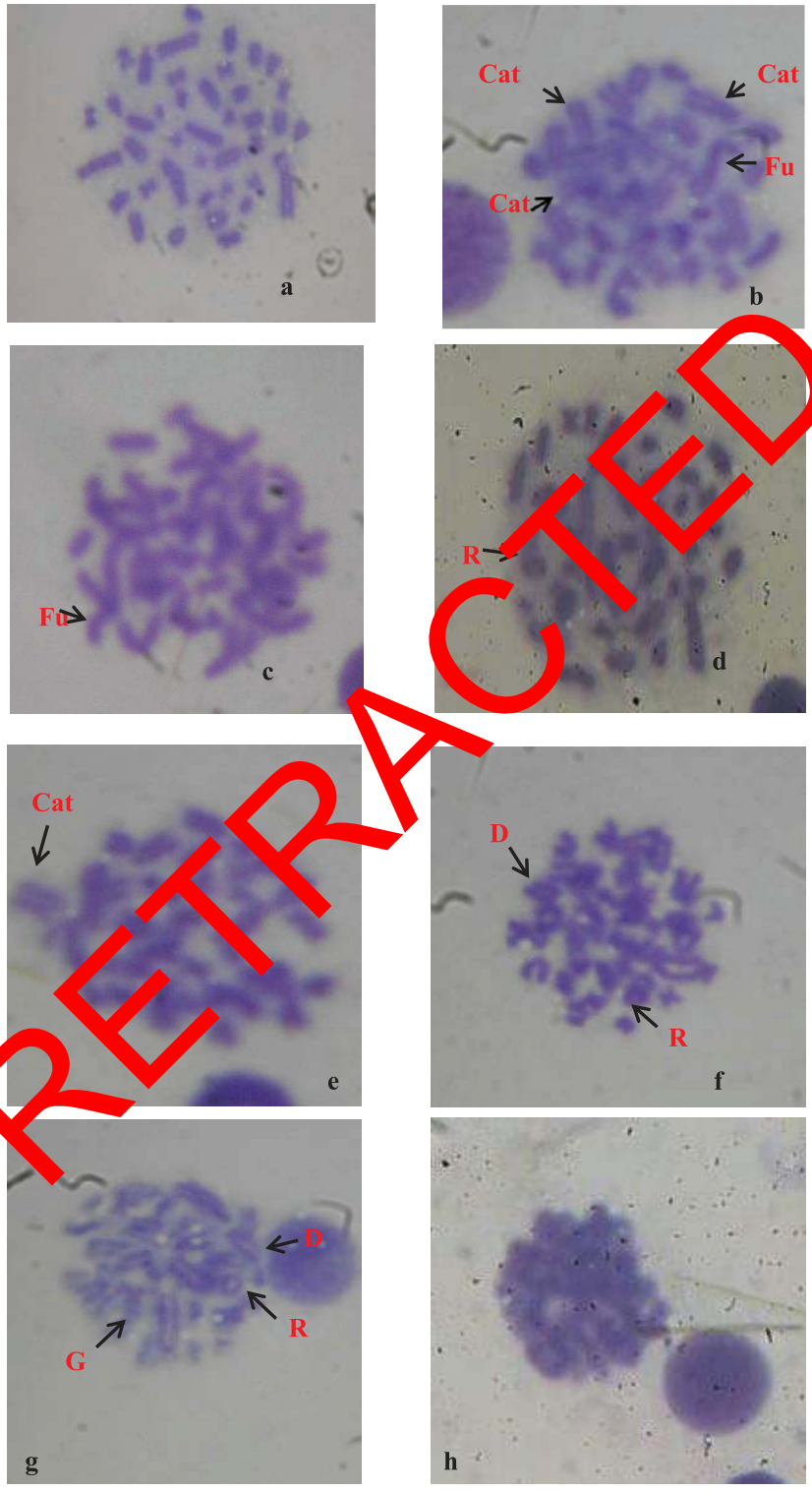

Fig 3. Types of chromosomal aberrations in rat bone marrow cells treared with lead acetate; a – control; b – centromeric attenuation (Cat) and fusion (Fu); c – fusion (Fu); d – chromosomal ring (R); e – centromeric attenuation (Cat); f – chromosomal ring (R) and fusion (F); g – deletion (D), chromosomal ring (R), gap (G); h – sticky chromosome.

Table 1. Effect of lead and vitamin E on body weight and reproductive organ weights of male rats (g).

	Control	Lead	Lead+vitamin E
Initial body weight	170±1.5	171±2	170±2
Final body weight	195±3	155±2.5	183. ±1.5
I.W. of testes	1.60±0.02	1.24±0.06*	1.48±0.04*
I.W. of epididymis	0.74±0.03	0.55±0.02*	0.64±0.01*
I.W. of accessory gland	0.91±0.03	0.68±0.02*	0.79±0.03*

The values are expressed as mean ± SEM (n=10). * *P<0.05*.

Table 2. Effect of vitamin E on the properties of sperm from lead acetate-treated male rats.

Experimental groups	Sperm character			
	Count (10^6/ml)	Motility (%)	Viability (%)	Sperm abnormalities (%)
Control	63.3±3.63	84.4±4.53	90.6±4.31	6.8±0.51
lead acetate	29.6±1.29**	43.1±3.73**	53.4±3.65**	19.4±0.93**
lead acetate+vitamin E	45.3±3.22*	59.4±2.16*	69.8±3.35*	13.2±0.85*

The values expressed as mean ± SEM (n=10). * *p0, 05*; ** *p<0, 01*.

Table 3. Incidence of sperm-head abnormalities per thousand cells after treatment with lead and vitamin E.

Groups	Abnormal sperm			
	Amorphous	Banana shaped	Without hook	Hammer shaped
Control	11.6±0.91	1.4±0.42	2.4±0.37	0.3±0.2
lead acetate	42.4±0.86**	27.8±0.51**	15.8±0.70**	2.4±0.51**
lead+vitamin E	18.4±0.67*	3.8±0.35*	2.4±0.4*	0.9±0.24*

The values expressed as mean ± SEM (n=10). * *p0.05*; ** *p<0.01*.

Table 4. Average numbers of chromosomal abnormalities observed in bone marrow cells of male rats treated with lead and vitamin E.

Groups	Structural aberrations								Total
	Deletions	Chromatid fragments	Ring	Centromeric attenuation	Centric fusion	Breaks	Gaps	Sticky	
Control	3.2±0.2	0.98±0.3	0.78±0.2	0.6±0.2	0.4±0.2	0.4±0.2	0.5±0.2	0.7±0.3	7.56±1.5
Pb 20 mg/kg	28.8±0.8	10. 6±0.3	6.2±0.3	3.4±0.6	2.8±0.4	6.8±0.3	1.6±0.4	1.4±0.5	61.6±3.6
Pb+Vit E	14.9±0.4	5.4±0.4	2.6±0.3	1.2±0.3	1.3±0.3	1.2±0.2	0.8±0.2	0.98±0.4	28.38±2.5

Organ weights

The index weight of the testis, epididymis and accessory sex glands was significantly decreased (P ≤0.05) in rats treated with lead compared to the control group. The reduction was less pronounced in the group treated with lead plus vitamin E (Table 1).

Differential sperm characteristics

Epididymal sperm concentration, sperm motility, viability and abnormal sperm are given in Table 2 for the lead- and vitamin E-treated groups. The lead-treated group had a highly significantly (p<0.01) lower sperm count, motility and viabil-

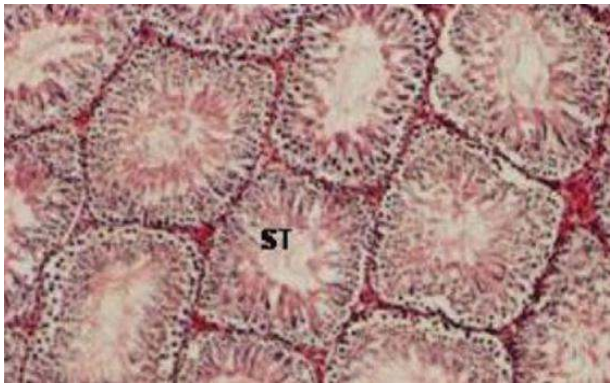

Fig 4. Testes of control male rats control showing normal structure of seminiferous tubules (ST) (H&E X 200).

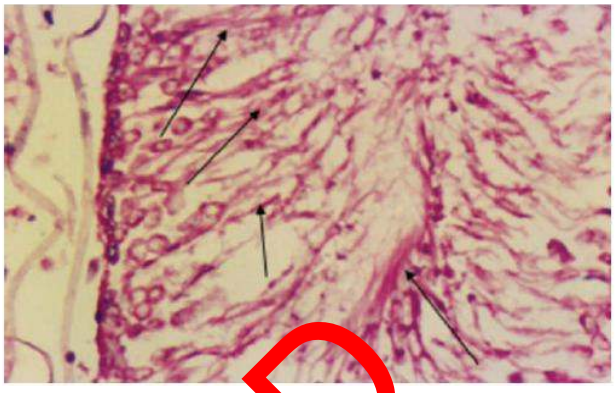

Fig 5. Testes of male rat treated with lead acetate showing complete testicular necrosis and sloughing of all layers, ischemic necrosis (arrows) (H&E X 200).

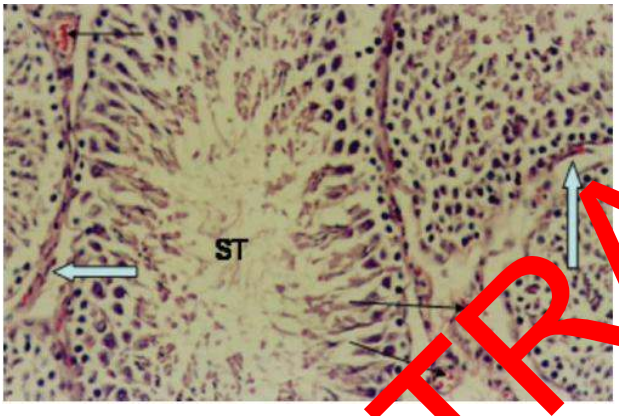

Fig 6. Testes of male rats treated with lead acetate and vitamin E showing mild degenerative changes of seminiferous tubules (ST), mild congestion (thin arrows) and edema (large arrows) (H&E X 200).

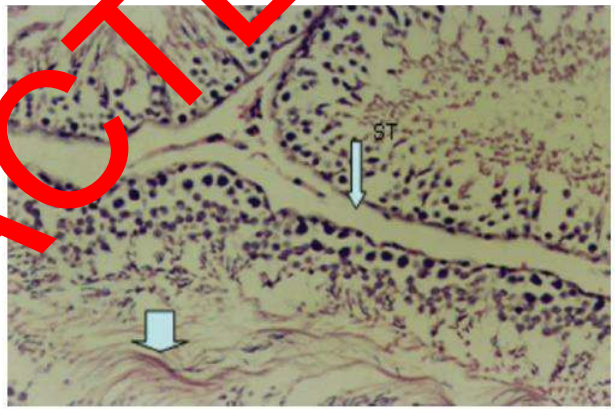

Fig 7. Testes of male rats treated with lead acetate ant vitamin E showing nearly normal seminiferous tubules with mild odema (thin hollow arrow) and necrosis (big hollow arrow) (H&e X 200).

ity than the control group. However, the vitamin E-treated group exhibited a significant increase in sperm concentration ($p<0.05$) compared to the control group.

Table 3 and Fig. 1 present the incidence of abnormal shaped sperm per 1000. Abnormalities included hammer shape (Fig. 1a), banana shape (Fig. 1b), lack of hook (Fig. 1c) and amorphous (Fig. 1d). The highest incidence of abnormality was observed in rats treated with lead acetate. Vitamin E administration had a protective role against sperm-head abnormalities induced by lead treatment.

Plasma testosterone, LH and FSH level

Results in Fig. 2 show a highly significant decrease in serum testosterone, LH and FSH concentration ($p<0.01$) in the lead-treated group compared to the control group, while oral treatment with vitamin E induced a significant elevation in serum testosterone, LH and FSH concentrations.

Chromosomal abnormalities in rat bone marrow cells

Various chromosomal aberrations were observed in the bone marrow cells of male rats treated with lead

and vitamin E. The structural and numerical types of aberration are presented in Table 4 and Fig. 3, respectively. Table 4 gives the average of chromosomal abnormalities in the bone marrow cells of male rats treated with lead and vitamin E. This represented a significant increase in the group treated with lead when compared to the control group. On the other hand, it indicates that vitamin E had a protective role against lead. The structural aberrations included chromatid deletion (Fig. 3g), centromeric attenuation (Fig. 1be), centric fusion (Fig. 1 cf), gap (Fig. 3 g) and chromosomal rings (Fig. 1df). Stickiness may give arise to sticky adhesion between two or more chromosomes and the formation of stick bridges at metaphase (Fig. 3h).

Histopathological results

The normal architecture of testicular seminiferous tubules and interstitial spaces were examined in the control rats (Fig 4). Rats that were treated with lead acetate showed complete necrosis and sloughing of all layers of seminiferous tubules (Fig. 5). The testes of rats treated with vitamin E showed mild to moderate edema, congestion with minute foci of necrosis and hemorrhage (Fig. 6-7).

DISCUSSION

The toxicity of lead has been studied for many years, but data related to the mutagenic, clastogenic and carcinogenic properties of lead and lead compounds are still conflicting. The IARC classified lead as a possible human carcinogen (IARC, 1987), on the basis of sufficient evidence for carcinogenicity in experimental animals but inadequate evidence for carcinogenicity in humans; inorganic lead compounds are classified as probable human carcinogens (IARC, 2006) on the basis of sufficient evidence for carcinogenicity in experimental animals but limited evidence for carcinogenicity in humans. However, in those studies that evaluated the induction of chromosomal aberrations by lead chromate (Douglas et al., 1980; Wise et al., 2003; Wise et al., 2004; Xie et al., 2005), the observed effects could be related to the toxic action of chromate and not to lead (Douglas et al., 1980). The variability reported in different studies could be due to the influence of different experimental variables that may act as confounding factors, such as the duration and route of lead exposure, cell culturing times following exposure, smoking habits and simultaneous exposure to other toxic agents that could act by modifying the genotoxic response of the cells to lead exposure and thereby modifying the results of the studies. Regarding this last factor, many epidemiological studies point to the possibility that multiple exposure in the occupational environment (and not only to lead), could be responsible for the obtained results (Garcìa-Lestòn et al., 2010). In the present study, we evaluated the protective effect of vitamin E against testicular damage induced by lead acetate toxicity in experimental animals. Lead administration caused a significant decrease in body weight. Nabil et al. (2012) found that lead caused a decrease in the growth rate in rats when fed with lead. These effects on body-weight could be associated with several factors, one of which is an imbalance in the metabolism produced by changing the zinc status in zinc-dependent enzymes that are necessary for many metabolic processes. Along with the decrease in body weight, a significant reduction in testicular weight was also found in lead acetate-treated animals. The weight of the testis is largely dependent on the mass of differentiated spermatogenic cells. Hence a reduction in its weight might be due to the decreased number of germ cells and elongated spermatids (Chapin et al., 1997). The weights of accessory sex organs were also decreased after the lead acetate treatment. The weight loss of accessory sex organs corresponds to the decrease in serum testosterone concentration as observed in this study. It has been reported that testosterone plays a major role in the maintenance of structural integrity and functional activities of the accessory sex organs (Moor et al., 1930a).

Serum testosterone, LH and FSH levels were decreased in the lead acetate-treated groups of animals compared to their respective controls. Significant alterations in testostrone LH and FSH levels have been reported after exposure to certain heavy metals (Gabuchyan, 1987; Chattopadhyay et al., 2005; Al-Attar, 2011). LH and FSH activity depends on both

the quantity of these hormones and the number of specific receptors in the testis. It has been shown that exposure to environmental contaminants adversely affects testicular function by decreasing pituitary LH secretion and reducing Leydig cell steroidogenesis (Akingbemi et al., 2004; Murugesan et al., 2007). Together with gonadotrophins, testosterone is a key hormone regulating spermatogenesis. The secretion of testosterone by the Leydig cells is dependent upon the secretion of LH by the pituitary gland (Uzun et al., 2009). This may be because lead induces pathological change in the Leydig cells in the interstitial tissues. In our study, the FSH and LH levels in lead acetate-treated rats were significantly lower than the levels in the control rats at the end of the 20 days. Notably, however, treatment with vitamin E has a protective effect on FSH and LH levels. These results may be explained by the androgenic activities of vitamin E, this activity being reflected by the increase of testis weight and serum testosterone, LH and FSH levels (Muthu and Krishnamoothy, 2012). The potential toxicity of lead caused alterations in sperm morphology, count, motility, as well as hormones (Roy Chowdhury, 2009). Lead has an adverse effect on sperm count and retards the activity of live sperm. Moreover, motility as well as prolonged latency of sperm melting both in exposed persons and experimental animals were observed after Pb exposure (Lancranjan et al., 1975; Roy Chowdhury et al., 1986). In the present study a significant decrease in the total sperm number was found in lead acetate-treated rats compared to control. Lead was reported to induce apoptosis in the testis (Nava-Hernandez et al., 2009). Moreover, the decreased motility and increased incidence of teratospermia at a higher dose of Pb exposure along with the inhibition of post-meiotic cells, mainly pachytene spermatocyte, were noted (Al-Attar, 2011). In the same experiment the detachment of the germinal cell layer from the basal membrane, atrophy of Leydig cells plus interstisial edema and low density of seminal plasma were also observed. Additionally, Madhavi et al. (2007) showed that lead induced cytogenetic damage in germ cells of mice. Testicular damage was also confirmed by histopathological lesions (Muthu and Krishnamoothy, 2012). The present study clearly demonstrated that lead acetate can seriously alter the testicular tissues that started the changes with vacuolar degeneration until necrosis and atrophy of seminiferous tubules. The treated groups displayed vacuolation and degenerative changes in spermatogonia, arrest of spermatogenesis and pyknotic changes in spermatocytes. The center of most seminiferous tubules showed a moderated amount of spermatozoa and edema, advenced degeneration and necrosis of spermatogonia and interstitial cells and abnormal distribution of spermatozoa. These results indicate that in male rats lead targets testicular spermatogenesis and sperm within the epididymis to produce reproductive toxicity. These findings support the results from other reports that lead acetate can seriously alter the testes and reproductive tract in male rats treated with lead (Jonasson and Bellicciari, 1988; El-Shafai et al., 2011). However, little is known about how vitamin E acts as a protective agent against lead-induced testicular toxicity. Administration of vitamin E to lead-treated rats restored testicular damage. Accumulating evidence suggests that the protective effect of vitamin E could be attributed to its antioxidative properties (Wang et al., 2004). From our results of the induction of chromosomal aberrations in bone marrow cells of rat treated with lead acetate, the aberrant type induced was only the structural type (chromatid gaps, deletion and fragment). Gaps are the most frequent type of aberration induced by lead exposure (Nordenson et al., 1978). In the present study, lead treatment caused increases in the percentage of chromosomal abnormalities in spermatocytes and sperm-head abnormalities, emphasizing a positive correlation between cytogenetic damage and sperm abnormality as was previously reported in mice (Lavu et al., 1985; El-Nahas et al., 1989). A significant increase in the percentage of sperm abnormalities occurred with lead acetate treated-animals. It can be mentioned in this context that the increase in the incidence of abnormal sperm has been reported after treatment of male mice with irradiation (Wyrobek and Bruce, 1978) as well as different chemical agents (insecticides) (Hassan et al., 1995). In the present study, the sperm-head abnormalities such as amorphous and banana-like had the highest incidence of aberration in the treated group, while the lack of hook and hammer-shape had the lowest frequency. This study showed that rats treated

with lead acetate revealed an increase in the frequency of total epididymal sperm-head abnormalities. These results are in accordance with García-Lestòn et al. (2010) who suggested that lead would induce the disruption of spermatogenesis in the testes causing a detoriation of motility and content of sperm as well as morphological abnormalities. In an attempt to explain the different mechanisms involved in the induction of the abnormal morphology of the sperm heads, Kaczmarski (1972) stated that the incomplete condensation of chromatin and the presence of large vacuoles and canals containing remnants of cytoplasm in various regions of the head are the cause of failure of the sperm to pass through the final steps of maturation occurring normally during spermatogenesis. Moreover, Topham (1980a) mentioned that the agents that accumulate in the testis can cause alterations in testicular DNA and disrupt the process of differentiation of spermatozoa directly. It is clear from the obtained results that vitamin E had a high protective role against sperm-head abnormalities induced by lead acetate.

In conclusion, lead acetate can be considered as an environmental genotoxic material. Vitamin E has a protective effect on lead acetate induced testicular damage..

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