

The Protective Effect of Vitamin E against Genotoxicity of Lead Acetate Intraperitoneal Administration in Male Rat

Nadia AIT HAMADOUCHE^{*}1, Nesrine SADI¹, Omar KHAROUBI¹

Miloud SLIMANI², Abderlkader AOUES¹

¹Department of Biology, Laboratory of Experimental Biotoxicology, Biodepollution and Phytoremediation, Dr Moulay Tahar Oran, Algeria; naithamadouche@live.fr

²Department of Biology, Faculty of Science and Technology, Saida, Algeria

Abstract

Lead is industrial pollutant that may have toxic effects on the male. The aim of this study was to further investigate the protective effects of vitamin E on lead acetate (Pb) induced reproductive toxicities and genotoxicity effects in male rat. Sexually mature male Wistar rats (weighing 120-160 g) were given Pb (20 mg/Kg) and vitamin E (600 mg/kg/rat) orally for 20 days. The sperm counts, sperm motility, sperm morphology, chromosomal aberrations, FSH, LH and testosterone levels, and histopathological changes in the testes of these rats, were investigated at the end of 20 days. Result revealed a statistically significant ($p < 0.01$) increase in the number of abnormal sperm in treated animal. Lead acetate increased the percentage of chromosomal abnormalities. Significant decrease in LH, FSH, and testosterone were observed in treated group compare with the control. Pathological examination of testicular tissues showed degenerative changes of spermatogonia and spermatocytes to advanced degeneration and vacuolation. The observation, dealing that lead acetate can be considered as an environmental genotoxic and cytotoxic effect in male rat and may contribute in reduction of fertility. We recommended that it must be administered of vitamin E as a protective agent to reduce the genotoxic effect of lead in the somatic and germ cells.

Keywords: lead, genotoxicity, chromosome aberration, vitamin E

Introduction

The humans are exposed to various types of environmental contaminants at different stages of their life span, majority of them are harmful. In recent years, there has been growing concern about the deleterious effects of chemical on developing male reproductive system. Exposure of heavy metals during pregnancy has been associated with adverse effects on development of gonads. These substances may act as testicular toxicants and correspond to different compounds, which are related to social habits, life conditions, working hazards or 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 human and animals, it is also well known that these metals form a crucial part innormal biological functioning of cells. Many heavy metals are classical testicular toxicants, 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 a disruption of the hypothalamic control of pituitary hormone secretion and in turn, spermatogenesis (Sokol, 1987). Since male do not possess accessory reproductive organs, reproductive potential relates to three factors: sperm availability, quality and quantity (Tsuji and Karagatzides,

2001). Vitamins are essential to maintain normal metabolic processes and homeostasis within the body. Vitamin C (Vit C) and vitamin 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 potential role of antioxidants to ameliorate lead toxicity. The natural antioxidants may be helpful in preventing or reducing the harmful effects of ROS on 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 Vit E increases total sperm output and sperm concentration in rabbits (Yousef, 2010) and rams (Yue *et al.*, 2010). Conversely, deficiency of Vit E may lead to detrimental effects on the reproductive organs, such as degenerative spermatogonium, testicular damage and degeneration of the seminiferous tubules (Wilson *et al.*, 2003). Influence of dietary Vit E on semen quality has been described in mice (Sánchez-Gutiérrez *et al.*, 2008), rat (Wu *et al.*, 1979), rabbit (Cesare *et al.*, 2002) and goats (Shi *et al.*, 2010).

Consequently, this study aimed to evaluate (1) the influence of lead acetate on reproductive organs and fertil-

ity of male albino rats (2) the protective role of Vit 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 12/12 h light/dark cycle prior to use in experimental protocols. All the procedure performed on animals were approved and conducted in accordance with the National Institute of health Guide (Reg. No. 488/160/1999/CPC-SEA).

Grouping of animals and treatment

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

Reproductive organs weights

All rats were euthanized at the end of the experiment. After animal dissection, the testes, epididymes and accessory sex organs (seminal vesicles and prostate glands) were removed, grossly examined and weighed. The index weight (I.W.) of each organ was calculated by Matousek (1969) $I.W. = \text{organ weight (g)} / 100 \times \text{body weight (g)}$.

Sperm motility

Sperm-progressive motility 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 $\times 400$ magnification.

Sperm abnormalities

A total of 300 sperm was counted on each slide under light microscope at $\times 400$ 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 the abnormal shape involving the head was recorded.

Determination of serum testosterone, LH and FSH levels

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

Chromosomal abnormalities in rat bone-marrow cells

After 24 h from the last treatment, rats were injected with 0.6 mg kg⁻¹ b.w. colcemid 2 h prior 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 student's t-test for unpaired means. A p value $< 0, 05$ was considered significant.

Results

Body weight changes

Tab. 1 shows that rats in control group had significant ($P < 0.05$) increase in weight. Both lead acetate group lost weight when compared with their initial weights. However the weight loss by the Pb rats that received vitamin E.

Tab. 1. Effect of lead and its combination with Vit E on body weight and reproductive organs weights of male rats (g)

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

The values expressed as mean \pm SEM (n=10). * $P < 0.05$

Organs weights

The index weight of testis, epididymis and accessory sex glands was significantly decreased ($P \leq 0.05$) in rats treated with lead compared to the control group. While the reduction was less pronounced in the group treated with lead plus vit. E (Tab. 1).

Differential sperm characteristics

Epididymal sperm concentration, sperm motility, viability and abnormal sperm are reported in Tab. 2 for Pb and vit. E groups. Pb group had highly significantly ($p < 0,01$) lower sperm count, motility and viability than the control group. However, vit. E group significantly increased the sperm concentration ($p < 0,05$), when compared to the control group.

Tab. 2. Effect of Vit E on sperm character of lead acetate intoxicated male rats

Experimental groups	Sperm character			
	Count (106/ml)	Motility (%)	Viability (%)	Sperm abnormalities (%)
Control	63,3±3,63	84,4±4,53	90,6±4,31	-6,8±0,51
Pb	29,6±1,29**	43,1±3,73**	53,1±3,65**	19,4±0,93**
Pb+Vit E	45,3±3,22*	59,4±2,16*	69,8±2,85*	13,2±0,85*

Tab. 3 and Fig. 1, represented the incidence of abnormality in the shape of sperms per 1000 for each rats treated with Pb and protected with vit. E, which include hammer shape. 1(Fig a), banana shape (Fig. 1b), lack of hook (Fig. 1c) and amorphous (Fig.1d). The highest incidence of abnormality in the shape of sperms of rats treated with lead acetate was amorphous while hammer shape was the lowest frequency. Vit E made a high protective role against sperm head abnormalities induced by Pb.

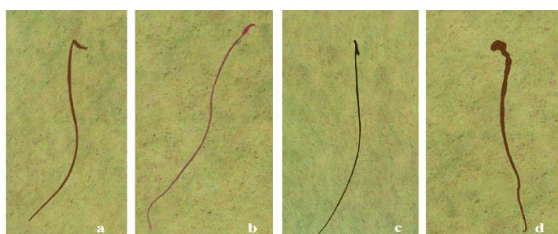


Fig 1. Types of sperm head abnormalities in rat treatment with Pb. a: hammer; b: banana like; c: lake of hook; d: amorphous

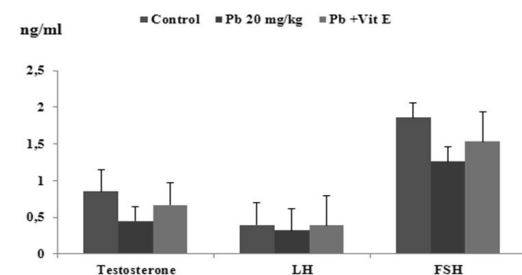


Fig. 2. Serum levels of testosterone, LH and FSH after intraperitoneal lead acetate. The values expressed as mean \pm SEM (n=10). * $p < 0,05$; ** $p < 0,01$

Plasma testosterone, LH and FSH level

Results in Fig. 2 showed highly significant decrease in serum testosterone, LH and FSH concentration ($p < 0,01$) in Pb group compared to control group, while orally treatment with Vit E induced a significant elevation in serum testosterone, LH and FSH concentration.

Tab. 3. Incidence of the sperm head abnormality per thousand after treatment with Pb and protection with vit. E

Groups	Abnormal sperm			
	Amorphous	Banana like	Without hook	Hummer shape
Control	11,6±0,91	1,6±0,42	2,4±0,37	0,3±0,2
Pb 20 mg/kg	42,4±0,86**	17,8±0,51**	15,8±0,70**	2,4±0,51**
Pb+Vit E	18,4±0,67*	3,8±0,35*	2,4±0,4*	0,9±0,24*

Chromosomal abnormalities in rat bone-marrow cells

Various chromosomal aberration are observed in the bone marrow cells of male treated with Pb and protected with Vit E. Structural and numerical types of aberration are elucidate in Tab. 4 and Fig. 3 respectively. Tab. 4, showed the average of chromosomal abnormalities of bone marrow cells of male rats treated with Pb and protected with Vit E.

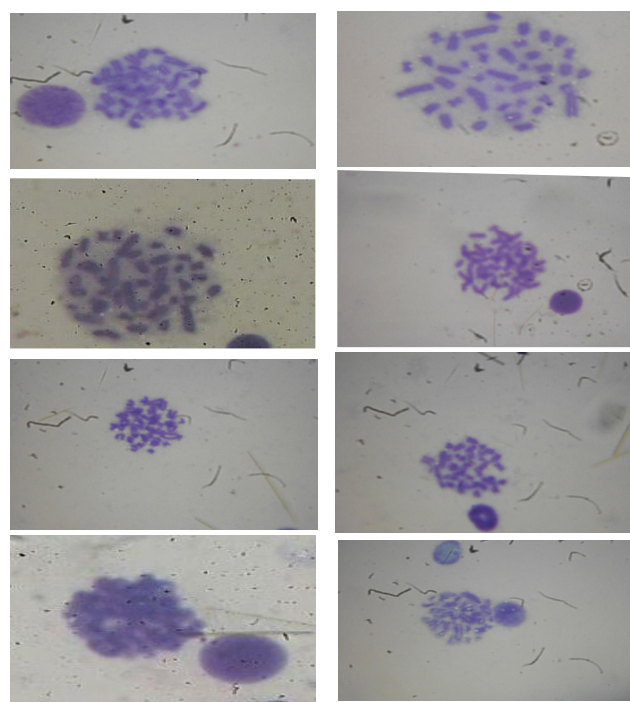


Fig. 3. Types of chromosomal aberrations in rat bone marrow cells treated with acetate lead. a: control; b: centromeric attenuation (Cat) and fusion (Fu); c: Fusion (Fu); d: chromosomal ring (R); e: centromeric attenuation (Cat); f: chromosomal ring (R) and fusion (F); g: deletion (D), chromosomal ring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

It represented a very high significant increase in treated group with Pb than control group, on the other hand it indicate that vit. E made a high protective role against Pb. The structural aberrations included chromatid deletion (Fig. 3g), centromeric attenuation (Fig. 1be), centric fusion (Fig 1. cf), gap (Fig. 3 g), and chromosomal ring (Fig. 1df). Stickiness may give arise to sticky adhesion between two or more chromosomes, and formation of stick bridges at metaphase (Fig. 3h).

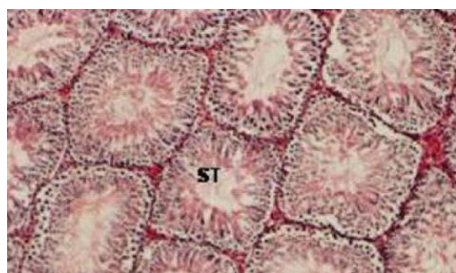


Fig. 4. Testes of male rats control showing normal structure of seminiferous tubules (ST) (H&E X 200)

Tab. 4. Average of chromosomal abnormalities observed in bone marrow cells of male rats treated with Pb and protected with Vit E

Groups	Structural aberrations								Total
	Deletion	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

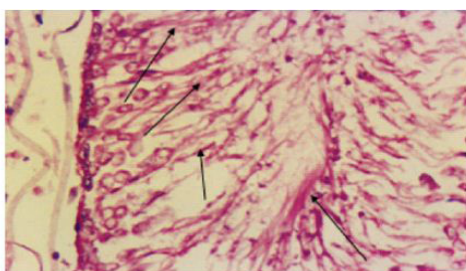


Fig. 7. Testes of male albino rats intoxicated with cadmium chloride alone showing complete testicular necrosis and sloughing of all layers, ischemic necrosis, (Arrows) (H&E .X. 400).

Fig. 5. Testes of male rat intoxicated with lead acetate showing complete testicular necrosis and sloughing of all layers, ischemic necrosis (arrows) (H&E X 400)

Histopathological results

The normal architecture of testicular seminiferous tubules and interstitial spaces were shown in the control rats (Fig 4). Moreover, in rats which were intoxicated with lead

acetate showed complete necrosis and sloughing of all layers of seminiferous tubules (Fig. 5). The testes of rats, treated with Vit E showed mild to moderate edema, congestion with minute foci necrosis and hemorrhage (Fig. 6-7).

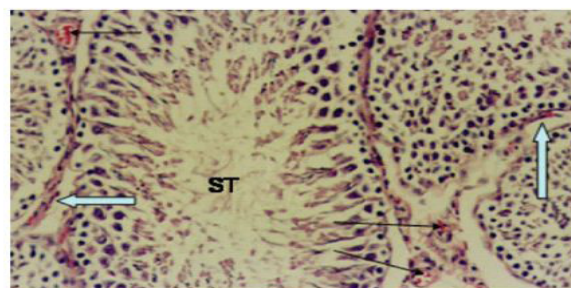


Fig. 6. Testes of male rats treated with lead acetate and vitamin E showing mild degenerative changes of seminiferous tubules (ST), mild congestion (thin arrows) and edema (hollow arrows) (H&E X 200)

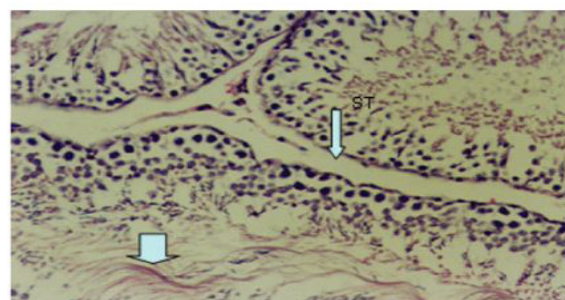


Fig. 7. Testes of male rats treated with lead acetate and vitamin E showing nearly normal seminiferous tubules with mild edema (thin hollow arrow) and necrosis (big hollow arrow) (H&E X 200)

Discussion

The toxicity of lead has been studied from many years throughout several end-points but data related to the mutagenic, clastogenic and carcinogenic properties of lead and lead compounds is still conflicting. The IARC classified lead as possible human carcinogen (IARC, 1987), on the basis of sufficient evidence for carcinogenicity in experimental animals but inadequate evidence for carcinogenicity in humans, and the 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 chromosomes aberration by lead chromate (Douglas *et al.*, 1980; Wise *et al.*, 2003; Wise *et al.*, 2004; Xie *et al.*, 2005), the positive results achieved may be related to the toxic action of chromate and not to lead, based on the results reported by Douglas *et al.* (1980). The variability found in the different studies could be due to

the influence of different experimental variables that may act as confounding factors, such as duration route of lead exposure, cell culturing time following the 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 similarly, modifying the results of the studies. Regarding to this last factor, many of the epidemiological studies reviewed suggest the possibility that multiple exposures present in the occupational environment, and not only lead, are responsible for the obtained results (García-Lestón *et al.*, 2010). To our knowledge, the present study planned to evaluate the protective effect of vitamin E against testicular damage induced by lead acetate toxicity in experimental animals. The effect of lead acetate on final body weight of intoxicated rats was significantly lower than that of the health normal group. These results clearly indicated that lead caused a significant decrease in the gain of body weight. Nabil *et al.* (2012) found that lead caused decrease in growth rate in rats when fed lead. These results in body weight gain which may be caused by the toxic ions could be associated with several factors, one of which is imbalance metabolism produced by impairing zinc status in zinc-dependent enzymes which 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. Weight of the testis is largely dependent on the mass of the 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). Weight of accessory sex organs were also decreased in lead acetate treatment. Weight loss of accessory sex organs corresponds with 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).

As mentioned earlier, in the present study serum testosterone level LH and FSH were decreased in lead acetate treated groups of animals as compared to their respective control. Significant alterations in testosterone LH and FSH levels have been reported after exposure to certain heavy metals (Gabuchyan, 1987; Chattopadhyay *et al.*, 2005; Atef 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 that regulates spermatogenesis. The secretion of testosterone by the Leydig cells is dependent upon the secretion of LH by the pituitary gland (Fatma Gokce 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. To 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 was reflected by the increase of testis weight and serum testosterone, LH and FSH levels (Muthu and Krishnamoorthy, 2012). The potential toxicity of lead caused alterations in sperm morphology, count, motility as well as hormones (Chowdhury, 2009). Lead has an adverse effect on sperm count and retarded the activity of alive sperm. Moreover, motility as well as prolonged latency of sperm melting both in exposed person and experimental animals were observed after Pb exposure (Lancranjan *et al.*, 1975; 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 decrease motility and increased incidence of teratospermia at higher dose of Pb exposure along with inhibition of post-meiotic cells mainly pachytene spermatocyte were noted (Attar, 2011). In the same experiment the detachment of germinal cell layer from basal membrane, atrophy of leydig cells plus interstitial edema and low density of seminal plasma were also observed. Additionally, Madhavi *et al.* (2007) showed that Pb induced cytogenetic damage in germ cells of mice. Testicular damage was also confirmed by histopathological lesions (Muthu and Krishnamoorthy, 2012). The present study clearly demonstrated that lead acetate can seriously alter the testicular tissues which started the changes with vacuolar degeneration till necrosis and atrophy of seminiferous tubules. Treated groups showing vacuolation and degenerative changes of most spermatogonia arrest of spermatogenesis and pyknotic changes of spermatocytes. The center of most seminiferous tubules showed moderated number of spermatozoa and edema, advanced degeneration and necrosis of spermatogonia and interstitial cells and abnormal distribution of spermatozoa. These results indicating 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 (Johansson and Pellicciari, 1988; El-Shafai *et al.*, 2011). However, little is known about vitamin E act as protective agents against lead induced testicular toxicity. Administration of vitamin E with Pb treatment clearly restored the testicular damage. Accumulating evidence suggest that the protective effect of vitamin E could be attributed to its anti-oxidative 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). It was clearly indicated that gaps are the most frequent type of aberration to be

induced by lead exposure as mentioned by Nordenson *et al.* (1978). In the present study, Pb increase in percentage of chromosome abnormalities in spermatocytes and sperm head abnormalities induced lead emphasize the positive correlation between cytogenetic damage and sperm abnormality which 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 may be mentioned in this context that, increase in the incidence of abnormal sperm have been reported after treatment of male mice with irradiation (Wyrobek and Bruce, 1978) as well as different chemical agents (insecticide) (Hassan *et al.*, 1995). In the present study, the sperm head abnormalities as amorphous and banana like was the highest incidence of aberration in treated group, while lack of hook and hammer shape was 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 in accordance with García-Lestón *et al.* (2010) who suggest that lead would induce disruption of spermatogenesis in the testes causing deterioration 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. Kaczmarek (1972) stated that incomplete condensation of chromatin and the presence of large vacuoles and canals containing remnants of cytoplasm in various regions of the head is the cause of failure of sperm to pass through the final steps of maturation occurring normally during spermatogenesis. Moreover, Topham (1980a) mentioned the agents which 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 made a high protective role against sperm head abnormalities induced by lead acetate.

Conclusion

In conclusion, the combination of laboratory tests, suggested that lead acetate can be considered as an environmental genotoxic material. Besides, the present study showed that vitamin E has protective effect on lead acetate induced testicular damage. This study therefore suggests that vitamin E may be a useful preventive agent against the effect on the studied lead acetate at least partly due to its antioxidant properties.

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