The Possible Protective Effect of Vitamin E on Lead Acetate Toxicity on the Testes of Adult Albino Rats

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Abstract: Introduction: Lead (pb) is considered to be one of the major environmental pollutants which have potential threat to human health. Exposure to lead is still a major medical problem in both environmental and occupational settings. **Aim of the work:** To detect the possible harmful effects of lead acetate on the testes of adult albino rat and to study the effect of lead acetate withdrawal and to detect whether vitamin E will reverse these harmful effects or not. **Material and Methods:** forty mal albino rats were maintained for 20 days as follows: Group1, control group, group 2, lead acetate group (20 mg/kg/day, intrapertonial (IP), Group 3, lead acetate withdrawal (20 mg/kg/day (IP)) then sacrified20 days after the last dose, group 4, lead acetate + vitamin E (600 mg/kg/day (IP)). At the end of experimental study, testicular tissues and blood samples were taken for histological and laboratory studies. **Result:** Comparing with the control it was found that, simultaneous administration of vitamin E with lead acetate and lead acetate withdrawal showed protective effect against histotoxicity and laboratory results of lead acetate, through increases in the thickness of germinal epithilum with significant increase in the size of the seminiferouse tubules, sperm count and significant increase in serum testosterone level. **Conclusion:** Lead acetate can be considered as an environmental genotoxic material that could destroy testicular tissues in short time duration while lead acetae withdrawal and vitamin E could produce partial protection through its antioxidant mechanism. Further studies are necessary to illuminate the other dark sides of lead acetae on infertility in human and to discover others protective agents against its toxicity. According to our results we recommend administration of vitamin E as a dietary supplement to human population exposed to lead toxicity.
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Key words: Lead acetate, Testes, Vitamin E, Fertility.

1. Introduction

Lead (Pb) 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 (1). The most common source of lead is storage batteries, as it was reported that, about 70% of current lead use occurs in these batteries. It is 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) (2).Air pollution by lead can be produced by combustion of leaded gasoline in vehicles and it is the major source of pollution in the developing countries. Other than, to contamination of air, lead also contaminates the water sources and the cultivated soils along the highways (3). Lead exposure can affect reproductive system. It had been shown to change sperm shape and reduce sperm numbers in seminal fluid and this observation may explain some of the causes of idiopathic male infertility (4). The possible molecular mechanism involved in lead toxicity is oxidative stress, which is a consequence of an unbalance between oxidants and the antioxidant systems (5). 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 (ROS) such as superoxide radicals, hydrogen peroxide and hydroxyl radicals and lipid peroxides (6). Vitamin E is a fat soluble vitamin and thought to protect tissues by reducing or preventing oxidative damage. Insufficient intake of vitamin E has been reported to produce deleterious effects on the process of spermatogenesis and production of normal sperms (7). Vitamin E regulates oxidation processes in the body as it acts as a powerful antioxidant preventing membrane damage mediated by free radicals (8).

2. Material and Methods

Animals used:

Forty adult male wistar albino rats of 8-10 weeks and weighting 200-250 gram (gm) were used. The animals were obtained from animal house, Faculty of Medicine, Cairo University. They were housed in spacious wire mesh cages in a well-ventilated room, and were kept under a constant day/night cycle in a climate controlled condition with an access to food

and water. All experiments were carried out according to the guidelines of the Institutional Animal Ethics Committee.

Chemical:

1- Lead acetate was obtained from Sigma-Aldrich company through Egyptian International Center. Cairo-Egypt, in the form of white powder. One gm of lead acetate was dissolved in 100 ml distilled water, so each 1ml contained 10 mg of lead acetate.

2-Vitamine E was obtained from Faculty of Pharmacy, Al-Azhar University, Egypt. One gm of vitamin E was dissolved in 10 ml of corn oil, so each 1ml contained 100 mg of vitamin E.

Experimental Design:

After two weeks of acclimatization, the rats were randomly divided into four groups:

Group 1 (control):

- Formed of 10rats.
- They received distilled water orally for 20 days (9).

Group 2 (lead acetate exposure):

- Formed of 10 rats.
- Animals were injected with lead acetate (20mg/kg body weight) intraperitoneal (IP) every day for 20 days and sacrified at day 21(9).

Group 3 (lead acetate withdrawal):

- Formed of 10 rats.
- Animals were injected (IP) with lead acetate (20 mg/kg body weight) every day for 20 days and sacrified 20 days after the last dose (9).

Group 4 (lead acetate and vitamin E treated):

- Formed of 10 rats.
- Animals were injected IP with lead acetate (20 mg/kg body weight) followed by oral administration of vitamin E (600mg/kg body weight) every day for 20 and sacrified at day 21(9).

Specimens collection:

At the assigned times, the rats were anaesthetized using ether inhalation, and blood samples were obtained by direct left ventricle puncture for serum testosterone measurement. Then the testes were dissected out, preserved in 10% buffered formalin and then processed for paraffin section.

Processing of the specimens for light microscopic examination:

The specimens were processed for paraffin sections by gradual dehydration using ascending graded concentrations of alcohol, cleared in xylene and embedded in soft and then in hard paraffin wax.

Transverse sections were cut at 5-6 µm and treated as follows:

1- Sections were stained with H & E for evaluation of histopathological changes.

2-Sections were stained with AgNOR for detection of mitotic activity.

3-Sections were stained with Mallory's Trichome stain for identification of collagen fiber.

Testosterone level assessment:

At the end of the experiment serum testosterone was measured in Ressalla lab Zgazig Egypt for all groups by VIDAS Testosterone. It is an automated quantitative test for use on the instruments of VIDAS family for the enzyme immunoassay measure of total testosterone in serum or plasma, using the ELFA technique (Enzyme Linked Fluorescent Assay). It is intended in the diagnosis and management of conditions involving excess or deficiency of this androgen.

3. Results:

1. Histological results

Group 1: (Control group)

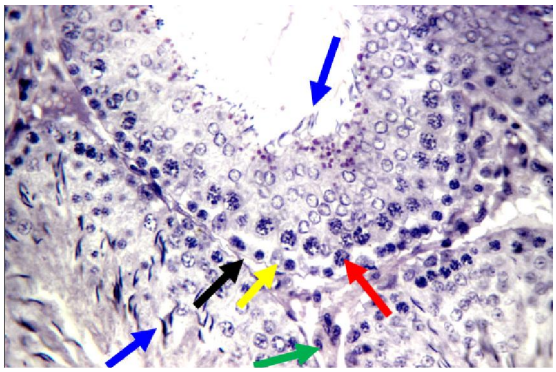

Fig 1: A photomicrrograph of a section of the testis of control adult albino rat showing semniferous tubule with average BM (black arrow), Sertoli cell (yellow arrow), average germinal lining with primary spermatocyte (red arrow), many spermatozoa (blue arrows), spermatid (violet arrow) and average interstitium showing Leydig cells (green arrow) (H & E X 360).

The testicular tissue of adult albino rat of control group showed well circumscribed circular seminiferous tubules with rounded or oval contour with regular basement membrane (B.M) and were lined with stratified germinal epithielium showing two types of cells, germ and sertoli cells. Sperms were seen in the lumina of the tubules. The stratified germinal epithelium revaled several types of spermatogenic cells. Spermatogonia appeared as small rounded cells with rounded nuclei. Primary spermatocytes appeared large in size with large rounded nuclei located closer to the lumen of the tubule than the spermatogonia. Spermatids were small rounded cells with pale nuclei. Spermatozoawere present free in the lumen of seminiferous tubutoli cells

were detected in between spermatogenic cells as pyrimidal cell resting up on the basement membrane, they have large ovoid vesicular nuclei, while cytoplosmic outlines cannot be seen distinctly. The interstitial tissue was lying in between the seminiferous tubules and containing Leydig cells. The Leydig cells were present singly or in groups in the connective tissue. They had oval or spherical nuclei. (fig.1).

Group 1(Control group)

The seminiferouse tubules surrounded by thin and regular basement membrane. (fig.2)

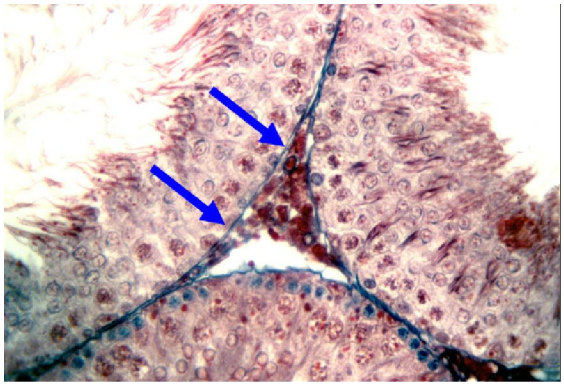

Fig 2: A photomicrograph of a section of the testis of adult control albino rat showing seminiferous tubules surrounded by thin and continuous basement membrane (blue arrows) (Mallory's stain X 360)

Group 1(Control group)

AgNOR dots were located strictly within the nuclei of the spermatocytes.

They were clearly visible as black dots.

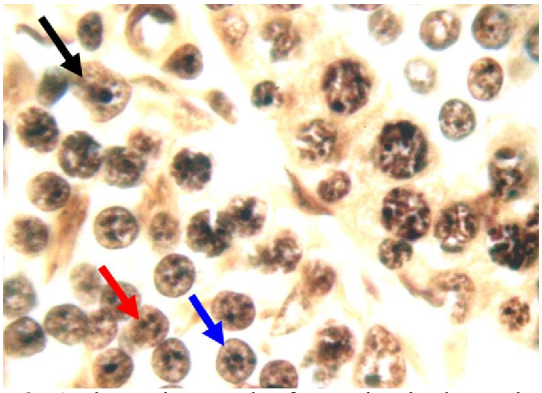

Fig 3: A photomicrograph of a section in the testis of control adult albino rat showing3 dots\ nucleus (red arrow), other one showing up to 4 dots\nucleus (black arrow), and other one showing 5dots\nucleus (blue arrow) (Ag NOR stain x 1000)

Group 2 (Lead acetate treated)

The testicular tissue of adult albino rat of lead acetate treated group showed shrinkage of seminiferous tubules and wide interstitial spaces between seminiferous tubules with sub capsular and interstitial edema. Some seminiferous tubules appeared small in diameter, distorted and degenerated while other S.T showing incomplete spermatogenesis and empty lumen. The germinal lining was necrotic with few sprermatids and no sperms. All these S.T were widely separated with distorted interstitial tissue containing few irregular spindle shaped leydig cells. Few seminiferous tubules appeared distorted with distorted lining formed mainly from sertoli cells and primary spermatocytes with no spermatids or sperms.

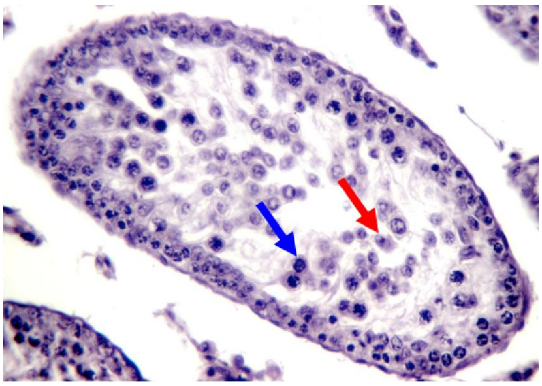

Fig 4: A photomicrograph of a section of the testis of adult albino rat treated with lead acetate for 20 showing distorted tubule with distorted lining formed mainly from sertoli cells (red arrow), and primary spermatocytes (blue arrow), no spermatids or sperms (H & E X 360)

Group 2 (Lead acetate treated)

The seminiferous tubules surrounded by markedly thick and irregular basement membrane.

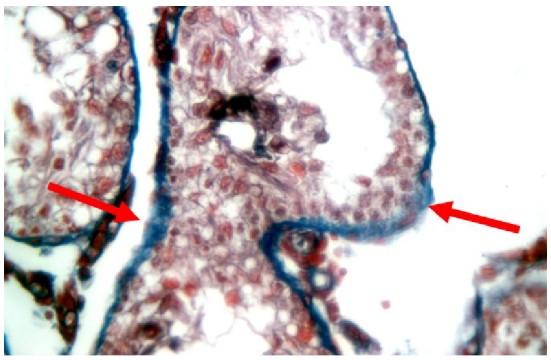

Fig 5: A photomicrograph of a section of the testis of adult albino rat treated with lead acetate for 20 days showing: distorted seminiferous tubules with thick and irregular basement membrane (red arrows) (Mallory's stain X 360)

Group 2 (Lead acetate treated)

The least number of AgNOR dots was present in lead acetate treated group indicating mitotic arrest and decreased cell proliferation.

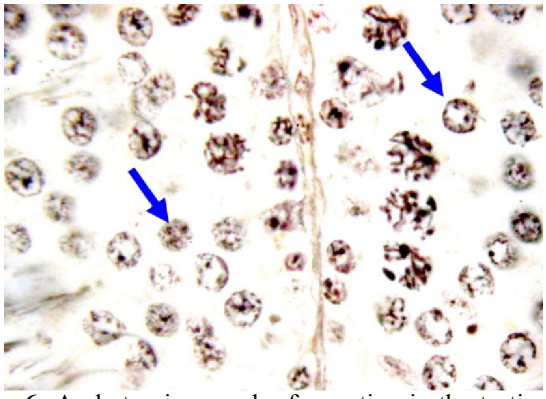

Fig 6: A photomicrograph of a section in the testis of adult albino rat treated with lead acetate for 20 days showing most of germinal cells showing1 dot\ nucleus (red arrows), other one showing 2 dots\nucleus (black arrow), and few showing more than 2 dots\nucleus (blue arrow) (Ag NOR stain x 1000)

Group 3 (Lead acetate withdrawal)

Most of the tubules were small in size, necrotic and empty with thin lining while some S.T appeared average in size with complete spermatogenesis. Seminiferous tubules were returning back to normal showing complete spermatogenesis with thin germinal lining and average interstitium showing increased number of polygonal Leydig cells

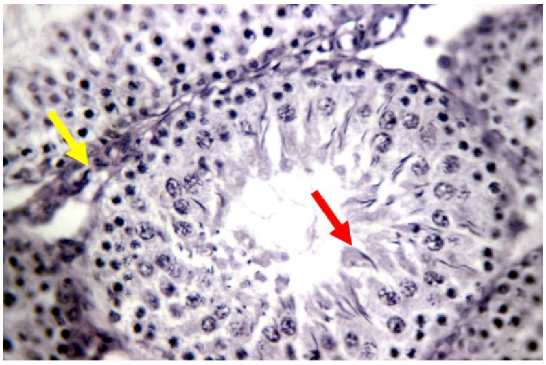

Fig 7: A photomicrograph of a section in the testis of adult albino rat treated with lead acetate for 20 days and scarified 20days after the last dose showing semniferous tubule with average lining and full spermatogenesis (red arrow), with average interstitium containing leydig cells (yellow arrow) (H & E X 360)

Group 3 (Lead acetate withdrawal)

The seminiferous tubules were surrounded by mildly thick and irregular basement membrane.

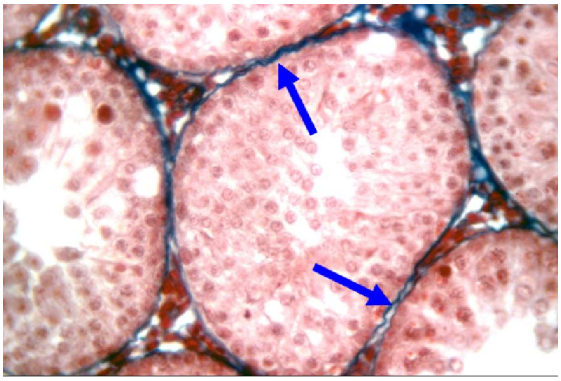

Fig 8:: A photomicrograph of a section in the testis of adult albino rat treated with lead acetate for 20 days and sacrified 20 days after the last dose showing: seminiferous tubules surrounded by mildly thick and irregular basement membrane (blue arrows) (Mallory's stain X 360)

Group 3 (Lead acetate withdrawal)

AgNOR dots were clearly visible as black dots and increased in number than that of lead acetate treated indicating mitotic activity and cellular proliferation

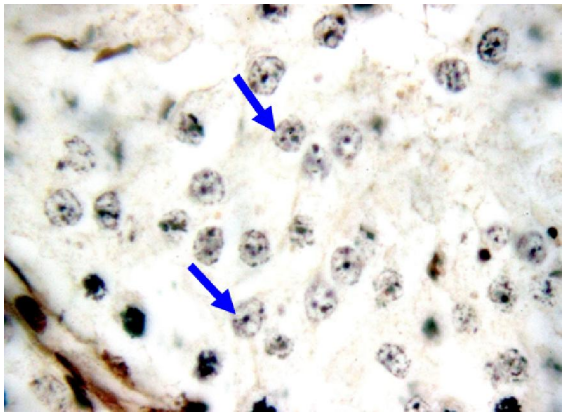

Fig 9: A photomicrograph of a section in the testis of adult albino rat treated with lead acetate for 20 days and sacrified 20 days after the last dose showing most of germinal cells showing more than 3 dots\nucleus (blue arrows) (Ag NOR stain x 1000)

Group 4 (Lead acetate and vitamin E)

Most of the seminiferous tubules appeared regular in their outline, they showed nearly normal architecture with average basement membrane and average interstitial tissue which contains polygonal leydig cells. The seminiferous tubules showed average size, average B.M, complete spermatogenesis in the form of spermatogonia, primary spermatocytes, spermatids and mature sperms. Sertoli cells were present separating the columns of germinal cells.

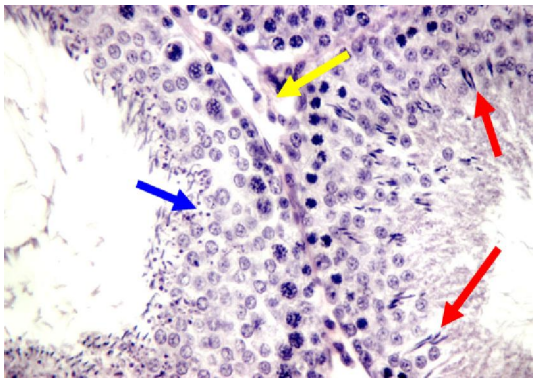

Fig 10: A photomicrograph of a section in the testis of adult albino rat treated with lead acetate and vitamin E showing average germinal lining with many sperms (red arrows) and many spermatids (blue arrow), with average interstitium containing polygonal leydig cells (yellow arrow) (H & E X 360)

Group 4 (Lead acetate and vitamin E)

The seminiferouse tubules were similar to control that surrounded by thin continuous basement membrane.

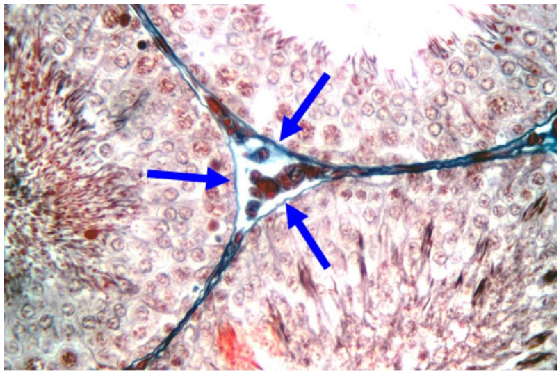

Fig 11: A photomicrograph of a section in the testis of adult albino rat treated with lead acetate and vitamin E for 20 days showing seminiferous tubules surrounded by thin and continuous basement membrane (blue arrows) (Mallory's stain X 360)

Group 4 (Lead acetate and vitamin E)

4. Discussion

Lead is a ubiquitous environmental and industrial pollutant that has been detected in every facet of environmental and biological systems (10)**.** The toxicity of lead remains a matter of public health concern due to its pervasiveness in the environment and the awareness about its toxic effects (11)**.** 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 (12).

AgNOR dots were clearly visible as black dots and increased in number than that of lead acetate treated indicating mitotic activity and cellular proliferation.

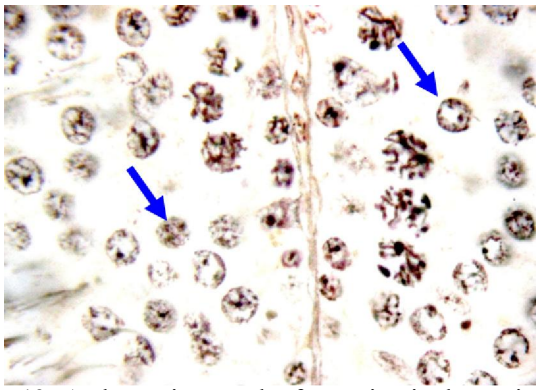

Fig 12: A photomicrograph of a section in the testis of adult albino rat treated with lead acetate and vitamin E for 20 days showing most of germinal cells showing more than 3 dots\nucleus (blue arrows) (Ag NOR stain x 1000)

Laboratory Results:

Testosterone hormone level measurements:

There was highly significant decrease in testosterone hormone obtained from lead acetate treated group for 20 days (0.21ng/ml) as compared to that of control group (0.35 ng/ml). On the other hand, there was insignificant decrease in testosterone hormone in lead acetate and vitamin E treated group for 20 days (0.31ng/ml) and withdrawal group (0.28ng/ml).

Table 1: Testosterone level measurements of all groups

Group	Testosterone (ng/ml)
Control	0.35
Lead acetate treated	0.21
Lead acetate withdrawal	0.28
Lead acetate + Vitamin E	0.31

The adult albino rat was used in this study due to its similarity to testicular architecture of human. The onset of puberty is around the age of 7-9 weeks and when the average weight is 200 gm **(**13**)**. In the present study the specimens were stained by Haemoatoxyline and Eosin, AgNOR stain and Mallory's trichome for histological study. Hamatoxyline and Eosin stain is the best stain that can demonstrate the acidophilic and basophilic components of the cell, AgNOR was used to demonstrate mitotic activity and Mallory's trichome for identification of collagen fiber. All animals were kept in animal house under the same environmental condition and were allowed to move freely in their

cages. The rats were fed on daily diet composed of milk powder, bread and vegetables; however five rats died from the treated group, and no deaths between the other group. In the present work, the animals exposed to some general signs of ill-health including loss of appetite (anorxia), diminished movement (lethargy), recurrent attacks of diarrhea, vomiting and reduction of weight, all these signs appeared in the lead treated group only. 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, the testes of the treated group showed that lead exposure caused testes atrophy manifested by distorted and shrunken seminiferous tubules with thick and irregular membrane, degenerated spermatogonia and spermatocytes, absence of spermatids and mature sperm and reduction in the interstitial cells of leydig, this could be due to distribution of lead in the seminiferous tubules via blood stream and then absorbed lead caused mechanism of toxicity in the seminiferoustubules. These results are in agreement with (14**)** who reported that lead exposure caused progressive vascular, tubular and interstitial testicular damage. These results also are in agreement with the finding of (15), (16), (17) who proved that exposure of lead induced testes injury represented by apoptotic changes in most of the germ cells and thickness in the basement membrane of the seminiferouse tubules might be a result of increase in the amount of collagenous fibers that could result from either over production of collagen fibers by fibroblasts or decrease the rate of collagen phagocytosis. In the collapsed cases the seminiferous tubules were characterized by absence of spermatozoa in the center. In agreement with **(**9**)** who reported that lead aceate induced reduction in sperm count, sperm motility, sperm viability and normal sperm cells. Also, our result were in agreement with the results obtained by (18), (1) who showed that lead acetate can seriously alter the testes and reproductive tract in male rats. The results of the present study showed that in lead intoxication, spermatids underwent degenerative changes, which may lead to a reduced number of spermatozoa. Lead 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 (19), (20) who showed that destruction of Leydig cells may cause testicular atrophy, gonadal dysfunction, and male factor infertility. The cause of these degenerative changes can be attributed to oxidative stress**.** In this study lead withdrawal resulted in improvement of the testicular histology in the form of complete spermatogenesis with normal spermatogonia, primary spermatocytes, spermatids and spermatozoa. This an agreement with (21) who reported that there were both regeneration of the germinal epithelium and restructuring of the interstitium towards normal in the recovery groups. 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 lead induced oxidative stress in rat male reproductive organs, in accordance with literature reports. Also our study demonstrated that vitamin E resulted in improvement of testicular histology in the form of complete spermatogenesis with normal spermatogonia, primary spermatocytes, spermatids and spermatozoa. This is an agreement with **(22)**, (**23**) who revealed that coadministration of vitamin E with lead prevented the degeneration of different generations of germ cells to some extent and significantly increased its number toward control. In agreement with **(24)** vitamin E improved the reduction in sperm characteristics, hormonal levels and testicular alteration observed in lead treated. The study shows that lead exerts significant deletrious effects on male reproductive system and the concurrent administration of vitamin E ameliorated these detrimental effects. The ameliorating effect of vitamin E noticed in the present study may be attributed to its antioxidant properties as reported by (**25).** Natural antioxidant as polyphenols of vitamin E have received much attention for treatment of oxidative-stress-related pathological condition **(26).** In the present study, serum level of testosteron hormone in the animals were altered in the lead exposed animals and these results are in agreement with those reported by **(27).** They explained these effects by the fact that, increased blood lead content has been associated with disruption of hypothalmic secretion of hormones and spermatogenesis. On the other hand, **(28)** showed no significant decrease in serum testosteron level in lead treated rats. In this study lead withdrawal results in partial improvement of serum testosteron level. This result are in agreement with **(21)**. Results of the present study also showed that, vitamin E treatment on lead intoxicated animals showed a significant increase in testosteron hormone and an ameliorative effect on the semen quality assessed. These result are in line with **(23)** who found a marked increase in the plasma level of testosterone hormone in male rats given vitamin E (150 mg kg day) concomitantly with lead acetate (60 mg kg day) for 6 weeks.

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