

The effect of vitamin E on lead induced gonadal dysfunctions in adult Wistar Albino rats

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- Lead, Vitamin E
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Abstract

Background: Lead exposure can cause adverse effects on the reproductive system. This study aimed to evaluate the protective and therapeutic effects of vitamin E on lead-induced pituitary and gonadal dysfunctions and the possible mechanisms underlying these effects. Material and methods: 104 Albino Wistar adult rats were divided into four groups: group I: 12 rats received vehicle of lead and 12 rats received vehicle of vitamin E; Group II: 40 rats subdivided into 2 subgroups: Group Iia: 20 rats injected with lead acetate (10 mg/kg/day 5 times/week, i.p. for 6 weeks) and Group Iib: 20 rats injected with lead as previous then stopped for 6 weeks; Group III: 20 rats injected with lead acetate as previous followed by oral administration of vitamin E (50 mg/kg/day 5 times/week); Group IV: 20 rats received vitamin E simultaneously with lead acetate as previous. At the end of the experiment the animals were scarified and blood samples were collected for measurement of gonadotrophic, gonadal hormones, malondialdehyde (MDA), total antioxidant capacity (TAC) and caspase 3 by ELISA kits. The pituitary gland, testes, and ovaries were processed for histopathological examination. Results: Lead administration significantly decreased the plasma levels of gonadotrophic, and gonadal hormones, and TAC but significantly increased the plasma levels of MAD and caspase 3. Meanwhile, vitamin E administration with or after lead exposure significantly increased gonadotrophic, gonadal hormones and TAC and markedly decreased MAD and caspase 3. Conclusion: Vitamin E has protective and therapeutic effects on lead-induced gonadal dysfunction. This effect mediated by inhibition of oxidative stress and apoptosis.

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INTRODUCTION

Alongside worldwide environmental pollution heavy metal poisoning became a progressively serious world health problem (1). There were innumerable sources of lead in our environment such as lead-acid batteries, water pipes, lead based paints, weapons lead shot and bullets, cosmetics, alternate and folk medicines and yet a number of low-cost toys (2). Regrettably, a major resource of lead exposure was air and soil pollution from leaded gasoline (3,4).

Exposures to lead affected various physiological processes as energy metabolism, apoptosis, cell adhesion, intercellular and intracellular signaling, protein maturation and genetic regulation. Therefore, lead poisoning exhibited significant manifestations for instance hypertension, anemia, nephropathy, infertility, behavioral changes besides severe conditions as acute encephalopathy and death (5). Infertility affects about fifteen percent of couples frustrating to conceive (6). The mechanisms of lead toxicity were multifaceted. Lead induced oxidative stress, altered membrane biophysics, deregulated cell signaling, and impaired neurotransmission (7). Oxidative stress (OS) was related to male and female infertility [8]. OS was a state of increased cellular damage occurred when the production of reactive oxygen species (ROS) exceeded the body's anti-oxidant defenses. ROS molecules bind with protein, lipid, carbohydrates and DNA within the cells caused pathological reactions that resulted in cellular damage included cell membranes and genetic materials (9).

Apoptosis is an active, exactly regulated, energy dependent cell death process (10). It was required for normal development and maintaining normal homeostasis (11). Caspases played vital roles in apoptosis. Caspases activation was followed by a chain of cellular modulation such as condensation of chromatin, fragmentation of DNA, blabbing and shrinkage of the cell membrane (10).

The majority of the chelating agents used in management of lead poisoning had serious side effects and their effects were frequently temporary (12). Thus, the use of agents such as vitamin E that treat lead poisoning is considered to be an important therapeutic strategy. The beneficial effect of vitamin E on reproductive system was demonstrated on several studies (13- 15).

So, the aim of this experimental study was to find out the effect of chronic administration of lead on gonadal functions in both male and female adult Westar albino rats and possible mechanisms of lead toxicity. Also, to find out the possible protective and therapeutic effects of administration of vitamin E on gonadal toxicity induced by chronic lead administration. Thus, levels of luteinizing hormone (LH), follicle stimulating hormone (FSH) in male and female rats, testosterone in males and estradiol and progesterone in female rats were measured as well as histological examination of pituitary gland, ovaries and testes were done. To investigate the possible mechanisms of chronic lead toxicity related to oxidative stress and apoptosis and effect of vitamin E administration on it, serum level of malondialdehyde (MDA) as a marker of oxidative stress and total anti-oxidative capacity (TAC) as a

marker of antioxidant and caspase 3 as a marker of apoptosis were measured.

MATERIALS AND METHODS

Animals

One hundred and four Westar White Albino adult rats (initial age ranged from 8 to 10 weeks), initially weighed 180–200 gram (52 males and 52 females), obtained from the animal house of the Faculty of Medicine, Assiut University, Egypt. The animals housed in clean, properly ventilated cages, 3-4 rats in each cage and maintained on standard laboratory diet, with free access to food and water throughout the study period. They maintained under a natural light–dark cycle and room temperature. All experimental protocols followed the guidelines of the Animal Committee of the Faculty of Medicine of Assiut University.

Experimental Design

The animals were divided into four groups as follow: **Group I** (normal control group): It consisted of 24 rats. Of them, 12 rats (6 males and 6 females) received distilled water (DW) (vehicle of lead), 12 rats (6 males and 6 females) received olive oil orally (vehicle of vitamin E) by intraperitoneal (i.p.) injection in the same volume as lead exposed groups. The animals sacrificed after 6 weeks; **Group II**: It consisted of 40 rats (20 males and 20 females). This group was subdivided into 2 subgroups: Group IIa (Lead exposed group): It consisted of 20 rats (10 males and 10 females) injected with lead acetate (10 mg/kg/day 5 times/week, i.p. for 6 weeks) (purchased from Al-Nasr pharmaceutical chemicals company, Egypt, and dissolved in distilled water). The animals sacrificed after 6 weeks; Group IIb (Lead

withdrawal group): It consisted of 20 rats (10 males and 10 females) injected with lead acetate (10 mg/kg/day 5 times/week, i.p. for 6 weeks). The animals sacrificed 6 weeks after stoppage of lead injection; **Group III** (Lead then vitamin E group): It consisted of 20 rats (10 males and 10 females) injected with lead acetate (10 mg/kg/day 5 times/week, i.p. for 6 weeks) followed by oral administration of vitamin E (50 mg/kg/day by stomach tube 5 times/week for another 6 weeks (purchased from Sigma-aldrich chemical company, St. Louis, Missouri 63103, USA and dissolved in olive oil). The animals sacrificed after 12 weeks; **Group IV** (Lead with vitamin E group): It consisted of 20 rats (10 males and 10 females) received oral vitamin E by stomach tube (50 mg/kg/day by stomach tube 5 times/week) simultaneously with i.p. injection of lead acetate (10 mg/kg/day 5 times/week, i.p. for 6 weeks). The animals sacrificed after 6 weeks.

Sample Collection

Immediately at the end of the experiments, rats sacrificed by cervical dislocation. Blood samples collected from the retro-orbital venous plexus in heparinized tubes, centrifuged at 3,000 revolutions per minute for 15 minutes, then the clear, non-hemolyzed supernatant quickly removed, and kept at -20 °C until used. Female rats sacrificed at the proestrous stage.

Plasma Measurements

Enzyme-linked immunosorbent assay (ELISA) kits were used for measurement of LH, FSH, testosterone, estradiol, progesterone (Biocheck, Foster City, CA), and caspase 3 (16) (Wkea Med Supplies Corp, New York, USA). TAC (17), and MDA (18) were measured using spectrophotometric measurement; kits purchased

from Research Bio-diagnostics, Giza, Egypt. The manufacturer's instructions of the kits were followed.

Histopathological Examination

Immediately after sacrifice the animals, the ovaries and testes were dissected and fixed in 10% neutral-buffered formalin, and then processed for paraffin sections. Sections (5 μ m thick) were stained with Hematoxylin and Eosin by the method of Durr and Wallington (19), For electron microscopy, specimens from the testes, ovaries and pituitary gland were fixed in 5% cold glutaraldehyde for at

least 24 hours and the sections were processed and photographed by electron microscopy (Jeol 100X) in Assiut University Electron Microscopy Unit.

Statistical Analysis

Data were analysed using SPSS version 20. All values were expressed as means \pm standard deviation (SD). Differences among the groups were compared by ANOVA test followed by least significance difference (LSD) comparison tests. The level of significance was considered at $P < 0.05$.

Table 1. Effect of vitamin E on lead-induced changes in the plasma levels of gonadotrophin and gonadal hormones in the different studied groups

Groups	Control groups		Lead exposed group (n=10)	Lead withdrawal group (n=10)	Lead then vitamin E group (n=10)	Lead with vitamin E group (n=10)
	Distilled water (n=6)	Olive oil (n=6)				
LH (mIU/ml)						
Males	2.21 \pm 0.17	2.20 \pm 0.14	1.82 \pm 0.22**	2.00 \pm 0.13	2.08 \pm 0.17*	2.20 \pm 0.30††
Females	2.09 \pm 0.09	2.15 \pm 0.17	1.73 \pm	1.88 \pm 0.28	2.03 \pm 0.16*	2.07 \pm 0.28††
FSH (mIU/ml)						
Males	7.45 \pm 0.05	6.74 \pm 0.27	6.27 \pm 0.69*	6.61 \pm 0.53	7.00 \pm 0.42	7.41 \pm 1.42†
Females	7.44 \pm 0.82	6.62 \pm 0.68	6.37 \pm 0.53*	6.52 \pm 0.37	6.67 \pm 0.96	7.33 \pm 0.86†
Testosterone (ng/ml)	0.90 \pm 0.05	0.90 \pm 0.03	0.27 \pm 0.19*	0.62 \pm 0.11	0.75 \pm 0.32†	0.91 \pm 0.67††
Estradiol (pg/ml)	4.29 \pm 0.61	3.87 \pm 1.02	2.01 \pm 0.66***	3.22 \pm 1.38††	3.79 \pm 0.69†††	4.26 \pm 0.43†††
Progesterone (ng/ml)	10.45 \pm 4.95	7.45 \pm 2.86	6.34 \pm 2.01**	7.57 \pm 1.45	9.47 \pm 0.45†	10.05 \pm 2.05††

Data were expressed as mean \pm SD. LH: Luteinizing hormone, FSH: Follicle stimulating hormone. Analysis between groups was done using one way ANOVA test (LSD equation). *: $P < 0.05$, **: $P < 0.01$, and ***: $P < 0.001$ compared to the control level with distilled water; †: $P < 0.05$, ††: $P < 0.01$, †††: $P < 0.001$ compared to the lead exposed group.

RESULTS

Plasma levels of gonadotrophic hormones and gonadal hormones

In comparison to the control group, lead administration significantly decreased the plasma levels of gonadotrophic hormones ($P < 0.01$ for LH and $P < 0.05$ for FSH) and gonadal hormones ($P < 0.001$ for estradiol, $P < 0.01$ for progesterone and $P < 0.01$ for testosterone). Lead withdrawal

increased the gonadotrophic hormones and gonadal hormone plasma levels but this increase didn't reach a significant level. Meanwhile, administration of vitamin E with or after lead poisoning significantly increased the gonadotrophic and gonadal hormones in comparison to lead exposed group with insignificant difference with lead withdrawal group (Table 1).

Table 2. Effect of vitamin E on lead-induced changes in the plasma levels of total antioxidant capacity (TAC), malondialdehyde (MDA) and caspase 3 in the different studied groups

Groups	Control groups		Lead exposed group (n=10)	Lead withdrawal group (n=10)	Lead then vitamin E group (n=10)	Lead with vitamin E group (n=10)
	Distilled water (n=6)	Olive oil (n=6)				
TAC (mM/L)						
Male	0.94±0.01	0.96 0.02	0.41±0.15***	0.53±0.15***	0.89±0.09†††§§§	0.94±0.25†††§§§
Female	0.84±0.01	0.84±0.03	0.45±0.19***	0.50±0.03***	0.89±0.05†††§§§	0.95±0.06†††§§§
MDA (µM/L)						
Male	0.40±0.06	0.38±0.02	0.55±0.11***	0.44 0.02††	0.4 ± 0.03†††	0.42± 0.04†††
Female	0.37±0.02	0.48±0.06	0.71±0.12***	0.51 ± 0.15†	0.48 ± 0.18††	0.46 ± 0.09††
Caspase 3 (ng/ml)						
Male	2.15±0.11	2.28 0.22	2.59 ± 0.27*	2.43 ± 0.39	2.22 ± 0.23†	2.16 ± 0.35†
Female	2.19±0.05	2.24±0.04	2.34 ± 0.07*	2.29 ± 0.17	2.23 ± 0.04†	2.21 ± 0.02†

Data were expressed as mean ± SD. Analysis between groups was done using one way ANOVA test (LSD equation). *: $P < 0.05$, and ***: $P < 0.001$ compared to the control level with distilled water; †: $P < 0.05$, ††: $P < 0.01$, †††: $P < 0.001$ compared to the lead exposed group, §§§: $P < 0.001$ compared to the lead withdrawal group.

Plasma levels of TAC, MDA and caspase 3

Lead administration significantly decreased the plasma levels of TAC ($P < 0.001$) while, significantly increased the plasma levels MDA ($P < 0.001$) and caspase 3 ($P < 0.05$) compared to the control group. Lead withdrawal significantly

decreased the plasma level of MDA ($P < 0.01$, $P < 0.05$ in males and females, respectively) with insignificant decreased in caspase 3 as compared to lead exposed group, however the plasma level of TAC is still significantly decreased than normal ($P < 0.001$). The administration of vitamin E after

lead or with lead exposure resulted in a significant increase in TAC ($P < 0.001$) and a significant

decrease in MDA ($P < 0.001$, $P < 0.01$ in males

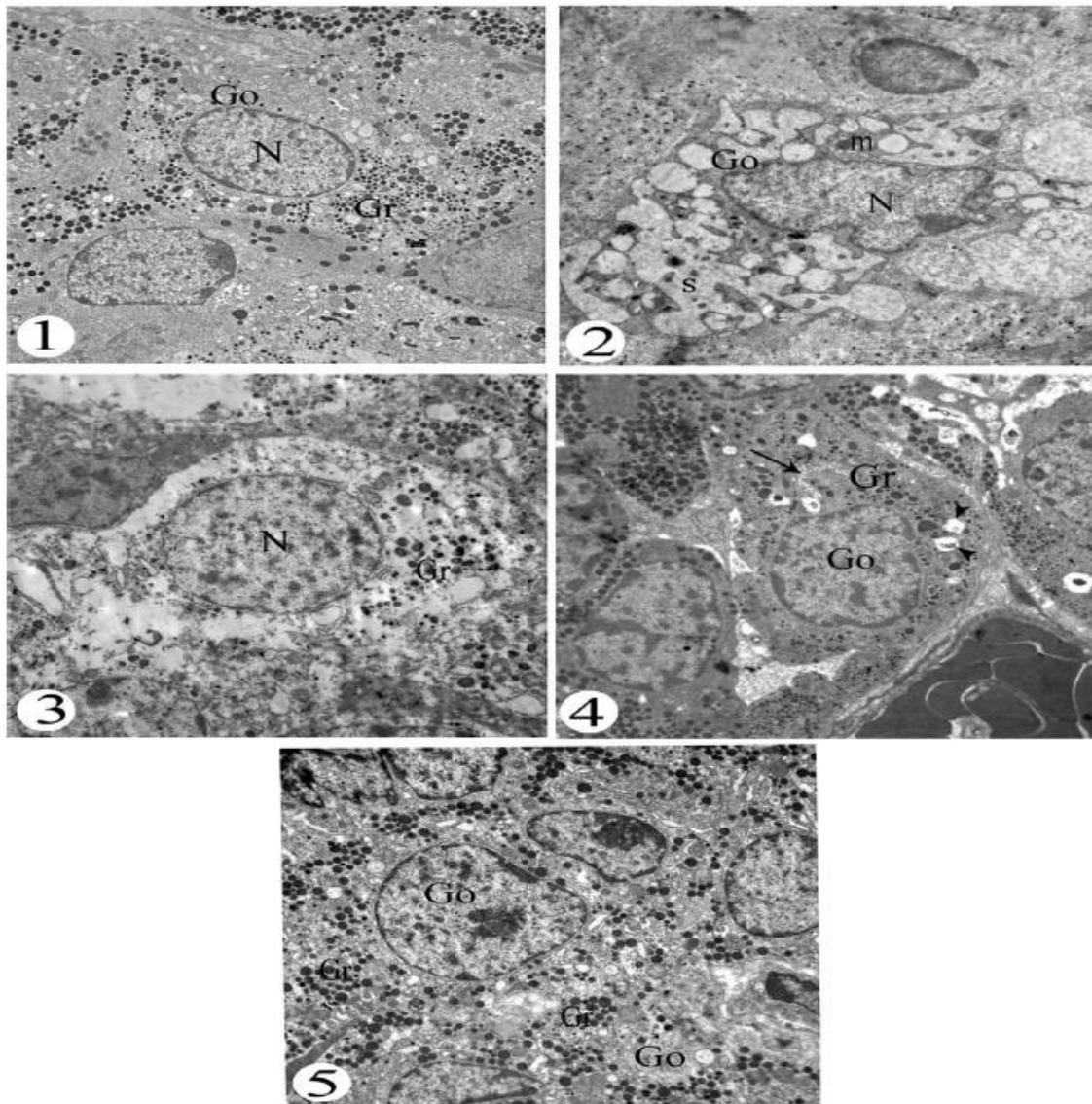


Figure 1. An electron micrograph of the pituitary gland of adult rat showing: (1) Control group: the gonadotrophic cell (Go) engorged with cytoplasmic granules (Gr). Round to oval nucleus (N) is observed. (2) Lead exposed group: gonadotrophic cell (Go) is devoid of cytoplasmic Gr and occupied with numerous empty spaces (s). The nucleus (N) is irregular and peripherally located. Degenerated mitochondria are noticed (m). (3) Lead withdrawal group: gonadotrophic cell with few cytoplasmic Gr and degenerated cytoplasm are noticed. Granular nucleus (N) is observed. (4) Lead then vitamin E treated group: the gonadotrophic cell (Go) shows numerous cytoplasmic Gr. Well-developed Golgi body (arrow) is observed. Few degenerated granules surrounded by empty space are noticed (arrowhead). (5) Lead with vitamin E treated group: group of Go with normal cytoplasmic Gr similar to control group. (X 4800)

and females, respectively) and caspase 3 ($P < 0.05$) compared to lead exposed group with insignificant difference with the lead withdrawal group as regard MDA and caspase 3. However, TAC level was significantly increased in vitamin E treated

group compared to lead withdrawal group (Table 2).

Histopathological electron microscopic (EM) examination

EM examination of the pituitary gland, testis and ovary showed that lead administration exhibited

marked degeneration in the general morphology of the pituitary gland, testis and ovary. Meanwhile, withdrawal of lead led to partial improvement of histopathological changes that occurred in anterior pituitary, testes and ovaries by lead exposure.

Treatment with vitamin E exhibited recovery of lead damaging effect on pituitary gland and gonads with marked improvement in rats given vitamin E with lead (Figures 1–3). Results

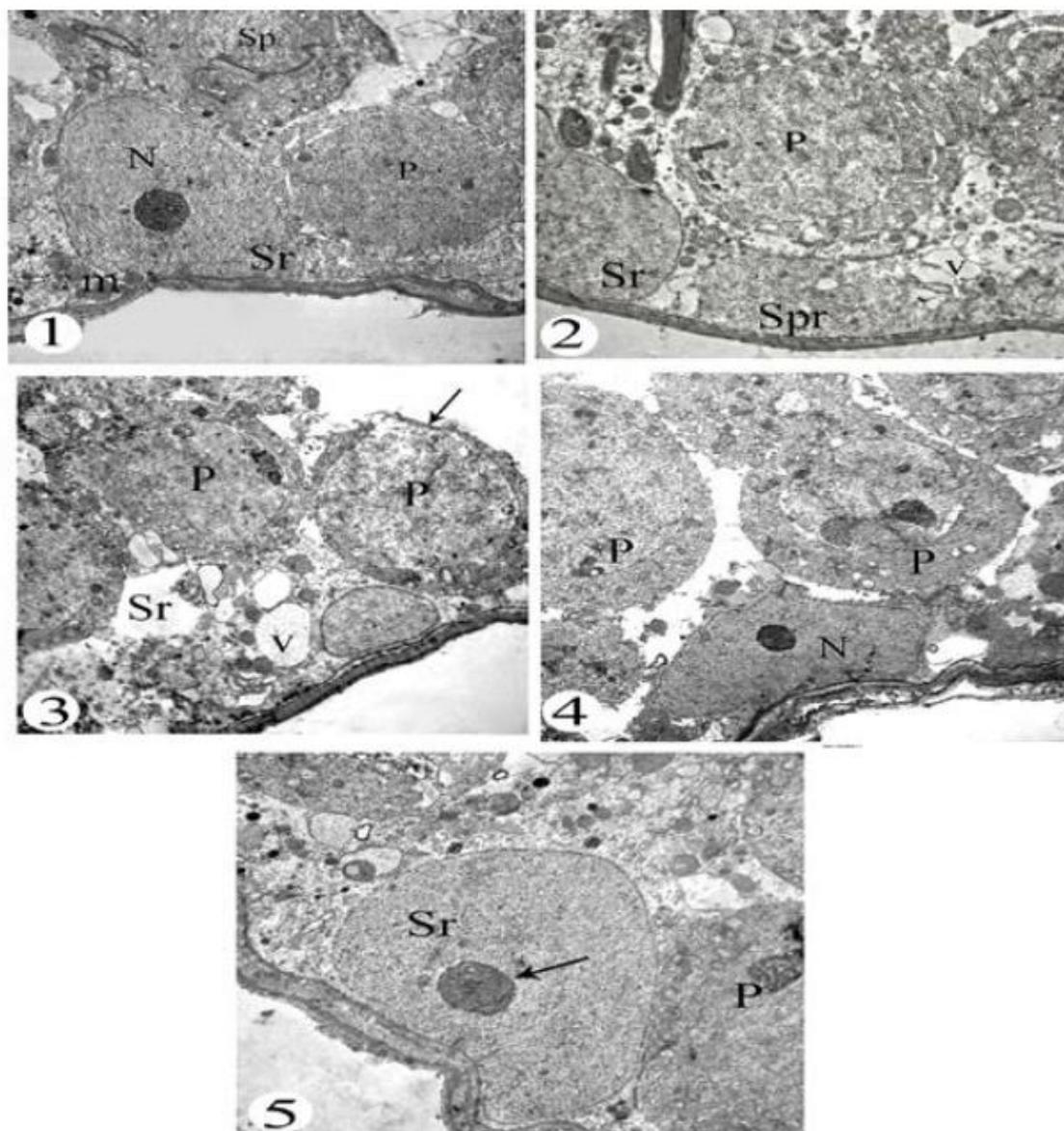


Figure 2. An electron photomicrograph of testis of albino rat showing: (1) Control group: it reveals Sertoli cell (Sr) resting on the basement membrane. The nucleus (N) is large and oval in shape, basely located with fine granular chromatin distribution. The cytoplasm is rich with mitochondria (m). Note the surrounding primary spermatocyte (P), has nearly rounded nucleus with evenly distributed chromatin. Spermatid cell (Sp) with prominent acrosomal cap is observed. (2) Lead exposed group: shows Sr with distorted N. The cytoplasm appears greatly disrupted and devoid of the organelles apart from few electron dense mitochondria. Spermatogenic cell with degenerated cytoplasm containing multiple vacuoles (v) is noticed. (3) Lead withdrawal group: shows degenerated Sr with cytoplasmic v. Primary spermatocytes appear with destructed cell membrane (arrow) and granular nuclei. (4) Lead then vitamin E treated group: shows disappearance of many degenerative changes observed in the previous group. (5) Lead with vitamin E treated group: shows normal appearance of Sr and primary P. (X 3600)

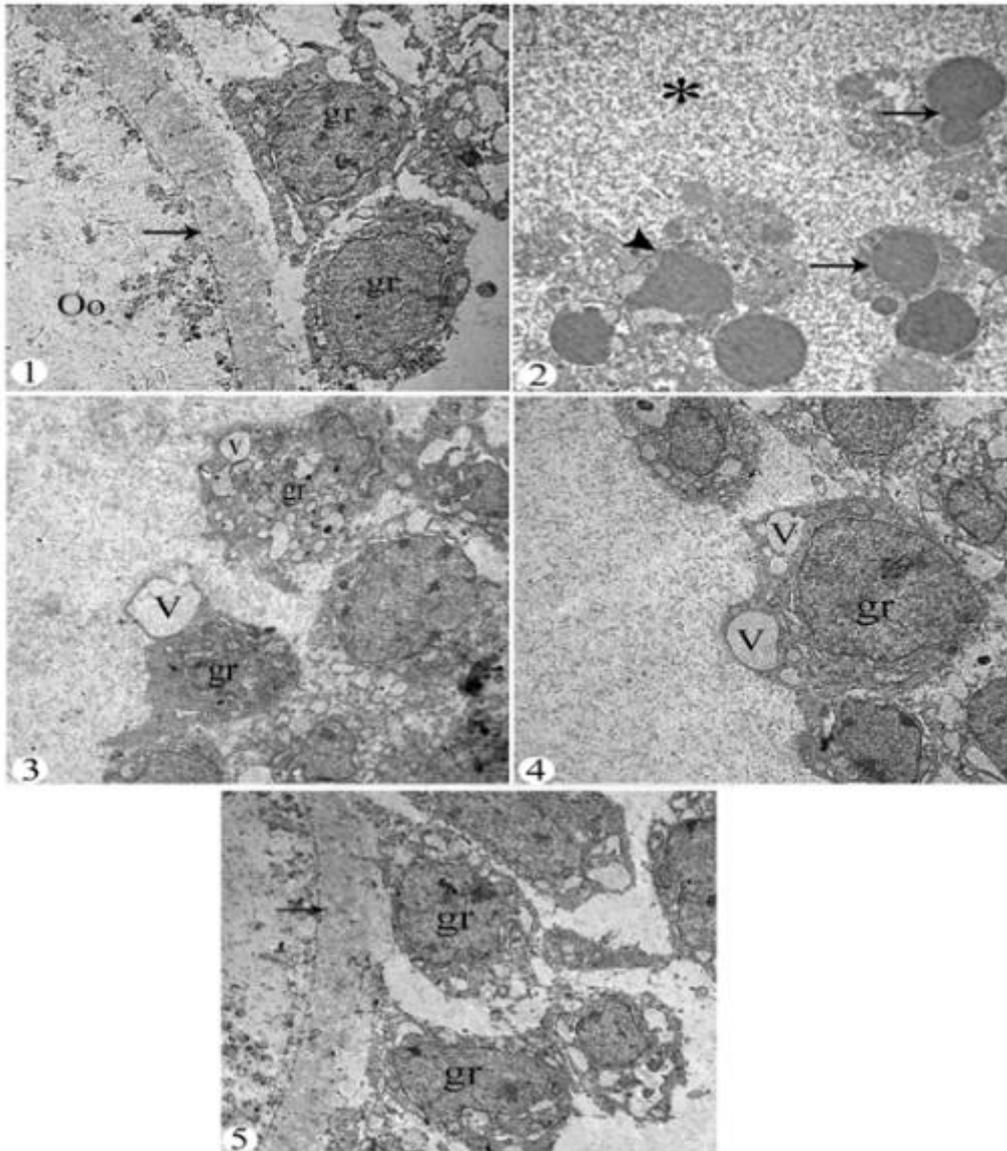


Figure 3. An electron microphotograph of ovary of albino rat showing: (1) Control group: shows normal oocyte (Oo) surrounded by intact zona pellucida (arrow) and granulosa cells (gr). (2) Lead exposed group: shows absent zona pellucida (*), degenerated gr (arrow), apoptotic nuclei of gr can be observed (arrowhead). (3) Lead withdrawal group: shows gr with multiple cytoplasmic vacuoles (v), shrunken degenerated gr (arrow) containing single cytoplasmic v is observed. (4) Lead then vitamin E treated group: shows gr with cytoplasmic v. (5) Lead with vitamin E treated group: shows oocyte with intact zona pellucida (arrowhead), gr appear similar to the control group. (X 3600)

DISCUSSION

Lead, a silvery, grey soft metal, was extensively disseminated geologically. The chief target of lead toxicity was the reproductive tract and the pituitary-gonadal axis resulted in infertility (20).

Our work showed that chronic administration of lead acetate to adult male and

female rats (10 mg/kg/day 5 times per week for 6 weeks) resulted in pituitary and gonadal dysfunctions manifested by a marked decrease in the plasma levels of LH, FSH, estradiol, progesterone and testosterone. Thus, lead induced gonadal impairment might due to the direct effect of lead on gonads and/or central effect on the pituitary gland. These findings confirmed by histopathological examination that revealed

changes in the morphology of the pituitary gland and gonads. Lead withdrawal resulted in partial improvement both at the hormonal level and in the structure of the pituitary gland and gonads. Taken together, ninety nine percent of the circulating lead after absorption extensively bound to circulatory erythrocytes with a half life of approximately 30-36 days. Only, 1-2% of blood lead was present in plasma. Also, it distributed to soft tissues (liver, kidney, lung, spleen, teeth, brain and gonads) with a half-life of approximately 40 days (21). We demonstrated that plasma level of TAC still lower in lead withdrawal group than the control group; this could be explained by the role of glutathione (GSH) in active excretion of lead via binding to the thiol group of GSH and then excreted in bile (21).

These results were in consistence with the finding of Mokhtari and Zanboori (23) who demonstrated that oral lead administration in adult male Wistar rat in different doses (25, 50 and 100 mg/kg/day for 28 days) resulted in a dose-dependent decline in the serum level of FSH and testosterone in adult male Wistar rats. Also, Ayinde and his coworkers (24) reported that exposure of Albino rats to lead (60 mg/kg/day for 6 weeks) exhibited a marked decrease in the serum levels of FSH and testosterone. Furthermore, Hamadouche and his colleagues (25) revealed that adult male rats given lead acetate (20 mg/kg/day for 20 days, i.p) provoked a reduction in the serum levels of LH, FSH and testosterone. Also, Pillai and his team (26) demonstrated a decline in the plasma levels of estradiol and progesterone in female rat's offspring whose mothers given subcutaneous lead acetate (0.025 mg/kg/day) during all gestation and lactation periods.

The reduction in the plasma levels of FSH and LH explained by Sokol et al. (27) who speculated that lead accumulated in the median eminence of the hypothalamus and interfered with gonadotropin releasing hormone (GnRH) secretion from the nerve terminals in the median eminence.

Mokhtari and Zanboori (23) explained the decline in testosterone level by increasing angiotensin II that bound to LH receptors in the Leydig cell resulting in decrease testosterone production. Wang et al. (28) suggested that reduction in the plasma level of testosterone was due to inhibition of testicular key steroidogenic enzymes. Meanwhile, Pillai et al. (26) explained the decline in the plasma level of estradiol and progesterone either by direct inhibition of ovarian steroidogenic enzymes or by indirect effect via decrease FSH and LH release. Furthermore, Ahmed et al. (29) explained this decrease by a direct damage of the ovary due to lead accumulation.

Contrary to our results, Munga and his coworkers [30] found that intraperitoneal doses of lead (0.0036, 2,61 and 4,95 mg/kg/day for 60 days) to mature male guinea pigs did not greatly affect estradiol, progesterone and LH level, however, testosterone level increased with low lead dose while the level decreased with high dose. The level of FSH markedly increased with high lead dose. The researchers claimed high level of FSH with low testosterone level to Leydig cells dysfunction. Whereas, Ayinde et al. (24) and Riaz et al. (31) found no obvious change in the plasma level of LH in male rats given lead at concentration 0.3% in drinking water for 2, 4 and 6 weeks and 60 mg/kg/day for 6 weeks,

respectively and they speculated that lead slightly affect pituitary gland.

In this experimental study, administration of vitamin E concurrently with lead or after lead exposure obviously increased the plasma levels of FSH, LH, testosterone, estradiol and progesterone. These results were in consistence with the results obtained with Ayinde et al. (24) who found a marked increase in the plasma levels of FSH and testosterone in male rats given vitamin E (150 mg/kg/day) concomitantly with lead acetate (60 mg/kg/day) for 6 weeks. Also, Hamadouche et al. (25) demonstrated a great increase in the plasma levels of LH, FSH and testosterone with oral administration of vitamin E (600 mg/kg/day) after lead exposure (20 mg/kg/day) for 20 days. Previously, Karanth et al. (32) claimed the increase in the plasma levels of FSH and LH with vitamin E treatment to the neurotransmitter effect of vitamin E in the hypothalamus as it activated nitric oxide synthase that form nitric oxide, diffused to the GnRH neurons, and stimulated guanylate cyclase that converted guanosine triphosphate to cyclic guanosine monophosphate (cGMP). Then, cGMP activated protein kinase G that released GnRH from its secretory granules caused increase in FSH and LH secretion from the anterior pituitary gland. Recently, Khan et al. (33) demonstrated that vitamin E exhibited a significant increase in the size and area of FSH and LH gonadotropes. In our study we observed that gonadotrophic cells engorged with secretory granules, euchromatic nuclei and numerous mitochondria and this could explain the increased plasma levels of LH and FSH with vitamin E treatment. Increased plasma levels of estradiol and progesterone explained by Cicek et al. (34) who

speculated that vitamin E had an anticoagulant effect resulting in adequate blood supply to ovarian follicles.

The hormonal assay findings obtained by this study confirmed by histopathological examination, lead acetate administration exhibited degeneration of gonadotrophic cells of the anterior pituitary gland that appeared devoid of secretory granules and occupied with numerous empty spaces. Their nuclei were irregular and peripherally located. Degenerated mitochondria were frequently noticed. Our results agreed with Hamadouche et al. (35) who described that the administration of lead acetate (0.1%, or 0.3% via drinking water for 30 days) to adult male rats provoked degenerative changes in endocrine cells.

In the present study, we observed numerous structural alterations of the rough endoplasmic reticulum (RER) of gonadotrophic cells including proliferation, fragmentation, dilation and degranulation. The dilation of RER cisternae explained by reduced secretory activity (36). The destruction of the RER and mitochondrial degeneration is considered an indication of single cell necrosis (apoptosis) or shrinkage necrosis as a result of lead toxicity (37).

The results of the current study clearly demonstrated that chronic exposure of adult male rats to lead acetate seriously altered the morphological appearance of the testes resulted in vacuolations and degenerative changes of most spermatogonia and pyknotic changes of spermatocytes. These changes were in accordance with Moniem et al. (38) who documented that i.p. administration of 20 mg/kg lead acetat for 5 days to adult male rats altered the testicular morphology. Also, Ahmed et al. (39) observed that

exposure of adult male New Zealand rabbits to different oral doses of lead acetate five days weekly for 12 weeks (15, 20 and 30 mg/kg) markedly changed the testicular tissues. Singh et al. [40] revealed that seminiferous tubules had depleted germ cells and arrest of spermatogenesis at the level of primary spermatocytes in male Albino rats treated with oral lead nitrate (40 and 80 mg/kg for 6 days a week for 2 months). They claimed these changes to their loss via apoptosis or differentiation failure or might due to reduced expression of Sertoli cell growth factor, glial cell line-derived neurotrophic factor (GDNF) or Sertoli cells cytoplasmic processes retraction. This retraction made the germ cells loosely arranged and easily sloughed out from their place.

The present research work showed observable morphological changes in the ovary of adult female rats after chronic lead administration. These changes were in the form of damage in germinal epithelium, cortex and inner medullary region, decreased numbers of primordial and primary follicles, graffian follicles lost its original shape, oocyte was not apparent, there was an apparent space between the granulosa cells and outer theca layer. Distorted zona pellucida and corona radiata were visible. Cells of stratum granulosum showed pyknosis of the nucleus and dissolution of cytoplasm. Frequent appearance of atretic follicles was observed. The same results were noticed by Dorostghoal et al. (41) who found that ovaries of the female offspring's whose mothers chronically exposed to lead acetate (20, 100, 300 mg/L/day via drinking water) during lactation showed reduced numbers of primary, secondary and antral follicles. Furthermore, the number of corpora lutea decreased and this

explained the decline in the plasma progesterone level.

In addition, Sharma et al. (42) who found that adult female Swiss mice given lead acetate (160 mg/kg/day) showed reduction in the number of primordial follicles and decrease the number of follicles that enter the growing phase as well as increased number of atretic follicles and congestion in stroma tissue.

The precise reason for such changes may be due to direct lead damage to the ovarian tissue as stated by Nampoothiri and Gupta (43) or alteration in gonadotrophin receptors and decreased steroid production in ovaries as stated by Qureshi and Sharma (44).

In our experimental study, administration of vitamin E simultaneously with or after lead showed recovery of most degenerative changes in the pituitary gland and gonads that appeared with lead exposure.

In the present study, lipid peroxidation as measured by the amount of MDA in plasma elevated and the plasma level of TAC was reduced in lead exposed group. Lipid peroxidation implicated in the cell membrane damage via inactivation of cell constituents by oxidation or causing oxidative stress through radical chain reaction (45). Lipid peroxidation induced by lead administration was related to free radical formation and anti-oxidant depletion.

These results were in consistence with Tangpong and Satarug (46) who found that adult male mice received lead acetate (1 g/L for 8 weeks) via drinking water had decreased plasma level of TAC and increased plasma level of MDA. Furthermore, Ayinde et al. (24) found that male rats given lead acetate (60 mg/kg/day for 6 weeks)

showed a marked decrease in the antioxidant enzymatic activity and greatly increased MDA in testis. On the other hand, Pillai et al. (26) found decreased ovarian glutathione content in female offspring's whose mothers treated subcutaneously with lead acetate (0.05 mg/kg per day) throughout gestation and lactation.

Yenhaw et al.(47) explained lead-induced OS by decreasing the cellular antioxidant defense system through inhibiting sulfhydryl dependant enzymes and interfering with some necessary metals required for antioxidant enzyme activities. Moreover, lead in ionic form displaced bivalent ions such as Zn^{+2} , Cu^{+2} and Fe^{+2} making the resulting enzyme useless. Also, lead displaced the selenocysteine group from the active site of glutathione peroxidase (48). Thus, oxidant/antioxidant imbalance induced by lead partly responsible for lead toxic effects on the reproductive system.

Our research showed that administration of vitamin E with or after lead administration greatly reduced the plasma level of MDA and markedly increased the levels of TAC. This was attributed to the antioxidant functions of vitamin E. This explained the ability of vitamin E to exhibit ameliorative effect on lead treated rats. As it is a major chain-breaking antioxidant. It acted as a free radical scavenger, increased the activity of some antioxidant enzymes, decreased nitric oxide and lipid peroxidation (24).

In a study conducted by Aybek et al. (49), treatment with vitamin E revealed a decline in MDA level and enhancement in GSH level in male Wistar rats. Also, adult female mice given vitamin E (2 mg/kg body weight) besides nickel chloride or potassium dichromate lowered lipid

peroxidation level and improved the antioxidant status (50).

Caspase 3 implicated as an “effector” caspase related to the beginning of the “death cascade” and is therefore a hallmark of apoptosis (51). The results of this study revealed elevation in the plasma levels of caspase 3 in lead treated group. These results were in partial agreement with Shan et al. (52) who detected marked elevation in the levels of caspase 3 and Fas/Fas-L in adult male mice testis treated with lead acetate (20 mg/kg). Jin and Feng (53) demonstrated an increase in the protein levels of caspase 3 and Bax of adult mice testis treated with oral lead acetate (0, 10, 50, 100 mg/kg every other day) for 4 weeks.

The studies in female rats showed similar results as Xiu-yuan et al. (54) who reported a dose-dependent increase in the apoptosis rate of granulosa cells in female mice injected abdominally with different lead doses (10, 20 and 40 mg/kg) for 2 days. Also, Ahmed et al. (55) demonstrated apoptosis and DNA fragmentation in ovarian cells of adult female rabbits given oral lead acetate (15 mg/kg and 30mg/kg five days a week) for 8 weeks. The researchers attributed these changes to either a direct effect of lead on DNA structure, or indirectly by another mechanism involved caspases activation.

The results obtained from this study also showed that administration of vitamin E with or after lead greatly reduced the plasma level of caspase 3. This is consistent with the result of Rzepczynska et al. (56) who found considerable decrease in the caspase 3 levels in ovarian theca-interstitial cells incubated for 24 hours with vitamin E. Furthermore, vitamin E effectively

prevented neuronal apoptosis in male New Zealand white rabbit fed high cholesterol diet (57). In conclusion, this study demonstrated that vitamin E, through its antioxidant and anti-apoptotic activity, has protective and therapeutic effects on gonadal dysfunction-induced by chronic lead administration through stimulating the proliferation of gonadotrophic cells of the anterior pituitary and increasing the number of hormone secreting granules inside them as well as

restoration of normal histological structure of testis and ovary, and reverting gonadal and gonadotrophic hormone levels. From these findings we recommend administration of vitamin E as a dietary supplement to human populations exposed to lead toxicity. Further experimental and clinical investigations are needed for using vitamin E as an adjuvant therapy in treatment of human gonadal dysfunction.

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