The effect of lead toxicity on male albino rats reproduction with ameliorate by vitamin E and pumpkin seeds oil

Amany Ramah, Ragab M. EL-shwarby, Nabila M.A., Elham A. El-shewey
Department of Forensic Medicine & Toxicology. Faculty of Veterinary. Medicine. Benha University.

A B S T R A C T

Lead is an industrial pollutant that may exert specific toxic effect on mammals. The aim of study was to investigate the protective effect of vit. E and pumpkin seeds oil on male reproductive system of albino rat induced by lead toxicity , the studying toxic effect of lead on body weight , testis weight, Glutathione, Glutathione-s- transferase, lipid peroxide, testosterone, lead residue , sperm gram, Nrf2 gene expression and histopathological change in testes of rat. Sixty male albino rats randomly divided into 4 groups. Group I was given olive oil 3 times a week orally, group II was given 1.5 g/L lead acetate daily in drinking water, group III was given lead acetate plus 600 mg/kg/ bwt vit. E orally 3 times a week, while group IV was given lead acetate plus 288 mg/kg/bwt pumpkin seeds oil orally 3 time a week. The experiment was extended for 8 weeks. Our results revealed significant decrease in body weight, GSH, GST, testosterone hormone level and sperm viability, and significant increase in MDA, concentration of lead, sperm abnormality and expression of Nrf2 gene in group II without any significant changes in testis and epididymis weight. Moreover, the pathological changes in testes showed focal degeneration with loss of spermatogenic series in the seminiferous tubules. All above mentioned result were significantly improved in group III & IV. In conclusion, vitamin E and pumpkin seeds oil have a protective effect on the testicular damage induced by lead.

Key words: Lead toxicity, vitamin E, pumpkin seeds oil, Nrf2, antioxidants.

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1. INTRODUCTION

Lead is a common environmental and industrial pollutant. The most important sources of lead exposure are industrial emissions, soil, car exhaust gases, and contaminated foods, such as spinach and cabbage can contain high levels when grown near lead sources (Gama et al., 2006). Lead is used in the production of lead acid batteries, alloys, cable sheathing, pigments, and rust-proof (Quinn and Sherlock, 1999). Lead has known to induce over production of reactive oxygen species (ROS) and enhance lipid peroxidation (Malecka, 2001). Also nuclear factor E_2 – related factor -Nrf2 is an important regulator of cellular oxidative stress response, which is closely related to antioxidant response elements (Kobayashi *et al.*, 2006). Lead may inhibit spermatogenesis and reduce young spermatids, spermatocytes, and mature spermatids (Kaushal et al., 1996). Pumpkin seeds oil had been used for improve sexual performance, sexual sensation, and copulatory efficiency (Gundidza *et al*., 2009). In fact vitamins are essential to maintain normal metabolic processes, and homeostasis in the body. Vitamin E is low molecular mass antioxidants that scavenge or quench free radicals (Janisch et al., 2005).

2. MATERIAL AND METHODS

2.1. *Experimental animals and dosing*

Sixty apparently healthy male albino rats (western strain) weighed 150-200 gm obtained from Veterinary National Research

Institute, Cairo, Egypt, were randomly divided into 4 groups each one contains 15 rats. Group I was given olive oil 3 times a week. Group II was given 1.5 g/L lead acetate (El-Gomhoria company, Egypt) daily in drinking water according to Wang *et al*., 2013. Group III was given 1.5 g /L lead acetate and 600 mg/Kg/bwt vit. E (Pharco.company Egypt) dissolved in olive oil according to Nadia *et al*., 2013 3 times a week. Group IV was given 1.5 g/L lead acetate plus 288 mg/kg/rat Pumpkin seeds oil (Arab company Egypt) dissolved in olive oil according to Hongji and Jongrogu,2009 **,** 3 times a week, The experiment was extended for 8 weeks.

2.2. Performance and growth measurement

Mean body weight was estimated every 2 weeks, while the body gain and the weight of testes and epididymis was measured at the end experiment.

2.3. Sampling

Serum samples were collected from 10 rats in each group at end of experiment for determination of testosterone hormone. Tissue specimen from testes were taken and placed in dry labeled eppendorf tubes and stored at -20 ^{0}C for the determination of antioxidant enzymes (GSH- GST- MDA). lead residue and other samples from testes stored at -80 C^{0} for gene expression, while another specimen were collected immediately after scarification and kept in Bowen's reagent for histopathological study.

2.4. Detection of testes oxidative cascade

Glutathione (GSH) was determined according to Beutler *et al*.,1963. Glutathione-s-transferase (GST) was determined according to Habig and Pabst 1974. Lipid peroxidation (malondialdehyde) was determined according to Satoh, 1978.

2.5. Detection of lead residue

Lead residue was determined by using Perkin-Elmer 2380 Atomic absorption spectrophotometer described by Ruhling and Tyler, 1973.

2.6. Determination of testosterone hormone

Testosterone was determined by using ELISA described by Ekins , 1998.

2.7. Sperm gram studies

Sperm viability and sperm abnormality was determined according to Bearden and Fluquary, 1980.

2.8. Gene expression

Total RNA isolated from testis tissue using total RNA purification kits, then total RNA concentration was determined using Nano drop then cDNA was synthesize from RNA by using Reverse transcriptase Kits. Finally Two-Step RT-PCR was performed by using The amount of change in gene expression was calculated from the obtained cycle threshold(CT) values provided from real-time PCR instrumentation using the $2^{-\Delta\Delta CT}$ calculation, where ΔCT indicates the CT changes in target gene Nrf2 in comparison with the reference (house-keeping) gene, which is GAPDH as described by Livak and Schmittgen, 2001.

a. The primer used to amplify nuclear related factor Nrf2 gene is: -F: 5- GGG AGG AAT TCT CCG GTC TC -3, R: 5- CCT CAC CTC TGC GCC AGT -3

b- The primer used to amplify GAPDH (housekeeping gene) is: F: 5- AGC TTG TCA TCA ACG GGA AG -3, R: TTT GAT GTT AGT GGG GTC TCG -3

2.9. Histopathological examination

Tissue samples were taken from the testis of rat in different groups and used for histopathological examination using H& E stain according to Banchroft *et al*., 1996.

2.10. Statistical analysis

Data were statically analyzed by using the statistical software package SPSS for windows (Version 18). The significance of differences between more than two groups was evaluated by one way analysis of variance (ANOVA) test and DUNCAN test according to Bailey, 2008.

3. RESULTS

Table (1) showed no statistical difference of mean body weight at 1th and 2nd week of experiment. While from 4th to 8th week significant decrease on mean body weight in group II (lead acetate). No statistical differences in body weight of rats in groups III and IV in compared to control group. Table (2) showed there was no significant change in right and left testis and right and cauda epididymis weight in all groups compared to control group. There was significant decrease in left cauda epididymis weight in all experimental groups compared to control group. Table (3) showed significant decrease in level of GSH and GST and significant increase in level of MDA in group II (lead acetate) compared to control group, while no significant change in group III and IV were detected comparing to control group. Table (4) showed the residue of lead in testes recorded a significant increase in group II (lead acetate) compared to control group. While, no significant difference in group III and IV compared to control group. Table (5) showed significant decrease in testosterone level in serum of rats in group II (lead acetate) compared to control group. While, no significance difference in group III and group IV compared to control group. Table (6) showed significant decrease in number of live sperm and increase in number of dead sperm in group II (lead acetate) compared with control group, while no significant difference in group III compared to control group, and less significant increase in live sperm and decrease in dead sperm compared to control group. Significant increase sperm abnormality in group II treated with lead acetate compared to control group, while no significant difference in group III and group IV compared to control group. Table (7) and figure (1) showed significant up-regulation of Nrf2 gene expression in group II treated to control group. While, no significant difference in Nrf2 gene in group III and group IV compared to control group. Histopathological examination of testes of rats in control group observed normal histological structure of the mature active semniferous tubules with complete spermatogenic series were recorded in (Fig. 2). Testis of rats administrated lead acetate group II showed focal degeneration of semniferous tubules with loss of spermatogenic series in some semniferous tubules as shown in (Fig. 3).While testes of group III (lead acetate plus vitamin E) and group IV (lead acetate plus pumpkin seeds oil) showed no histological alteration (Fig. 4) and (Fig. 5).

4. DISCUSSION

Regarding to the effect of lead acetate on body weight, there was significant decrease in body weight of group II (lead acetate) compared with control group. These results agreed with Djebli *et al.,* 2004 and Nabil et al., 2012, who recorded that loss of body weight due to impaired intestinal absorption of some essential trace elements, these results disagreed with El-sayed et al., 2010 due to the dose, experimental animals and duration of the experiment, while group III (lead acetate plus vit. E) revealed increase in body weight compared with group II, these results agreed with Nadia et al., 2013**.** Result may be related to vitamin E is essential to maintain normal metabolic processes and hemostasis within the body (Jan-Ischi et al., 2005). Group IV (lead acetate plus pumpkin seeds oil) revealed increase in body weight compared with group II. This result agreed with Alan, 2006 that said that pumpkin seeds oil is excellent source of magnesium, phosphorus, manganese, copper, iron and zinc which vital in growth. Concerning to the effect of lead acetate on male reproductive organ, there was no significant change in weight of right, left testes and right epididymis in all groups compared to control group.

These results agreed with Ronis et al.,1998, result may be due to no reduction in the number of germ cells in testes due to blood-testes barrier may protect the germinal

Table (1): Effect of administrated lead acetate plus vit. E or pumpkin seeds oil on body weight of male albino rats (mean ± SD).

Groups / Weeks	Group I Olive oil	Group II Lead acetate	Group III Pb+vit.E	Group IV Pb +PSO
Zero week (gm)	176.40±5.6[a]	175±4.7[a]	176.40±3.3[a]	167.60±2.6[a]
2nd week (gm)	190.40±3[a]	182.40±4.7[a]	186.80±3.5[a]	181.80±2.4[a]
4th week (gm)	207.20±1.4[a]	187±2.5[c]	195.40±1.2[ab]	187.40±1.1[c]
6th week (gm)	274.80±6.9[a]	218±5.9[c]	262±5.9[b]	250±8.7[b]
8th week (gm)	293±5.4[a]	248±8.3[c]	277.80±5.7[ab]	273.20±4.3[b]
Weight gain	116.6gm	73gm	101.4gm	105.6gm

-Mean with different letters at the same raw differ significant (P<0.05).

Table (2): Effect of administrated lead acetate plus vit. E or pumpkin seeds oil on male reproductive organ weight of male rats (mean ±SD).

Parameters	Group I Olive oil	Group II Pb	Group III Pb+vit.E	Group IV Pb+PSO
Right testis (gm)	1.23±o.48[a]	1.24±0.06[a]	1.23±0.06[a]	1.3±0.05[a]
Left testis (gm)	1.25±0.02[a]	1.23±0.52[a]	1.29±0.05[a]	1.3±0.05[a]
Right epididymis(gm)	0.23±0.01[a]	0.21±0.03[ab]	0.25±0.02[a]	0.23±0.08[a]
Left epididymis (gm)	0.27±0.03[a]	0.19±0.02[b]	o.24±0.02[a]	0.22±0.01[a]

-Mean with different letters at the same raw differ significant (P<0.05).

Table (3): Effect of administrated lead acetate plus vit.E or pumpkin seeds oil on antioxidants of male albino rats (mean ±SD).

Parameters	Group I Olive oil	Group II Pb	Group III Pb +vit. E	Group IV Pb +PSO
GSH (mg/g)	61.7±1.6[a]	45.8±o.7[b]	53.7±2.1[a]	48.2±1.5[ab]
GST (u/g)	7.3±0.3[a]	3.6±0.5[b]	7.5±0.3[a]	7.4±0.2[a]
MDA(nmol/g)	13±2.4[a]	26±1.8[c]	17.6±1.6[ab]	20.4±1.7[bc]

-Mean with different letters at the same raw differ significant (P<0.05).

Table (4): Effect of administrated lead acetate plus vit. E or pumpkin seeds oil on lead residue in testis of male albino rats (mean ± SD).

Parameter	Group I Olive oil	Group II Pb	Group III Pb + vit. E	Group IV Pb + PSO
Lead residue (ppm)	0.08±0.3[a]	1.5±0.4[b]	0.7±0.01[a]	0.6±0.01[a]

-Mean with different litters at the same raw differs significant (P<0.05).

Table (5): Effect of administrated lead acetate plus vit. E or pumpkin seeds oil on serum testosterone hormone level of male albino rats (mean± SD).

Parameter	Group I Olive oil	Group II Pb	Group III Pb+vit.E	Group IV Pb+PSO
Testosterone (ng/ul)	25.1±4.2	7.5±2.1	18.2±.3.5	13.8±3

-Mean with different litters at the same raw differs significant (P <0.05).

Table (6): Effect of administrated lead acetate plus vit. E or pumpkin seeds oil on sperm gram of male albino rats (mean ±SD).

Groups Parameters	Group I Olive oil	Group II Pb	Group III Pb + vit. E	Group IV Pb + PSo
Sperm live%	56±1.3[a]	16±2.6[c]	46±2.1[ab]	20.1±2.3[c]
Sperm dead%	44±1.4[a]	84±2.6[c]	53±2.1[b]	79±2.3[c]
Headabnormal%	2.7±0.57[a]	8.8±1[b]	2.2±0.46[a]	3.1 ±0.59[a]
Tail abnormal%	1±0.3[a]	1.8±0.4[b]	0.9±0.23[a]	0.6±0.16[a]

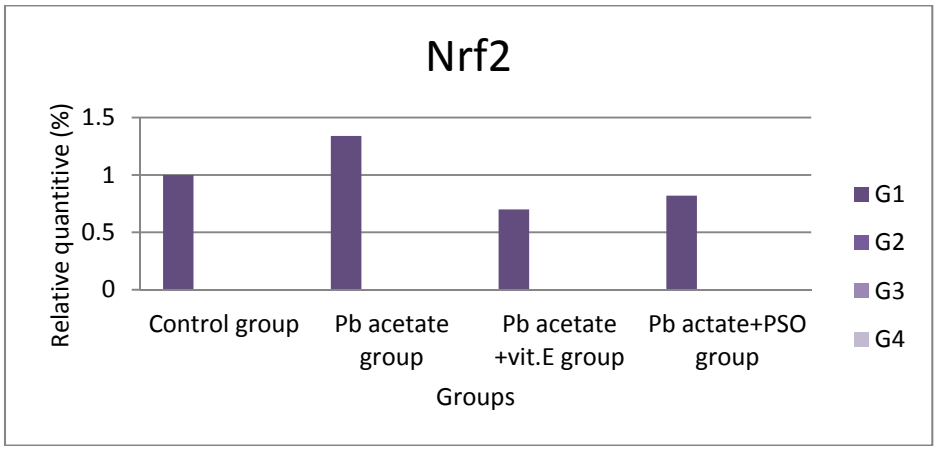
-Mean with different litters at the same raw differs significant (P<0.05).

Table (7): Effect of administrated lead acetate plus vit.E or pumpkin seeds oil on expression of nuclear related factor (Nrf2) of male albino rat (mean ±SD).

Groups	Nrf2 (%)
Group I	1.0±0.10[a]
Group II	1.34±0.14[c]
Group III	0.7±0.07[ab]
Group IV	0.82±0.11[ab]

Mean with different litters at the same raw differs significant (P<0.05).

Fig. (1): Effect of administrated lead acetate plus vit. E or pumpkin seeds oil on expression of nuclear related factor (Nrf2) of male albino rat (mean ±SD).



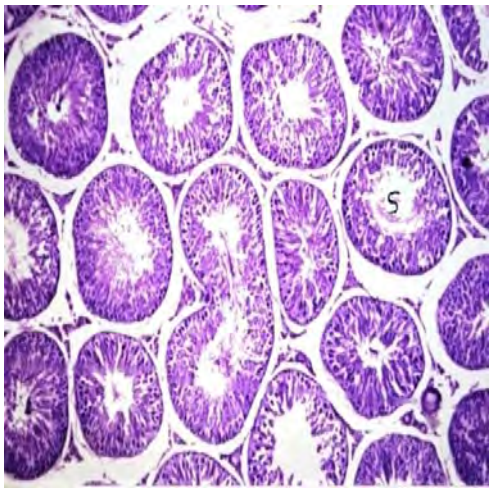


Fig. 1: Testes of control rat showing normal histopathological structure of mature active seminiferous tubules with complete spermatogenic series. H&E stain x16

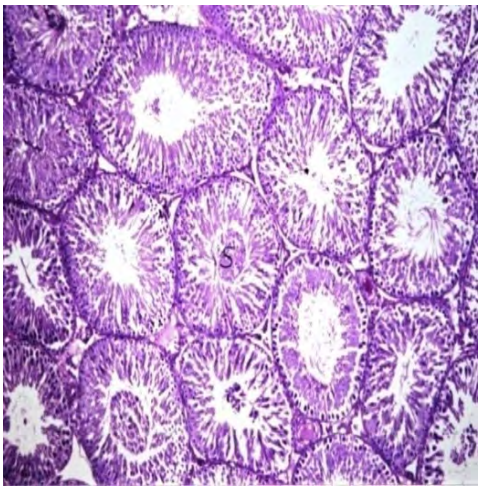


Fig.2: Testes of rat administrated of lead acetate for 8 weeks showing focal degeneration with lose of spermatogenic series in the same seminiferous tubules. H&E X16.

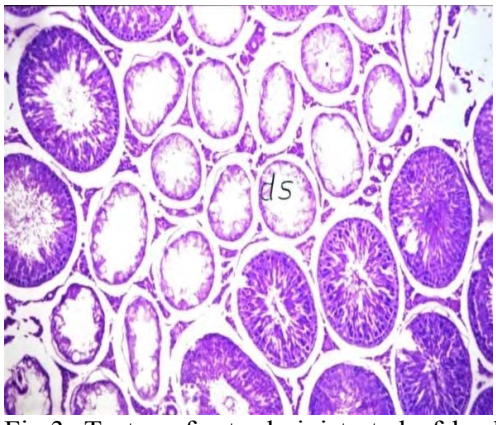


Fig.3: Testes of rat administrated of lead plus vitamin E for 8 weeks showing normal histological structure of seminiferous tubules with complete spermatogenic series. H&Ex10.

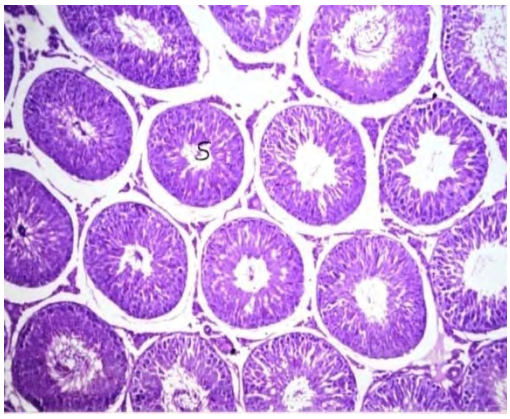


Fig.4: Testes of rat administrated of lead plus pumpkin seeds oil for 8 weeks showing normal histological structure of seminiferous tubules . H&E x 16.

epithelium of the testes from the salts of heavy metals as lead. These result disagreed with Sokol, 1990 due to the dose, experimental animals and duration of the experiment, while there was significant decrease in left cauda epididymis weight in all groups compared to control group. These result agreed with Kempinas et al., 1994, these result may be due to toxic effects of lead occured after releasing of the sperm from testes, probably in the epididymis (Barratt et al.,1989). Regarding to the effect of lead acetate on antioxidant there was a significant decrease in level of GSH and GST and significant increase in level of MDA in group II (lead acetate) compared to control group. While no significant change in group III (lead acetate plus vit. E) and group IV (lead acetate plus pumpkin seeds oil) comparing to control group. These results agreed with Abdel Moniem et al., 2010. These results may be due to heavy metal- induced oxidative damage by increased lipid peroxidation and inhibitions of enzymes required to prevent such oxidative damage. The result of the protective effect of vit. E in group III showed increase GSH and GST compared with group II. These results agreed with Madiha et al., 2008 due to vitamin E inhibit oxidation and oxidative damage to membrane polyunsaturated fatty acid

reported by Sugiyama, (1992). The result of group IV (lead plus pumpkin seeds oil showed increase in level of GSH, GST, and decrease level of MDA compared with group II. These result agreed with Bourse et al., (2004), these results may be due to pumpkin seeds oil is rich in vital trace elements (Kamal et al., 1996). Regarding to residue of lead in testes lead residue was detected in group II (lead acetate), no detection of lead in group III (lead acetate plus vit. E) and group IV (lead acetate plus pumpkin seeds oil). These results agreed with Marchlewicz et al., (2009). These results may be due to lead could be reserved in many positions of testes after penetrating blood-testis barrier so accumulate in testis (Gorbel et al., 2002). Regarding to the effect of lead acetate on testosterone hormone in serum showed significant decrease in group II (lead acetate) compared to control , these results agreed with Al- Attar, 2011, which may be attributed to depression of the secretory activity of the testes, and changes in peripheral metabolism (Katsiya et al., 1989). These results disagreed with Johansson et al., (1986) due to the dose, experimental animals and duration of the experiment, Testosterone level of group III (lead acetate plus vit. E) agreed with Mishra and Acarya,2004 and group IV(lead acetate plus pumpkin seeds oil) agreed with Banks, et al., 2004. These results revealed to vit.E act as antioxidant and pumpkin have rich source of zinc, which promote prostate health (Bach, 2000). Sperm viability significant decrease in sperm live and increase in sperm dead in group II (lead acetate) compared to control group , these result agree with Sokol et al., (1985) due to the accumulation of lead in many organs and fluids specially the gonads and seminal fluid (Silbergeld, 983). These results disagreed with Hsu et al., (1997) due to the dose, experimental animals and duration of the experiment, while group III (lead acetate plus vit. E) significant increase live sperm% and decrease dead sperm% compared with group II. These results agreed with Wang et al., (2004), related to vit. E acts as free radical scavenger as mentioned by Kartikeya et al., (2009). Group IV (lead acetate plus pumpkin seeds oil) revealed that oil significant increase in live sperm and significant decrease in dead sperm compared with group II and significant decrease in live sperm and significant increase in dead sperm compared to group I and group III. These results agreed with Jamilah, (2013), which attributed to pumpkin seeds oil is rich in Zinc plays an important role in the structure of proteins and cell membranes and protect against damage so plays important roles reproduction (Bataineh et al., 2002). The effect of lead acetate on sperm abnormality showed significant increase in sperm abnormality in group II (lead acetate) compared to control group, these results agreed with Chowdhury, (2009), due to lead would induce disruption of spermatogenesis in the testes causing detoriation of motility and content of sperm as well as abnormalities as reported by Gracia-Leston et al., (2010), these results disagreed with Hsu et al., (1997) due to the dose, experimental animals and duration of the experiment, while group III (lead acetate plus vit. E) significant decrease in sperm abnormality compared with group II. These results agreed with Acharya and Mishra 2003, these revealed to antioxidant property of vit. E (Rodriguez et al., 2011).The result of group IV (lead acetate and pumpkin seeds oil) agreed with Nkang et al., (2003), due to PSO contains B-carotene, a potent free radical quencher, singlet scavenger and lipid antioxidant. Regarding to effect of lead acetate on Nrf2 gene expression of treated testes showed significant increase gene expression of Nrf2 up-regulation in group II (lead acetate) compared to control group, these results agreed with Juang et al., (2008), due to exposure to lead induced oxidative stress which Nrf2-ARE pathway was activated because of it is regulation of the cellular oxidative stress response Wang et al., (2013), while no significant difference in group III (lead acetate plus vit. E) and group IV (lead acetate plus pumpkin

seeds oil). Regarding to histopathological examination of testes of rats administrated lead acetate revealed that group II showing focal degeneration with loss of spermatogenic series in the same seminiferous tubules (fig. 2). These results agreed with Madiha et al., (2008), these results may be related to zinc reduction. While testes in group III (lead acetate plus vit. E) in (fig. 3) showing normal histological structure of seminiferous tubules with complete spermatogenic series compared with group II (lead acetate). These results agreed with Nadia et al., (2013). These results may be due to vit. E with act as anti-oxidative properties as reported by Wang et al., (2004). The histopathologial finding of group IV (lead acetate plus pumpkin seeds oil) (fig. 4) revealed that normal histological structure of seminiferous tubules. These results agreed with Abdel-Ghany et al., (2010). Theses results may be due to pumpkin seeds oil is rich in essential fatty acid are required consisituents of health of cell membrane and rich in vitamin E which acts as a powerful antioxidant (Ryan et al., 2007). In conclusion, our study has demonstrated that vitamin E and pumpkin seeds oil amelorate lead induced testicular damage.

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