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Original Article

Effect of Ascorbic Acid on Reproductive Function of Male Rats Exposed to Lead Acetate

Ashraf S. El-Sebeay[1 ¥], Alaa F.M. Ibrahim[2], Amenh B. Yousif[3]

[1] Food and Human Nutrition Department, Regional Center for Food and Feed, Agriculture Research Center, Alexandria, Egypt.

[2] Food Toxicology Department - Regional Center for Food and Feed, Agriculture Research Center, Alexandria, Egypt.

[3] Family and Community Medicine Department, Faculty of Medicine, University of Benghazei, Libya.

Abstract

Background: The anti-oxidant ascorbic acid (AA) is known as a chelating agent in treatment of lead (Pb) toxicity, and has been reported to protect the cells from oxidative stress.
Objective(s): This work aims to study the efficiency of ascorbic acid on semen quality, sex hormone, antioxidant parameters and testis histology in rats treated with lead acetate.
Methods: A total of 50 male rats were divided into five equal groups; control group (received tap water only), Pb group (received 0.2% lead acetate/kg, BW) and the other three groups (received 500, 1000 and 1500 mg/kg BW AA along with 0.2% lead acetate/kg BW), respectively. Doses (as solutions) were orally administered every day for 8 weeks. Motility, validity, abnormal and dead sperm were assessed. Testosterone, luteinizing (LH) and follicle- stimulating (FSH) hormones were measured. Antioxidant activity [glutathione (GSH), thiobarbituric acid reactive substances (TBARS), total antioxidant capacity (TAC) and the level of nitric oxide (NO)] were determined. Histopathological examination was done for testis.
Results: The results showed that Pb caused a significant increase in number of abnormal and dead sperms in Pb group (43.0%, 67.2%) comparing to the control group (6.0%, 18.6%) respectively. Motility and validity of the sperm were significantly decreased in Pb group (16.0%, 32.8%) comparing to the control group (84.2%, 81.4%) respectively. Pb caused a significant increase in FSH (1.99 mIU/L) and LH (1.2 mIU/L) and a decrease in Testosterone hormones (0.86 nmol/L) comparing to the control group (0.64 mIU/L, 1.2 mIU/L, 5.24 nmol/L) respectively. On the other hand, AA caused a significant decrease in numbers of abnormal and dead sperms than in Pb group. AA also caused an increase in motility and viability of the sperms at all levels. Testosterone hormone showed a marked increased with AA and the best effect was found with the high level (1500 mg /kg BW). For antioxidant activity it was found that Pb caused a significant increase in NO and TBARS levels comparing to the control group, while it decreased significantly GSH and TAC levels. The significant effect for AA was found with the high level (1500 mg) on NO (28.5 µmol/ml) and GSH (4.9 µmol/ml). Also, it was found that AA significantly affected TBARS and TAC at all levels. Histopathological examination showed the presence of AA reduced the harmful effect of lead acetate on testis.
Conclusion: High daily intake of AA from rich sources or from supplementation can protect reproductive system of male rats from lead toxicity.

***Keywords*:** Ascorbic acid, Lead, reproductive function, antioxidant activity

Available on line at:
www.jhiph.alexu.edu.eg

¥*Correspondence*:
Email: elsebeay1969@gmail.com

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INTRODUCTION

The toxicity of heavy metals may cause pathological and physiological dysfunction of organs.(1) Lead (Pb) content in the air, food and tap water has increase folds during recent years due to extensive use of this metal in petrol, paints, battery and other industry.(2) Many animal studies showed that Pb can adversely affect the mammalian male reproductive system.(3) The results of previous studies suggested that relatively high occupational exposure to Pb, as indicated by blood Pb levels can reduce human semen quality (decreased number, motility and alter morphology) of sperm, whereas reproductive endocrine function is either not affected or is only marginally affected.(4) Low Pb exposure could alter human semen quality and sperm chromatin condensation.(5) Lead exposure may have reproductive consequences. Some authors have reported a reduction in spermatogenesis among battery workers as one of the findings in

symptomatic lead poisoning.[6] Pb could induce apoptosis in the germ cells within the seminiferous tubules.[7] Although adverse effects of Pb compounds on the testis have long been studied[8], effective drugs reversing the toxicity of Pb on male reproduction are still scarce and insufficient.

The anti-oxidant ascorbic acid (AA) is known as a chelating agent in treatment of Pb toxicity, and has been reported to protect the cells from oxidative stress and sperm from loss of motility.[9]

Spermatozoa are extremely sensitive to per oxidative damage due to low antioxidant capacity.[10] The antioxidant AA is a powerful reducing agent influencing many oxidation reactions in the biological systems.[9] AA protect male reproductive organs from most chemicals and /or drug - induce damages.[11]
Supplementation with vitamin E and/or AA reduced reactive oxygen species generation, prevented loss of motility and capacity of oocyte penetration in Pb-exposed rats.[12]

Previous study data suggested that high serum levels of AA are independently associated with a decreased prevalence of elevated blood Pb levels. If these associations are related causally, AA intake may have public health implications for control of Pb toxicity.[13] This work aim to study the efficiency of Ascorbic acid on semen quality, sex hormone, antioxidant parameters and testis histology in rats treated with lead acetate.

METHODS

Experimental animals: Male albino rats (*n:* 50) averaging 180±5g of BW were obtained from the animal house of the Medical Research Institute, Alexandria University, Egypt. The local committee approved the design of the experiments and the protocol follows the guidelines of the National Institutes of Health (NIH).[14] Animals received human care, and had adequate stable diet and water *ad lipitum*. Animals were acclimatized to the laboratory conditions for two weeks before being experimented.

Experimental design: After two weeks of acclimation, animals were classified into five equal groups, ten rats in each. A control group (received tap water only), lead (Pb) group (received 0.2% lead acetate/ kg BW) and the other three groups (received 500, 1000 and 1500 mg ascorbic acid/ kg BW along with 0.2% lead acetate/ kg BW), respectively. Doses were orally administered (as solutions) every day for 8 weeks. All chemicals were obtained from El-gomhouria company- Alexandria – Egypt.

Body weight and organs weight: Body weight of rats was recorded in the beginning and at the end of the experimental period. Animals were sacrificed by decapitation, then testis, and epididymis were immediately removed and weighed. Relative organ weights were calculated as g/100g BW. Testis and epididymis were excised immediately.

Blood sample: Blood samples were collected from the sacrificed animals in heparinized tube. Plasma samples were obtained by centrifugation at 4000 rpm for 20 minutes, and then samples were stored at–20ºC until used for further analyses.

Sperm parameter: The testis from each rat were carefully exposed and one of them was removed together with its epididymis. The spermatozoa were obtained from caudal epididymis. Caudal epididymis sperm density (count), grade degree of sperm classified and abnormal sperms percent were determined.[15]

Sex hormones: Testosterone, luteinizing hormone (LH) and follicle- stimulating hormone (FSH) were assayed in plasma.[16, 17, 18]

Antioxidant enzymes activity and free radicals: The activity of glutathione (GSH), thiobarbituric acid reactive substances (TBARS), total antioxidant capacity (TAC) and the level of nitric oxide (NO) were assayed in testis homogenates using commercial kits.[19, 20, 21, and 22]

Histological study: Testis specimen used for histological study was fixed in neutral formalin for a week at room temperature, dehydrated then cleared in xylene and embedded in paraffin wax. The paraffin sections were cut at 20 microns thickness and stained with hematoxylin and eosin for histological examination using the light microscope.[23]

Statistical analysis: All data were expressed as mean ± SD. Statistical Analyses System (SAS) software program version 9.1 (SAS, 2003)[24] was used for one-way analysis of variance (ANOVA), at ≤0.05 to compare the statistically significant difference between groups.

Ethical considerations

The study was approved by the institutional review board and the ethics committee. The study conformed to the international guidelines on research ethics of animal experimentation. All laboratory biological specimens and hazardous waste were disposed of safely

RESULTS

Table (1) showed that, there was no significant change in the body weight in all groups, while relative weight of testis and epididymis were decreased significantly in Pb group comparing to the control group. AA had no significant effect on the relative weight of both testis and epididymis irrespective of the tested dose (500, 1000 and 1500 mg/kg BW). Reproductive functions were summarized in table (2). It was noticed that the number of sperms were significantly decreased in Pb group (21.8 million/ml) than in the control group (84.4 million/ml). Also, the number of abnormal sperms was significantly increased in Pb group (43.0%) comparing to the control group (6.0%). The motility and

viability of the sperms were significantly decreased in Pb group (16.0 % and 32.8% than in the control group (84.2 % and 81.4%) respectively. The number of dead sperms was significantly increased in Pb group (67.2%) than in the control group (18.6%). The presence of AA along with Pb caused improvement in reproductive function in rats; AA caused a significant increase in the number of sperms at all the tested levels (33.4, 48.4 and 77.8% at 500, 1000 and 1500 mg/kg BW respectively) comparing to the Pb group (21.8%). The number of abnormal sperms was significantly decreased with increasing the level of AA (24.4, 18.6 and 9.6% at 500, 1000 and 1500 mg/kg BW respectively). Also, the number of dead sperms was significantly decreased at high level of AA (24.6% at 1500 mg/kg BW) than in the Pb group (67.2% mg/kg BW). AA had a positive effect on motility and viability of the sperms. It significantly enhanced sperm motility at all the tested levels (38.8, 51.6 and 73.4% at 500, 1000 and 1500 mg/kg BW) comparing to the Pb group (16.0%) and significantly increased their viability at high level (75.4% at 1500 mg/kg BW). Sex – hormones were significantly affected by Pb. As showed in table (3), it was found that Pb caused a significant increase in FSH (1.99 mlU/L) and LH (1.2 mlU/L) and a decrease in Testosterone (0.86 nmol/l) comparing to the control group (0.64 mlU/L, 1.2 mlU/L, 5.24 nmol/l respectively). AA significantly decreased the level of FSH at all the tested levels. Regarding the LH, the significant effect of AA was found at 1000 mg, but at 1500 mg/kg BW it did not make a difference. Testosterone hormone level was significantly increased with AA and the best effect was found at a high level (1500 mg/kg BW). Antioxidant activity was described in table (4). It was found that Pb significantly increased the levels of NO and TBARS comparing to the control group (36.8 µmol/ml and 26.2 nmol/ml in Pb group and 25.0 µmol/ml, 5.9 nmol/ml in the control group respectively), whereas it significantly decreased GSH and TAC levels than in the control group (3.2 µmol/ml and 0.9 mmol/ml in Pb group and 5.9 µmol/ml and 1.6 mmol/ml in the control group respectively). There was no difference in the of AA at level of 500 and 1000 mg/kg BW of in reducing the level of NO or increasing the level of GSH. However, a a significant effect was found at a high AA level (1500 mg/kg BW) on NO (28.5 µmol/ml) and GSH (4.9 µmol/ml). Also, it was found that AA had a significant effect on TBARS and TAC at all the tested levels. Histological study showed that Pb caused peritubular edema and congested blood vessel (Fig. 1B) comparing to the control group (Fig 1A). Different dosses of AA improved the damage caused by Pb (Fig. 1C, 1D and 1E). The best effect of AA was showed at 1500 mg.

Table 1: Changes in body weight gain (g) and relative weight of organs (g/100g BW) in the control and different treatment groups

Parameters	Experimental groups (mean ± SD)				
	Control	Pb	500 mg AA	1000 mg AA	1500 mg AA
Initial weight(g)	178.5 ± 5. 3[a]	183.0 ± 6. 7[a]	181.7 ± 5.9[a]	185.7 ± 7. 2[a]	185.8 ± 9.4[a]
Final weight(g)	215.5 ± 6.9[a]	215.4 ± 14.7[a]	210.0 ± 11.0[a]	207.1 ± 13.3[a]	211.7 ± 14.7[a]
Body weight gain(g)	37.0 ± 6.4[a]	32.4 ± 6.8[a]	28.3 ± 7.1[a]	21.4 ± 10.6a	25.8 ± 10.1[a]
Testis g/100g BW	1.3 ± 0.03[a]	1.1 ± 0.04b	1.2 ± 0.05[a]	1.2 ± 0.04[ab]	1.1 ±0.06b
Epididymis g/100gBW	1.42 ± 0.06[a]	1.27 ± 0.05ab	1.2 ± 0.08[b]	1.24 ± 0.07[ab]	1.28 ± 0.06[ab]

Results are expressed as mean ± SD; Means with different letters in the same row imply significant differences at P≤0.05 according to F test.

Table 2: Changes in morphology of semen of male rats in the control and different treatment groups

Parameters	Experimental groups (mean ± SD)				
	Control	Pb	500 mg AA	1000 mg AA	1500 mg AA
Count (million/mL)	84.4 ± 3.07[a]	21.8 ± 1.9[d]	33.4 ± 2.3[c]	48.4 ± 4.71[b]	77.8 ± 2.43[a]
Abnormal (%)	6.0 ± 0.7[d]	43.0 ± 3.16[a]	24.4 ± 1.94[b]	18.6 ± 1.21[c]	9.6 ± 0.68[d]
Motility (%)	84.2 ± 2.71[a]	16.0 ± 0.7[e]	38.8 ± 2.8[d]	51.6 ± 2.13[c]	73.4 ± 1.5[b]
Validity (%)	81.4 ± 2.87[a]	32.8 ± 1.85[d]	53.2 ± 2.59[c]	58.4 ± 2.38[c]	75.4 ±2.62[b]
Dead (%)	18.6 ± 2.87[d]	67.2 ± 1.85[a]	46.8 ± 2.59[b]	41.6 ± 2.38[b]	24.6 ± 2.62[c]

Results are expressed as mean ± SD; Means with different letters in the same row imply significant differences at P≤0.05 according to F test.

Table 3: Changes in plasma FSH, LH and Testosterone levels of the male rats in the control and different treatment groups

Parameters	Experimental groups (mean ± SD)				
	Control	Pb	500 mg AA	1000 mg AA	1500 mg AA
FSH (mIU/L)	0.64 ± 0.02^{d}	1.99 ± 0.11^{a}	1.67 ± 0.14^{b}	1.06 ± 0.08^{c}	0.71 ± 0.03^{d}
LH (mIU/L)	0.28 ± 0.04^{c}	1.2 ± 0.17^{a}	1.25 ± 0.09^{a}	0.75 ± 0.1^{c}	0.37 ± 0.05^{c}
Testosterone (nmol/L)	5.24 ± 0.36^{a}	0.86 ± 0.05^{d}	2.2 ± 0.23^{c}	3.3 ± 0.19^{b}	4.74 ± 0.48^{a}

Results are expressed as mean ±SD; Means with different letters in the same row imply significant differences at P≤0.05 according to F test.

Table 4: Changes in antioxidant activity of testis of male rats in the control and different treatment groups

Parameters		Experimental groups (mean ± SD)				
		Control	Pb	500 mg AA	1000 mg AA	1500 mg AA
NO	µmol/ml	25.0 ± 0.5^{d}	36.8 ± 0.73^{a}	31.3 ± 0.62^{b}	30.5 ± 0.61^{b}	28.5 ± 0.56^{c}
GSH	µmol/ml	5.9 ± 0.22^{a}	3.2 ± 0.12^{c}	4.4 ± 1.16^{b}	4.6 ± 0.17^{b}	4.9 ± 0.18^{b}
TBARC	nmol/ml	17.7 ± 0.46^{d}	26.2 ± 0.69^{a}	23.0 ± 0.61^{b}	22.1 ± 0.58^{bc}	20.5 ± 0.54^{c}
TAC	mmol/ml	1.6 ± 0.01^{a}	0.9 ± 0.01^{e}	1.2 ± 0.01^{d}	$1.2 \pm 0.0.01^{c}$	1.4 ± 0.01^{b}

Results are expressed as mean ±SD; Means with different letters in the same row imply significant differences at P≤0.05 according to F test.

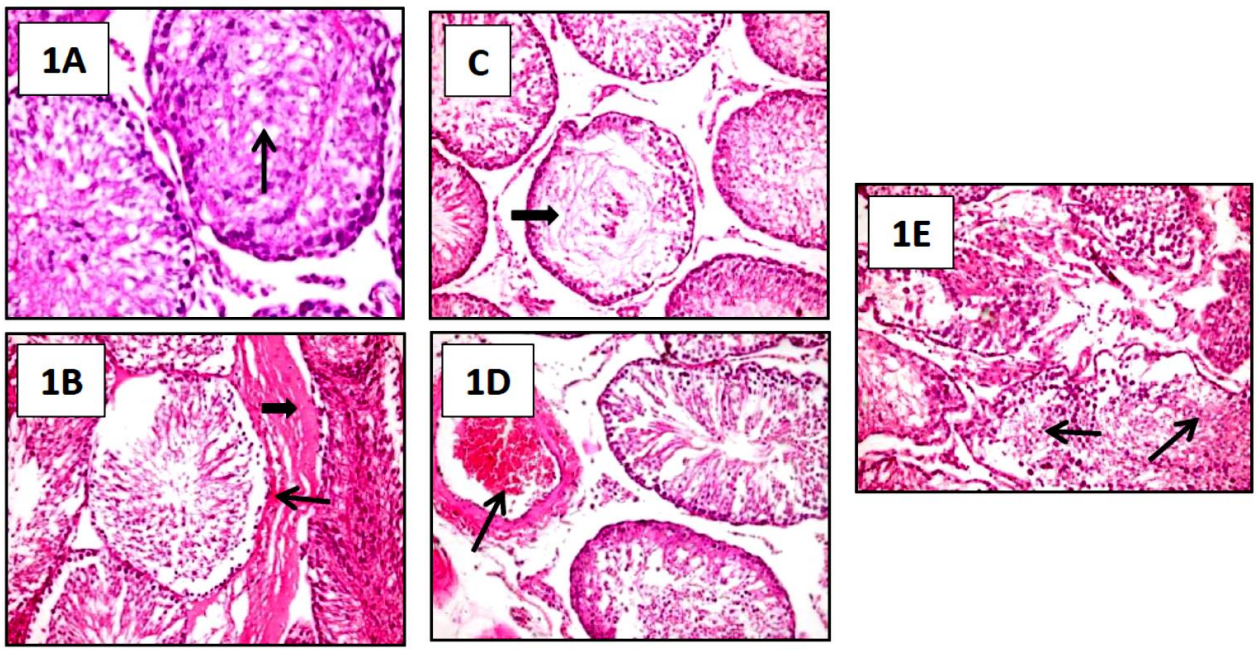

Figure (1): Photomicrograph of rat testis stained with hematoxylin and eosin: (1A) control group showing sections of seminiferous tubules and vacuolation, (1B) Testis showing peritubular edema (arrow head), and congested blood vessel (arrow), (1C) testis showing marked lysis and necrosis of spermatogonia cells (arrow head), (1D) testis showing congested blood vessel (arrow) and (1E) testis showing disintegrations of a massive number of semineufrous tubules (arrows), (H&E X 200).

DISCUSSION

Occupational and environmental exposures to heavy metals are known to have deleterious effects on human reproduction. In rats Pb has been implicated in male reproductive dysfunction.[25] Ascorbic acid is an essential component in the diet of human, and animals. It has been associated with fertility for many years, but its precise physiological role in reproduction has been uncertain.[26]

The results of the current study clearly demonstrate that lead acetate exposure can seriously alter the testis and reproductive function of male rats. The results revealed that, relative weight of testis and epididymis were decreased significantly in Pb group compared to control group. Al-Harrby *et al.,* also reported a significant decrease in all reproductive organs weight compared with a control group.[27] Other studies indicated that the weights of the testis and accessory sex organs were significantly decreased by some toxic substances, and the administration of AA reserved this reduction.[28]

In the present study it was found that Pb had harmful effect on reproductive functions, Pb caused decrease in sperm counts, also number of abnormal sperms was significantly increased. Motility and viability of the sperm were decrease significantly. Sokol *et al*., indicated that experimental exposure to Pb had adverse effects on sperm parameter (sperm counts, lower and a lesser motile and increase sperm abnormality).[29] Other studies showed that exposure to Pb greatly impaired male reproductive function by reducing sperm count or changing sperm motility and morphology.[6] Other studies showed many effects including a decrease in semen volume, sperm motility, sperm count and abnormal sperm morphology.[4]

Sex-hormones were affected significantly by Pb in this study, it was found that Pb caused significant increase in FSH and LH and decrease in Testosterone hormone compared to the control group. On the other hand, AA caused a significant increase in the number of sperms irrespective of the dose compared to the Pb group. The numbers of abnormal sperms were significantly decreased by increasing the level of AA. Also numbers of dead sperms were significantly decreased at high level of AA compared to Pb group. AA had positive effect on motility and viability of the sperms. Intervention with AA in the presence of Pb enhanced the levels of sex hormones. The significant effect of AA on LH was found at 1000 mg, but at 1500 mg it did not make a difference although this level had the best effect on testosterone hormone. The results of the present study are in agreement with the findings of Yousef *et al*., who reported that AA supplementation in drinking water increased sperm concentration of male rabbits.[30] The increase in the epididymis sperm concentration might be explained with the activation of both FSH and LH by AA, since the activated FSH and LH stimulate spermatogenesis, and increases the sperm concentration.

For antioxidant activity it was found that Pb caused a significant increase in NO and TBARS levels comparing to the control group, while it significantly decreased level of GSH and TAC than in the control group. AA play an important role in antioxidants activity, the significant effect was found at high level (1500 mg) on NO and GSH. Also we found that AA had a significant effect on TBARS and TAC at all the tested levels. AA, the known chelating agent with antioxidant features, was widely reported with the capability of protecting cells from oxidative stress.[31] Previous study showed that Pb exposure affected the testicular cells of mice these effects can be reversed by AA. The administration of AA in Pb-exposed rats prevented the Pb-induced spermatogenic cell apoptosis and protected the testicular histology and structure. This protective effect of AA was associated with activation of several pathways of cell apoptosis in the animals.[32]

In the present study AA treated testis showed a reduction in the various stages of developing spermatogonial cells but their cell populations were quite high as compared to Pb and Pb+ AA treated groups. These finding were in agreement with the hypothesis that the testicular damage in mice was also improved significantly when treated with AA and thiamine.[33] The results showed that daily doses of lead acetate cause significant alterations in the histology of the rats' testis in the different developing periods. The testicular tissue architecture of Pb treated animals showed serious damages within the seminiferous tubules. These findings were in agreement with other studies which found that Pb exposure caused progressive vascular, tubular and interstitial testicular damage. It also caused a reduction in the testicular volume and seminiferous tubules diameter.[34] Daily doses of Pb acetate cause a significant decrease in the average body weight and significant alterations in the histology of the mice testis.[35]

Conflict of Interest: None to declare.

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