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Ameliorative Effect of L-Arginine as Antioxidant Agent on Hematological and Testicular Functions in Lead-Induced Toxicity in Male Rats

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Author's contribution

The sole author designed, analysed, interpreted and prepared the manuscript.

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ABSTRACT

Aims: The present study aimed to elucidate the protective role of L-Arginine on lead – induced suppression of the pathway of heme synthesis and the toxicity of lead on testis.
Methodology: The current study included two experiments; the first was carried out on two groups to follow up the changes that could occur in iron status and reproductive system of male rats because of the lead exposure. The second experiment, three comparisons were made between three groups of rats with lead treatment only and other three groups rats with lead treatment followed by additional of daily injection of L-Arginine intraperitoneal about 500 mg L-Arginine / kg.b.wt. / day for 10, 20 and 30 days after the end of lead treatment.
Results: Lead significantly abolished heme synthesis presented by decrease in Hb, Fe and ceruloplasmin (Cp) and elevation in total iron binding capacity (TIBC) and serum ferritin. Furthermore, testosterone (total and free) significantly decreased after rats treated with 1% lead acetate while, estradiol (E_2) and acid phosphatase (total and prostatic) were elevated. The concentration of dehydroepiandrosterone sulphat (DHEA-S) did not change after lead treatment.
Conclusion: Daily treatment with 500 mg L-Arginine/kg body weight prevented the suppressive

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effects of lead on hematological parameters and testicular functions. The mechanism of L-Arginine action on lead induced changes was attributed to protection of antioxidant capacity in cells in addition to the ability of L-Arginine to scavenge free radicals and acts as cell membrane stabilizer.

Keywords: Lead; L-Arginine; hematological status; testicular functions.

1. INTRODUCTION

The rate of pollution with lead compounds is increased with civilization. The animal body can store some lead that enter the body and excrete it without adverse health effects, but when the animal is over exposed either accidentally or occupationally to high level of lead, symptoms of lead toxicity result. The total body burden of lead includes: a rapidly exchangeable pool in blood and soft tissue (like reproductive organs), an intermediate pool of exchangeable lead in skin and muscles and a more stable pool in the skeleton [1]. About 95% of the total body burden of lead is lodged in the bone and its accumulation in the bone can lead to elevated blood lead levels for long periods [2].

Anemia is common in lead poisoning. It is usually of mild to moderate severity of hemoglobin falling. The red blood cells are characteristically normocytic and normochromic or microcytic and hypochromic and may show mild polychromosia [3,4]. Also, in rats, it was noticed that a pronounced decline in the electrophoretic mobility of erythrocytes, a decrease in the number of RBCs and significant decline in hemoglobin (Hb), hematocrit (Ht), mean cell volume (MCV) and mean cell hemoglobin concentration (MCHC) after exposure of the animal to lead [5].

Lead exposure increases concentration in several organs of importance in reproduction such as hypothalamus and forebrain, the pituitary gland and the gonads [6,7]. Lead has a toxic effect on testicular spermatogenesis causing terato-spermia. This defect displays a positive correlation to blood lead levels [8]. The lead level of whole semen is normally much lower than in blood indicating the presence of a protective barrier [9]. Reported effect of lead exposure on men include reduced libido reduced motility and numbers of sperms, chromosomal damage, infertility, abnormal prostatic function and reduction of testosterone synthesis [10]. Moreover, there was a significant reduction of testosterone synthesis in the rat at steady state blood lead of 48 - 67 µg Pb/100 ml [11]. It also recorded the enzymatic alterations and reduced sperm counts associated with marked increase in the abnormal forms and significant changes in the pathological studies of testis rats after the animals treated with lead. They attributed these results to the disturbance of the androgen synthesis, which is responsible for enhanced testicular injury in lead induced stressed rats.

Recently, testicular dysfunction was observed with lead exposure manifested as oligospermia, azospermia decreased sperm motility testicular atrophy and increased apoptosis at specific stages of seminiferous epithelial cycle [9-11]. They also reported a significant loss of testicular proteins and testicular failure as manifested by decreased testosterone. FSH, LH, estradiol and testicular gamma glutamyl transpeptidase (GTP) levels were increased significantly as a result of lead exposure. They attributed this disturbance in the hormonal profile due to loss of inhibitory effect of sertoli cell on FSH level.

Arginine is a basic amino acid which is considered semi-essential. Although it can be synthesized by the body, it cannot be produced at rates enough to support growth and must therefore be ingested in the diet. L-arginine has many important biochemical functions, including immunoreactivity; ameliorate some clinical sequelae of Hb, enhancing release of growth hormones, and amino acid detoxification. L-arginine is also an important source for polyamine synthesis and is the only source of amino groups in the formation of creatine, which is involved in the regeneration of ATP [12-15]. Alterations in L-arginine bioavailability and subsequently NO synthesis have been linked to the pathogenesis of a wide range of cardiovascular and renal diseases [16].

The amino acid L-arginine is metabolized to NO and citrulline, unless diverted to ornithine by arginase. Arginine is depleted in SCD patients at baseline, and more so in patients with additional complications [17]. Plasma arginase, which degrades L-arginine, may serve as a marker for poor outcome in sickle cell disease [12,18,19]. L-arginine supplementation, in mice and in humans [12,13], has been reported to ameliorate some clinical sequelae of HbSS. This amelioration may be mediated by decreasing red cell density (mean corpuscular hemoglobin concentration

(MCHC) or by increasing red-cell glutathione (GSH) levels, both of which have been reported following L-arginine supplementation in transgenic mouse models of sickle cell disease [13,20].

Moreover, L- arginine supplementation led to increase in the activity and the motility of sperms and prolonged their life - span. Also, L- arginine tends to reduce oxidative stress in torsioned testes and helps maintain the seminiferous epithelium, thereby preserving the cells of the spermatogenic series [21]. Furthermore, other experiment showed that dietary L-Arginine at 2.33 g/kg improved testes weight, semen volume and sperm forward motility in roosters ($P<0.05$). Besides, serum concentration of testosterone was increased in roosters fed 2.33 g/kg L-Arg ($P<0.05$). The results of testes histology indicated that seminiferous tubules lumen diameter, leydig cells, spermatides and sperm cells counts were greater in birds received 3.22 g/kg dietary L-Arg ($P<0.05$). However, the birds fed diet supplemented with 2.33 g/kg L-Arg had greater seminiferous tubules diameter, sertoli and spermatogonia cell counts than other groups ($P<0.05$). According to the results of this experiment, it is concluded that dietary L-Arg had positive effects on reproductive traits in roosters [22].

The current study is an attempt to investigate the harmful effects of exposure to lead on haematological parameters and testicular functions in male albino rats, and the important role of the administration of L-Arginine to reduce or cure these harmful effects.

2. MATERIALS AND METHODS

Eighty growing male albino rats aged about 8 weeks were employed in this work. The animals were housed in a well-ventilated animal house and kept under the same managerial and environmental conditions. They were fed to appetite on a standard laboratory animal diet according to NRC [23] and fresh tap water was always available. The animals were divided at random into eight equal groups (ten rats each) on the base of body weight (120 ± 10 g in average) and caged in wire bottom galvanized metal wall boxes.

The current study included two experiments; the first was carried out on two groups to follow up the changes that could occur in iron status and reproductive system of male rats because of the lead exposure. To achieve this purpose, a comparison was done between a group of ten control rats received orally normal saline (0.9% NaCl) for 30 days and other ten animals were daily treated with 1% lead acetate (distributed by El- Nasr pharmaceutical and chemical company according to the methods of Prolabo) in drinking distilled water for 30 days.

The second experiment, three comparisons were made between three groups of rats with lead treatment only and other three groups rats with lead treatment followed by additional of daily injection of L-Arginine (Sigma Chem. Co., St Louis Mo., USA) intraperitoneal about 500 mg L-Arginine / kg.b.wt. / day for 10, 20 and 30 days after the end of lead treatment. Investigation has been made to evaluate the possible ameliorating effect of L-Arginine on iron status and reproductive system of rats at various intervals.

At the end of each experiment, blood samples were collected from each group by decapitation. Hemoglobin was determined using kits provided by Randox (U.K.). Iron (Fe) and total iron binding capacity (TIBC) were estimated using commercial kits purchased from (Bio-Merieux Co. Mary-L-Etoile, chorbonnieres, Les-Brain, France). Serums unsaturated iron binding capacity (UIBC) was calculated by subtract iron from total iron binding (TIBC). Hormonal assay (testosterone, free testosterone, estradiol and dehydroepiandrosterone-sulphate) and ferritin levels were estimated by using radioimmunoassay technique (Solid phase component system, ICN Co, USA). Total acid phosphatase (T.A.P.) and prostatic acid phosphatase (P.A.P.) were determined using commercial kits (Bio-Merieux Co. Marcy-L-Eloile, Chorbonnieres Les-Brain, and France).

Plasma Ceruloplasmin (Cp) level was measured by using O-dianisidine dihydrochloride as substrate using kits purchased from (Biochem Co. USA).

Student's "t" test was used for statistical analysis of the data at various intervals in both experiment No.1, and No.2 according to Snedecor and Cochran [24].

3. RESULTS AND DISCUSSION

In the current investigation, it has been established that the treatment of rats with 1% lead acetate in the drinking water for 30 days led to a significant ($p>0.01$) decrease in the

hemoglobin (Hb) content, iron (Fe) level and ceruloplasmin (Cp) level (Fig. 1). The significant decline of serum iron in particular is judged as the best clue ensuring that the problem is actually iron deficiency. These results are not surprising because lead toxicity led to pronounce reduction in the RBCs formation and because the life-span of RBCs was decreased. Many authors reported that anemia is common in lead poisoning [1,2]. Also, it was showed a significant decline in the RBCs, Hb, Ht, MCV and MCHC after the animal treated with lead. They attributed these results to mal-absorption of iron and decrease in the synthesis of globulin [3,4].

On the other hand, total iron binding capacity (TIBC), unsaturated iron binding capacity (UIBC) and ferritin level were increased ($p>0.01$) significantly as a result of lead toxicity (Fig. 1). This result defines the onset of iron deficiency and indicates that some impairment had taken place in the status of iron stores. Since, there is a direct relationship between ferritin content and the amount of iron stored in the body as mentioned by many authors [25,26].

In the present work, the reduced level of ceruloplasmin because of lead toxicity may reflect a decreasing iron turnover for needs of erythropoiesis and hence, a limitation of RBCs production [27] or may be due to elevation of erythrocyte protoprophyrin concentration [28]. As ceruloplasmin is considered a preventive plasma antioxidant and because it sequesters transition metals thereby preventing them from participating in free radical reduction as a result of metal toxicity [29,30]. The TIBC variable is a measure of iron binding capacity within the serum. It reflects the availability on iron binding sites on serum transferring. Thus, TIBC increases when serum iron concentration and stored iron are low [31,32].

Regarding to the hematological impairment observed in this study due to lead toxicity, L-Arginine administration led to a significant amelioration in the hemoglobin content and iron status of these toxic rats (Table 1). These improvements in the hemoglobin content and iron status may be due to the ability of L-Arginine to elevate the formation of RBCs count and help in the reduction of iron ion from ferric state to ferrous state [33-38].

In the present study, a testicular dysfunction occurred after lead intoxication in the adult rats and represented by a significant decline in the concentration of both total and free testosterone (Fig. 2). Moreover, a significant elevation in the levels of E_2, total and prostatic acid phosphatase was reported and not a remarkable fluctuation was recorded in the concentration of DHEA-S (Fig. 2). These results are in harmony with these obtained by [6,9]. They attributed these results to the testicular atrophy and interstitial cell hyperplasia because of lead exposure. It was illustrated that the lead administration led to a significant elevation of abnormal sperm count and a decrease in the sperm-span. They endorsed these results to the dysfunction in the hypothalamic-pituitary-testis axis (HTPTA) by the elevation of FSH and LH levels due to a remarkable damage in both Sertoli cells and Leydig cells [9].

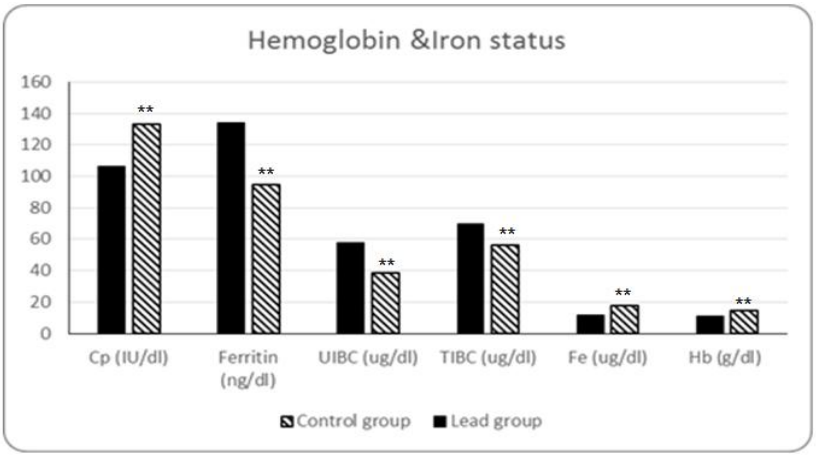

Fig. 1. Showed the effect of administration of 1% lead acetate in drinking water on Hemoglobin and iron status in male rats for one month

*Significant from normal control, * P < 0.05; ** P < 0.001*

Table 1. The protective effect and curative role of antioxidant L-Arginine (500 mg/kg b.wt./day) i.p on Hemoglobin and iron status in male rats for one month

Hemoglobin & iron status	Duration by days	Recovery group	L-Arginine group
Hb (g/dl)	10	11.42 ± 0.19^{a}	12.29 ± 0.17^{b}
	20	11.79 ± 0.16^{A}	12.91 ± 0.18^{B}
	30	12.08 ± 0.18^{A}	13.51 ± 0.16^{B}
Fe (ug/dl)	10	12.29 ± 0.08	12.35 ± 0.08
	20	12.83 ± 0.09^{A}	13.90 ± 0.08^{B}
	30	13.11 ± 0.08^{A}	14.13 ± 0.10^{B}
TIBC (ug/dl)	10	68.27 ± 1.14	66.17 ± 1.11
	20	67.21 ± 1.12^{A}	62.41 ± 1.02^{B}
	30	66.35 ± 1.11^{A}	58.11 ± 1.09^{B}
UIBC (ug/dl)	10	56.12 ± 1.03	54.46 ± 1.02
	20	54.43 ± 0.98^{A}	48.39 ± 0.97^{B}
	30	53.30 ± 0.96^{A}	43.89 ± 1.04^{B}
Ferritin (ng/dl)	10	133.09 ± 2.12	131.23 ± 2.04
	20	131.88 ± 2.17^{A}	123.51 ± 2.23^{B}
	30	128.67 ± 2.11^{A}	114.83 ± 2.16^{B}
Cp (IU/dl)	10	106.87 ± 2.01	110.39 ± 2.04
	20	108.42 ± 2.12^{a}	114.76 ± 2.17^{b}
	30	111.09 ± 2.07^{A}	122.43 ± 2.03^{B}

Data are expressed as means ± standard error (S.E).
Means bearing different superscript (A,B) within the same row differ significantly (p<0.01).
Means bearing different superscript (a,b) within the same row differ significantly (p<0.05).

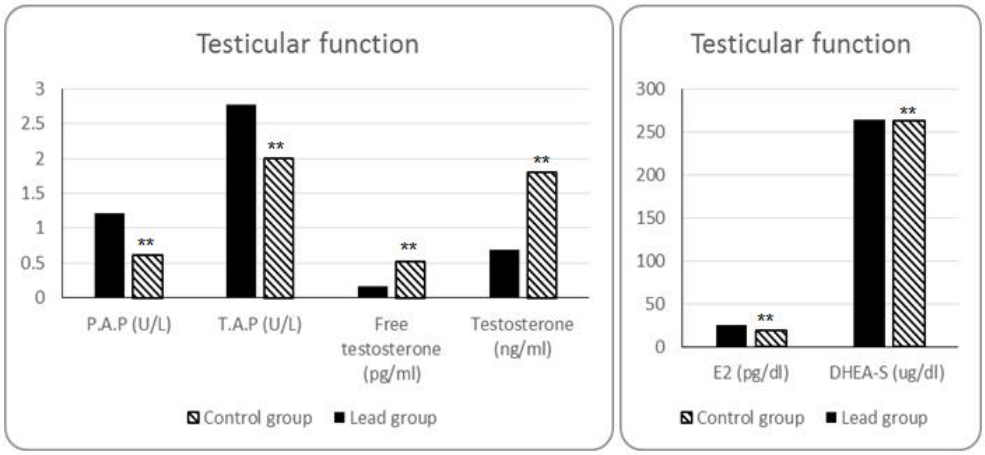

Fig. 2. Showed the effect of administration of 1% lead acetate in drinking water on testicular and prostatic functions in male rats for one month
*Significant from normal control, * P < 0.05; ** P < 0.001*

On other hand, L-Arginine supplementation led to a significant amelioration in the endocrine system, which related to the testis after lead exposure (Table 2). So, L-Arginine acts as free radical scavenger (reduce the harmful effect of lead) and prophylacting the hormonal and cellular damage in the testis of toxic rats (Table 2). It was pointed to L-Arginine administration as therapeutic or prophylactic beneficial, agent against the harmful effects of toxic compounds on testicular function of rats [39-43].

Table 2. The protective effect and curative role of antioxidant L-Arginine (500 mg/kg b.wt./day) i.p on testicular and prostatic functions for one month

Testicular & Prostatic Function	Duration by days	Recovery group	L-Arginine group
Testosterone (ng/ml)	10	0.72± 0.02	0.74 ± 0.03
	20	0.75 ± 0.01^{a}	0.80 ± 0.02^{b}
	30	0.79 ± 0.02^{A}	0.93 ± 0.03^{B}
Free testosterone (pg/ml)	10	0.17 ± 0.01	0.18 ± 0.02
	20	0.19 ± 0.01	0.20 ± 0.02
	30	0.22 ± 0.02^{A}	0.26 ± 0.01^{B}
DHEA (µg/dl)	10	263.69 ± 2.19	264.28 ± 2.31
	20	265.68 ± 2.37	266.39 ± 2.18
	30	267.83 ± 2.26	268.28 ± 2.29
E_2 (pg/ml)	10	25.11 ± 0.44	25.02 ± 0.49
	20	24.08 ± 0.38	23.51 ± 0.31
	30	23.66 ± 0.46^{A}	20.18 ±0.51^{B}
T.A.P (U/L)	10	2.71 ± 0.07	2.66 ± 0.06
	20	2.65 ± 0.07^{a}	2.40 ± 0.05^{b}
	30	2.51 ± 0.08^{A}	2.12 ± 0.07^{B}
P.A.P (U/L)	10	1.21± 0.07	1.18 ± 0.07
	20	1.16 ± 0.08^{a}	1.07 ± 0.08^{b}
	30	1.02 ± 0.08^{A}	0.88 ± 0.11^{B}

Data are expressed as means ± standard error (S.E).
Means bearing different superscript (A, B) within the same row differ significantly (p<0.01).
Means bearing different superscript (a, b) within the same row differ significantly (p<0.05).

4. CONCLUSION

In conclusion, based on the above results and discussion, it could be concluded that the exposure to lead induce severe damage in hematological parameters and destruction in testicular systems. However, the treatment with L-Arginine lessened all these damages and play an important role as anti-oxidative agent.

ETHICAL APPROVAL

The author hereby declares that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

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COMPETING INTERESTS

Author has declared that no competing interests exist.

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