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Protective Effect of Vitamin C against Lead Toxicity of the Reproductive System of Male Rats *

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ABSTRACT. This study assessed the significance of vitamin C in the protection against lead toxicity of the reproductive system of male rats. The treatment of rats with lead acetate (2%) for six weeks, caused a significant decrease in the sperm motility, sperm count, weight of seminal vesicles and prostate gland and serum LH. Moreover, sperm abnormalities were corrected to a large extent in the treated group. The abnormality of the sperm head was more than that of the tail. Some seminiferous tubules of the rats given lead appeared devoid of spermatozoa and with few spermatocytes. Vitamin C reduced the effects of lead on the sperm count and sperm morphology and the structure of the seminiferous tubules.

Key words: Antioxidant, gonads, lead, LH, sperm, vitamin C

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Introduction

Metals including mercury, lead (Pb) and cadmium are chemicals released into the environment following industrial use (Alegria *et al.* 1990). Once released into the environment, metals in various forms will contaminate the air, soil, water and food (Rossmann and Barres, 1988). Lead is not only present in the environment through leaded paint and drinking water; leaded gasoline and acidic food / beverages can also be a source of poisoning when kept in glazed containers.

Lead can be absorbed both through the gastrointestinal tract (ingestion) and respiratory tract (inhalation) tract. Clinical and animal studies indicate that abnormalities of spermatogenesis result from toxic

lead exposure (Hilderbrand *et al.* 1973; Eyden *et al.* 1978). Intra-testicular testosterone values, sperm counts and follicle stimulating hormone (FSH) levels were significantly decreased after lead treatment (Sokol *et al.* 1985). The weights of the body, testes and epididymes diminished by about 13% and seminal vesicle and ventral prostate weights by about 29% in lead exposed males (Pinon-Lataillade *et al.* 1995). A significant reduction of the intra-testicular testosterone levels was observed in mice after 30 days of exposure to lead acetate (Rodamilans *et al.* 1988). Due to lead poisoning, children showed growth retardation and growth hormone (GH) values were low (Huseman *et al.* 1992; Ronis *et al.* 1996).

Some vitamins play a protective role through direct or indirect mechanisms which interfere with the intestinal absorption of heavy metals by increasing urinary excretion (Pace and Iannucci, 1994). Ascorbate acts as an antioxidant by protecting human serum from lipid peroxidation (Dasgupta and Zdunek, 1992). Some investigators have reported that antioxidants inhibit chemical carcinogenesis when the antioxidants are administered either prior to or with the carcinogen (Novi, 1981 and Ames, 1983). One of the principal biochemical reactions of ascorbic acid is to destroy toxic free radicals resulting from the metabolic products of oxygen (Sapper *et al.* 1982).

The main aim of this study was to examine the protective effect of vitamin C against the deleterious effects of lead upon the testes of rats.

Materials and Methods

Animals and experimental design

Thirty-two male Wistar rats were used in this study. The average weight was 151±3.52 gm. The animals were maintained under standard laboratory condition (12h light, temperature 22±1^{O}C). Access to dry ration and water was *ad lib*. The animals were assigned to one of four groups, each of which contained 8 rats. The rats were treated daily for 6 weeks as follows: group (a) not treated and kept as control; group (b) treated with 2% lead acetate in drinking water (Bajorklund *et al.* 1981); group (c) administered only vitamin C (100mg/kg) orally (Paget and

Barnes, 1964) and group (d) given vitamin C (100mg/kg) and 2% lead acetate. The treatment of the rats was continued for 6 weeks.

Evaluation of spermatozoa

One epididymis was removed six weeks after the study began, cleaned of fat, dissected in 10 ml of normal saline (0.9% NaCl) and incubated at 37^{O}C for 30 min. Sperms were counted using haemocytometer (Neubauer) and percentage of sperm motility was determined. Smears were prepared from the suspension, stained with 1% eosin solution and examined for sperm abnormalities (Baloch and Cohen, 1964).

Histological study

The testes of the different rat groups were removed, weighed and fixed in 10% formol saline. Paraffin wax sections (5µm thick transverse section) of testes were stained with haematoxylin and eosin (Drury and Wallington, 1980) and examined under the light microscope. The weights of vasa deferentia, epididymes, seminal vesicles and prostate glands were recorded.

Hormonal assay

LH was assayed in serum samples of the different groups by enzyme immunoassay (Bio Merieux, France). The assay is based on a sandwich technique using two monoclonal antibodies.

Statistical analysis

Statistical comparisons between control and experimental groups were by One-Way Analysis of Variance, followed by the Least Significant Difference (LSD) range test.

Results

The body weight of the treated rats with lead was significantly lower ($P \leq 0.01$) at any time interval (after 2, 4 and 6 weeks) as compared to the control group (Table 1). Body weight of rats given lead and vitamin C

together also declined significantly ($P \leq 0.01$) after four and six weeks. Moreover, the treatment of the animals with vitamin C only had significant ($P \leq 0.01$) influence on the body weight at the sixth week. On the other hand, the body weights increased in all groups as the study progressed relative to the initial values. The body gains of the different groups in a descending order were control (+162.6%), lead with vitamin C (+113.1%), vitamin C (+100.5%), and lead (+71.4%). The weights of testes, epididymes and vasa deferentia were not significantly different in the lead group or lead and vitamin C in combination group (Table 2). The weight of the seminal vesicles of the two previous groups declined significantly ($P \leq 0.01$), by 36.96% and 10.86% respectively, relative to the control value. Similarly, the weight of the prostate gland was lowered significantly in the lead group ($P \leq 0.01$) or the lead with vitamin C group ($P \leq 0.01$), by 45.45% and 27.2% respectively, compared with the control group. The weight of the epididymis, prostate gland and seminal vesicles increased significantly ($P \leq 0.01$) in animals treated with vitamin C only, by 34.78%, 12.12% and 30.43% respectively, compared with the control group.

Sperm quality

The sperms were affected by lead (Table 3). The sperm motility in lead or lead with vitamin C treated groups decreased significantly ($P \leq 0.01$), by 38.7% and 13.71% respectively, compared with the control group. Similarly, the sperm count of the aforementioned groups lowered significantly ($P \leq 0.01$), by 33.94% and 22.55% respectively, relative to the control group values. The treatment of rats with vitamin C only resulted in a significant elevation ($P \leq 0.01$) of sperm motility and a decrease ($P \leq 0.05$) in tail abnormalities compared with the control group.

Hormonal assay

The plasma luteinizing hormone (LH) level of rats treated with lead was lowered significantly ($P \leq 0.01$), by 14.97%, as compared to the control group (Table 4). The hormone level of the rats given lead and vitamin C in combination also showed a significant reduction ($P \leq 0.01$) of 10.2%.

Histological study

Some seminiferous tubules of the rats given lead appeared devoid of spermatozoa or with few spermatocytes (Figs. 2, 6). Moreover, Leydig cells were deformed (Fig. 5). The other seminiferous tubules of lead treated rats appeared normal. No histological changes in the testes of animals treated with vitamin C and lead were observed (Figs. 3, 7).

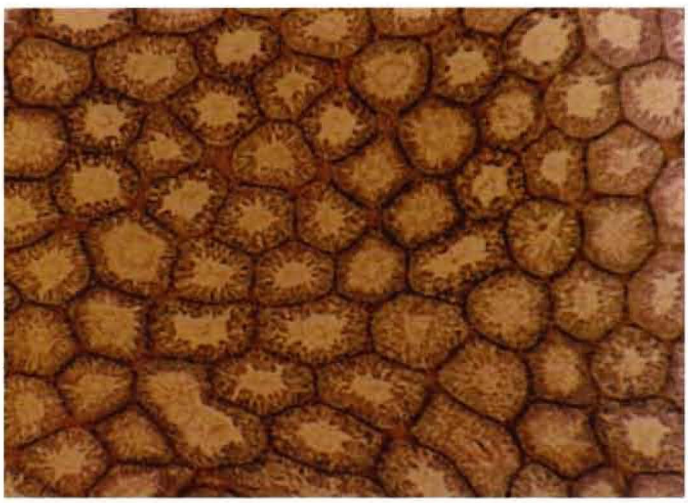

Fig 1 T.S. of the testis of the control rat showing normal structure (Hx. E. x 80)

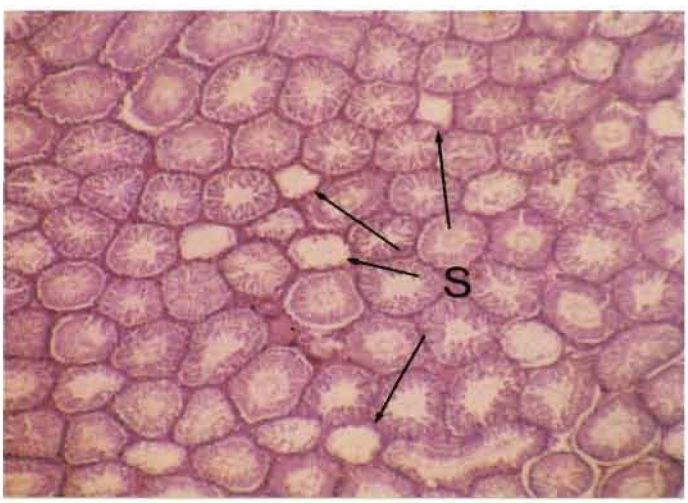

Fig 2 T.S. of the testis of lead treated rat. Some seminiferous tubles (S) appear devoid of spermatoza (Hx. E. x 80)

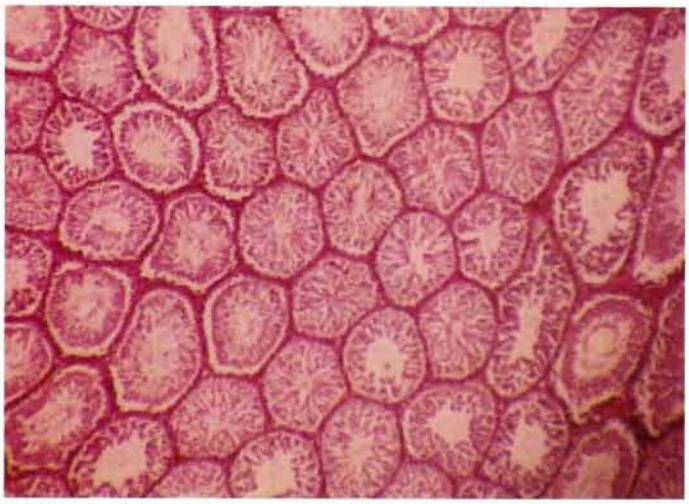

Fig 3 T.S. of the testis of rat treatedwith both vitamin C and lead, showing normal structure. (Hx. E. x 80)

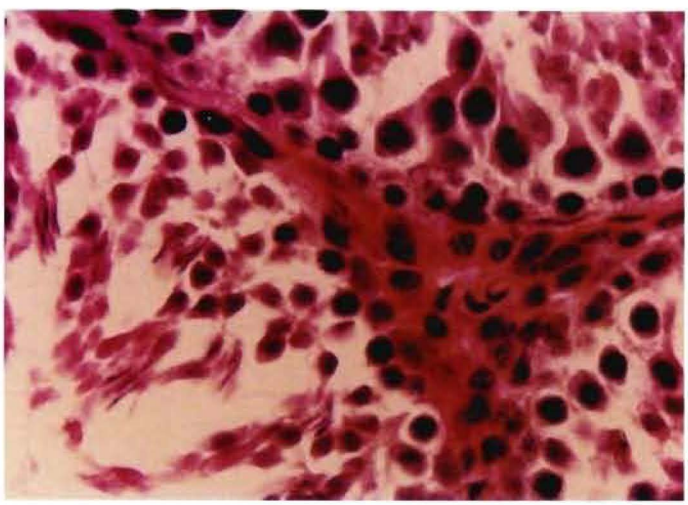

Fig 4 T.S. of the testis of the control rat showing normal structure (Hx. E. x 400)

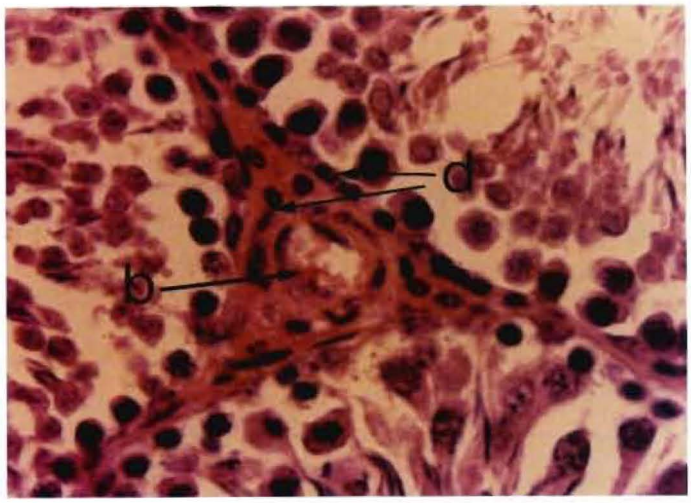

Fig 5 T.S. of the testis of lead treated rats showed congested blood vessels (b) and deformed Leydig cells (d). (Hx. E. x 400)

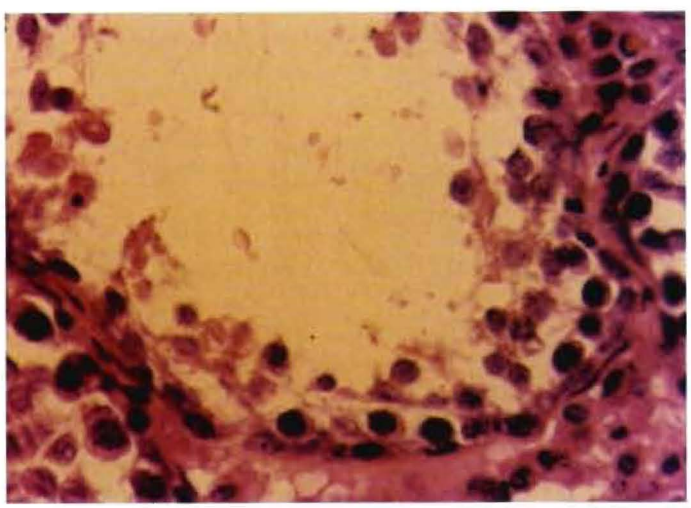

Fig 6 T.S. of the testis of lead treated rats showed arrest in spermatogenesis. (Hx. E. x 400)

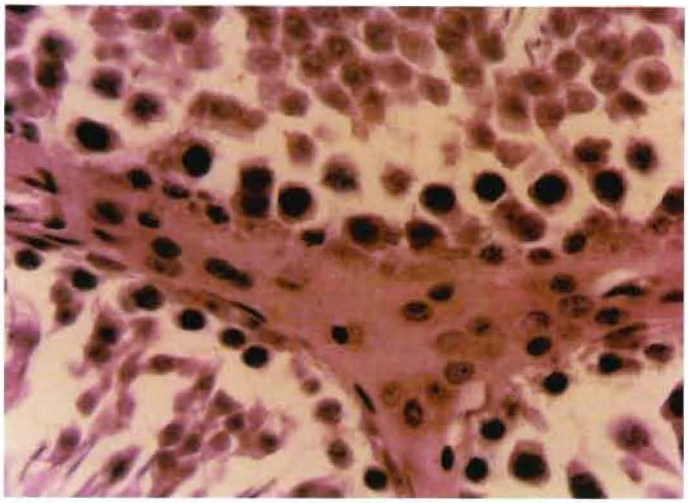

Fig 7 T.S. of the testis of rat treated withvitamin C and lead in combination. No histological changes were observed. (Hx. E. x 400)

Table 1. Effect of rat treated with lead and vitamin C, singly and in combination, on body weight (g) of male rats (mean±S.E.). Values are means of 8 replicates.

Treatment	Weeks after treatment						
	0	2	% of change▲	4	% of change	6	% of change
Control	145.1±1.81	208.8±5.49	+43.22	300.6±10.88	+106.3	382.8±10.69	+162.6
Lead (2%)	153.9±1.88	172.5±3.41**	+12.1	205.1±2.81**	+33.31	263.8±4.98**	+71.4
Vitamin C (100mg/kg)	158.8±2.45	210.3±3.9	+32.44	284.8±2.75	+79.37	318.25±5.66**	+100.47
Lead & Vitamin C	145.8±1.73	201.9±4.11	+38.51	260.6±6.44**	+78.82	310.6±6.16**	+113.12

Values are means of 8 replicates

** : Significantly difference from control at P≤0.01.

▲ : The percentage of the change is relative to Zero time.

Table 2. Effect of lead and vitamin C, singly and in combination, on weights (g) of testes and accessory sex organs of male rats relative to body weight (mean±S.E.). Values are means of 8 replicates.

Treatment	Sperm motility (%)	Sperm count per epididymis ($x 10^5$)	Sperm abnormalities (%)		
			Head	Tail	Total
Control	83.9±0.55	24.45±0.56	2.48±0.09	3.18±0.11	5.53±0.15
Lead (2%)	50.52±1.35**	16.23±0.27**	20.36±0.81**	11.91±0.61*	28.39±1.2**
Vitamin C (100mg/kg)	91.29±0.47**	26.6±0.63**	2.85±0.05	2.63±0.07*	5.37±0.17
Vitamin C & Lead	71.1±1.23**	19.03±0.18**	9.7±0.24**	4.9±0.23*	13.9±0.36**

Values are means of 8 replicates

*,** : Significantly difference from control at P≤0.05 and P≤0.01, respectively.

Table 3. Effect of lead and vitamin C, singly and in combination, on sperm count and sperm quality of male rats (mean±S.E). Values are means of 8 replicates.

Treatment	Testes	Epididymis	Vasa deferentia	Seminal vesicles	Prostate gland
Control	1.26±0.02	0.23±0.007	0.06±0.005	0.46±0.01	0.33±0.01
Lead (2%)	1.25±0.03	0.22±0.01	0.04±0.002**	0.29±0.01**	0.18±0.01**
Vitamin C (100mg/kg)	1.26±0.02	0.31±0.004**	0.06±0.003	0.6±0.01**	0.37±0.01**
Lead & Vitamin C	1.26±0.02	0.23±0.01	0.05±0.003	0.41±0.01**	0.24±0.01**

Values are means of 8 replicates

** : Significantly difference from control at P≤0.01.

Table 4. Effect of lead and vitamin C, singly and in combination, on serum LH level (mlU/ml) of male rats (mean±S.E). Values are means of 8 replicates.

Treatment	LH level (mlU/ml)
Control	6.68±0.09
Lead(2%)	5.68±0.13**
Vitamin C (100mg/kg)	7.18±0.13*
Vitamin C & Lead	6.00±0.11**

Values are means of 8 replicates

** : Significantly difference from control at P≤0.01.

Discussion

Lead, a ubiquitous environmental contaminate, continues to represent a serious health concern. In the present study, the body gain of the rats given lead was less than that of control rats. It has been suggested that the growth depression induced by lead involved a depression of appetite rather than reduced release of growth hormone or thyroxine (Hammond *et al.,* 1989). It has been found that postnatal lead exposure is negatively correlated with infant growth rate (Shukla *et al.,* 1991).

Animals treated with lead (2%) in our experiment, showed a significant decrease in the weight of the seminal vesicles and prostate gland relative to body weight. On the other hand, the weights of the testes, vas deferentia and epididymes were not affected by lead. Pinon-Lataillade *et al.* (1995) reported similar results in mice exposed to 0.5% lead acetate in drinking water for 60 days. Sokol *et al.* (1985) found that rats treated with 0.1% or 0.3% lead acetate for 30 days had significantly lowered the prostate weight, but the weights of the testes and seminal vesicles were not affected by lead. In this study, the rats were treated with 2% lead acetate because the previous report indicated that the exposure of the animals to low doses of lead acetate for long time periods did not affect the structure of the testis (Bosclo, *et al.,* 1988).

The sperm motility and count significantly decreased in the rats subjected to lead. Moreover, sperm abnormalities were elevated in the lead-treated rats. Decreased sperm counts have been demonstrated in male subjects occupationally exposed to lead (Lancranjan *et al.,* 1975;

Lerde, 1992). Clinical and animal studies indicate that abnormalities of spermatogenesis result from toxic lead exposure (Petrusz *et al.,* 1979; Eyden *et al.,* 1978). Male occupational exposure to lead on a long-term basis is reportedly linked with fertility reduction (Lancranjan *et al.,* 1975). This has been correlated to increased frequency of asthenospermia, hypospermia or teratospermia.

Some seminiferous tubules showed degenerative changes and arrest in spermatogenesis due to treatment of rats with lead. Pinon- Lataillade *et al.* (1995) reported that the exposure of mice to 0.5% lead acetate in drinking water showed no significant influence on testicular histology. Moreover, male fertility was not affected.

The treatment of rats with 2% lead in drinking water for six weeks resulted in a significant decrease in LH level. Sokol *et al.* (1985) reported that the exposure of rats to 0.3% lead in drinking water had no significant effect on LH level. A similar result was observed in rats treated with 0.6% lead (Sokol, 1990). Gonadotropin releasing hormone was significantly lower in male monkeys exposed to lead (Foster *et al.* 1993).

In the present study, the treatment of rats with lead and vitamin C reduced many deleterious effects of lead on the male reproductive system. Vitamin C exerts a protective effect against cadmium chloride induced alteration in heme biosynthesis (Somashekharaiah and Prasad, 1991). Some vitamins play a protective role against heavy metals through interference with intestinal absorption (Pace and Iannucci, 1994). The important mechanism of the acute toxic effects of lead compounds is owing at least in part, to metal-catalyzed peroxidation of lipids (Yiin and Lin, 1995). Vitamin C inhibits lipid peroxidation (Rifici and Khachadurian, 1993) which results in many deleterious effects on the tissues. One of the possible mechanisms by which vitamin C antagonizes the effect of lead is inhibition of lipid peroxidation.

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الدور الوقائي لفيتامين (ج) في الحد من التأثير الضار للرصاص على المناسل الذكرية في الجرذان

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المستخلص: أظهرت الدراسات أهمية المواد المضادة للأكسدة (ومنها فيتامين ج) في حماية الجسم من كثير من الملوثات. ويهدف هذا البحث إلى دراسة التأثير الوقائي لفيتامين (ج) ضد أثر الرصاص على مناسل ذكور الجرذان. وقد أدت معاملة الحيوانات بخلات الرصاص (2%) لمدة ستة أسابيع إلى انخفاض حركة الحيامن وكذلك عددها الكلي في البربخ، ،انخفض وزن الحويصلات المنوية وغدة البروستات، بينما زادت نسبة الحيامن غير الطبيعية، وكانت نسبة تشوه رؤوس الحيامن أعلى من نسبة تشوه الذيول. وبالإضافة إلى انخفاض مستوى الهرمون الليوتيني أوضحت النتائج بعض التغيرات النسيجية في الخصى المعالجة بالرصاص. وبالمقابل، فقد قلل فيتامين (ج) من التأثيرات الضارة للرصاص على المناسل الذكرية.