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Ascorbic Protects Testicular Oxidative Stress and Spermatozoa Deformationsin Male Swiss Mice Exposedto Lead Acetate

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Abstract:

Lead is the known environmental contaminant adversely affecting the male reproductive system in human and experimental animals. The cytotoxic effects of lead on male reproductive system involve the production of reactive oxygen species (ROS) and oxidative damage in tissue. The higher quantities of ROS in testicular tissue detrimentally affected developing germ cells so as to produce verities of morphological abnormal sperm and finally reduced sperm count. The present study shows that lead exposure reduce testes weight and increase lipid peroxidation in the testes of mice in comparison to control. The sperm counts were significantly reduced in number. Importantly, lead treated mice show deformations in sperm morphology and testicular injuries. Supplementation of vitamin C daily to lead treated mice increase testes weight and partially protects testis from oxidative stress. In addition, vitamin C causes an increase in sperms count and significantly lowers the morphologically deformed sperms population. We conclude that taking daily consumption of vitamin C could be useful in lowering oxidative stress and sperm deformations in male mice exposed to lead.

Keywords: Lead Acetate, Sperm Count, Sperm Morphology, Testes, Vitamin C.

1.0 Introduction:

Reproductive hazards from metal exposure in males are one of the fastest growing areas of concern in toxicology today. Exposure to different heavy metals like lead, cadmium and mercury causes irreversible toxic insult to male reproductive system and produce cellular impairments at structural and functional level and can generate a big variety of harmful effects on cells, tissues, or organs (Institoris*et al*., 2001). Accumulated data suggest there is a close relationship between declining reproductive health and environmental pollutants like lead. Lead is a heavy soft metal, occurs in nature as an oxide or salts. Lead is a ubiquitous environmental and industrial pollutant that hasa long environmental persistence and never losses its toxic potential (Bonde*et al.,* 2002)**.** Lead acetate elicits toxic pathological changes in the testes, leading to atrophy of the organ (Saxena*et al.,* 1986). Seminal cytology of lead intoxicated animals normally depicts asthenospermia, hypospermia, teratospermi and remarkable changes in sperm count (Bell and Thomas, 1980). Lead-induced hypofertility, particularly, among the workers of lead factories, has been thought to be due to the direct toxic effects of lead on male gonads (Bauchinger*et al.,* 1976). Reproductive dysfunction by lead has distinct morphological and biochemical features such as disorganized epithelia, decrease sperm quality, and alter sperm morphology, and low androgen levels (Hsu *et al.,* 1997).Like all other heavy metals, lead is known to induce oxidative stress in testes due to its higher lipid content (Quinlan *et al.,* 1988) which is extremely damaging to cells and exerts its devastating effects by directly damaging cellular proteins, lipids, and DNA (Bartsch and Nair, 2000). Moreover, lead ions are believed to decrease the fidelity of DNA synthesis and affecting normal cellular signaling and gene regulation (Sirover and Loeb, 1979). In addition, lead deposits in many organs as kidney (Loumbourdis, 2003) ovary (Taupeau*et al.,* 2001) liver (Galleano and Puntarulo, 1997) brain (Schwartz *et al.,* 2002) blood (Mishra *et al.,* 2003) and endocrine system (Gorbel*et al.,* (2002). Accumulated evidence have revealed that testicular physiology which is basically characterized by spermatogenesis process, gets disrupted, at least

in part, by oxidative stress mechanisms (Koizumi and Li, 1992).

The damaging effects of oxidative stress are believed to be nullified in part by a variety of cellular antioxidant vitamins (Heffner and Respine, 1989). Vitamin C is known to be protective anti-oxidants. They cause the inhibition of peroxidation, mopping up of free oxygen radicals and disorganization and breakage of peroxidation chain reactions by an inhibition of gluthathione peroxide, Protein Kinase C (PKC) and calcium metabolism (Das & King, 2007), thus resulting in the blockade of oxidative mechanisms (Murray *et al.,* 2000). Furthermore, Vit.C has long been known to participate in spermatogenesis process of rodents and ameliorate oxidative stress related to testicular impairments in animal tissues (Steinberger and Steinberger, 1966). Extraneous antioxidant therapy has proved fruitful in improving sperm quality in smokers and male infertile patients (Dawson *et al.,* 1990).Vitamin C intake was associated with semen quality in human (Eskenazi*et al.,* 2005), in rabbit (Yousef*et al.,* 2003) and in rat (Sonmez*et al.,* 2005). Moreover, dietary ascorbic acid was found to protect against endogenous oxidative DNA damage in human sperms (Fraga*et al.,* 1991).In view of above findings, I have hypothesized that effect of lead intoxication on sperm might also have an impact on ascorbic acid level in the testes. Till date there is no evidence elucidating the role of ascorbic acid correlating the effects of lead toxicity on male reproductive function. Thus the present study was undertaken to evaluate the effects of lead induced oxidative damage on mice testes and their possible protection with ascorbic acid.

2.0 Material and Methods:

Healthy adult male swiss albino mice (*Musmusculus*) weighing 35 to 40 g. were used for the experiment. Animals (80 to 90 days) were maintained under standard laboratory condition and provided them balance diet and water ad-libitum daily. Animals were divided into control, experimental and recovery groups. The control group was given vehicle only. The experimental groups were given lead acetate (1.25 mg/kg) daily for 45 days by gavage (0.2 ml/animal). The recovery groups received lead acetate (1.25 mg/kg) and Vitamin C (2 mg/kg) for the same period by the same route. The treatment duration of 45 days was selected as the length of the complete spermatogenicepididymal maturation cycle in mice.

2.1 Sperms count and detection of sperms abnormality:

Animals of all groups were sacrificed after their respective treatment and sperm samples were collected from the vas deferens, and appropriately processed for the study of sperm counting and sperm abnormality testes as recommended earlier (Hollarden and Fredrick, 1978). Thesperm suspension was prepared and centrifuged at 1000 rpm for 1 min. Sperm smears were drawn on clean and grease free slides, dried overnight and then stained with 10% Giemsa diluted with sorresons buffer (pH 7.0) for one hour and subsequently observed. Morphologically abnormal sperm were recorded as per Wyrobek and Bruce (1975). Percentage of sperm abnormality was calculated by scanning abnormal sperm. The same suspension was used for sperm counting using a haemocytometer.

2.2 Histological study:

Animals of all groups were sacrificed after their respective treatment and the testes were dissected out, free from other accessory tissues and testes were fixed in Bouin's fixative, embedded in paraffin and 5 mμ thick sections were stained with routine hematoxylin-eosin. Histological changes in the testes were examined under optical microscope.

2.3 Lipid peroxidation determination (LPO):

The tissue was homogenized using a manual homogenizer. The unbroken cells were removed by centrifugation at 3,000 rpm for 10 min and the obtained supernatant was used for the estimation of lipid peroxidation level. This assay is based upon the reaction of thiobarbituric acid (TBA) with malondialdehyde (MDA). Values were expressed as nanogram of MDA/gm tissue (Kartha and Krishnamurthy, 1978).

2.4 Morphometric study:

Diameter of different developing cells of spermatogenesis was carried out from 5 perfect transversely cut tubules of spermatogenesis from each testis of respective groups. Seminiferous tubules diameter were determined at 400X magnification by ocular micrometer. The measurement of diameter of spermatogonia, primary and secondary spermatocytes, spermatids, sperm, sertoli cells and interstitial cells were measured by ocular micrometer at 600X.

2.5 Statistical analysis:

The data of tables were expressed in Mean±SEM and were evaluated for statistical significance with the student " t" test .

3.0 Results and Discussion:

3.1 Body and Testes weight:

There were no significant difference in the body weight of mice in control and experimental groups. Testicular weight significant decreases (P<0.05) in LA treated group compared to control while vit. C supplemented group did not effect on treatment with lead acetate (Table 1).

3.2 Morphometric analysis:

The diameter of seminiferous tubules and all cellular elements of spermatogenesis were significantly(P ≤ 0.001) reduced in LA treated group. In contrast, LA injected mice supplemented with Vit. C encountered significantly greater diameter of spermatogenic cells than LA treated group (Table 1).

3.3 Lipid Peroxidation:

Results of the present study emphasizes a sharp and significant rise (P ≤ 0.001) in LPO content in the testicular tissue of LA treated mice compared to vehicle control group. In the LA + Vit.C group, the levels of LPO in the studied tissues were significantly reduced compared to LA-groups, but the parameter did not come down to the control level (Table 2).

3.4 Sperm Abnormality and Sperm Count:

The analysis of sperm samples from LA treated mice depicted various types of sperm shape morphologies, which included hook-less, lance-shaped, banana- shaped, flower like, balloon shaped (amorphous sperm). In addition double-headed sperms were found with two normal and sometimes abnormal heads joined to a common-mid-piece. Furthermore, certain sperms were clearly double tailed. Occurrence of abnormal sperm population (P ≤ 0.001) and sperm counting profile significantly increases (P ≤ 0.01) in LA treated mice as compared to control. In contrast, LA injected mice supplemented with Vit. C encountered significantly decreased percentage of sperm abnormality and sperm count compared to LA treated mice (Table 2).

3.5 Histological Study:

Testes of control mice exhibited compact appearance of seminiferous tubules and other spermotogenic cells inside them (Fig.A) while in treated mice testes showed much pronounced changes in all the testes constituents. The seminiferous tubules were disorganized and highly vacuolaied. Germ cells were sloughed off from the seminiferous epithelium and were located in the luminal region of the tubules. Most of the seminiferous tubules of testes in this group showed complete absence of spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa and loss of spermatogenesis process. The Sertoli cells and interstitial cells were damaged (Fig.B). Administration of vitamin C protected the testis of mice exposed to LA as evidenced by appearance of normal structures of seminiferous tubule of testis (Fig.C).

Table 1: Body weight (g), Testes weight (mg) , diameter of seminiferous tubules and different spermatogenic cells of Control and Experimental Animals.

Parameters	Control	Treated (LA) for 45 days	Treated (LA+Vit.C) for 45 days.
Body weight (g)	38.8 ±1.5	43.6± 1.3^{NS}	40.3± 1.6^{NS}
Testes weight (mg)	123.5 ± 4.2	112.3 ± 3.2*	125.4 ± 5.2^{NS}
Seminiferous tubules	8.85±0.01	6.65±0.05***	7.45±0.07**
Spermatogonia	0.45±0.06	0.30±0.03***	0.38±0.06**
Primary spermatocyte	0.39±0.05	0.25±0.02***	0.33±0.06***
Secondary spermatocyte	0.38±0.07	0.24±0.1***	0.35±0.04**
Spermatids	0.35±0.08	0.24±0.04***	0.26±0.08^{NS}
Sperm	0.34±0.01	0.23±0.02***	0.30±0.06**
Seetoli cells	0.40±0.07	0.25±0.03***	0.32±0.06**
Interstitial cells	0.38±0.05	0.24±0.02***	0.32±0.06*

All values are expressed + SEM, Significant level, NS= Non significant, * = (P<0.05), ** = (P <0.01), *** = (P<0.001)

Table 2: Effect of Vitamin C (2 mg/kg) on LPO on the basis of TBA-Rs, Sperm Count (×10^6) and Sperm abnormality (%) in Lead Acetate (1.5 mg/kg) treated mice.

Parameters	Control	Treated (LA) for 45 days	Treated (LA+Vit.C) for 45 days.
Lipid peroxidation (nmoles MDA formed/100mg	0.210 ± 0.08	0.292 ± 0.21***	0.269 ± 0.17**
Sperm abnormality	2.37 ± 0.29	9.5 ± 1.01***	6.3 ± 0.08*
Sperm count (million /ml)	22.54 ± 1.04	13.7 ± 0.81**	15.07 ± 0.06**

Significant level, NS= Non significant, * = (P<0.05), ** = (P <0.01), *** = (P<0.001)

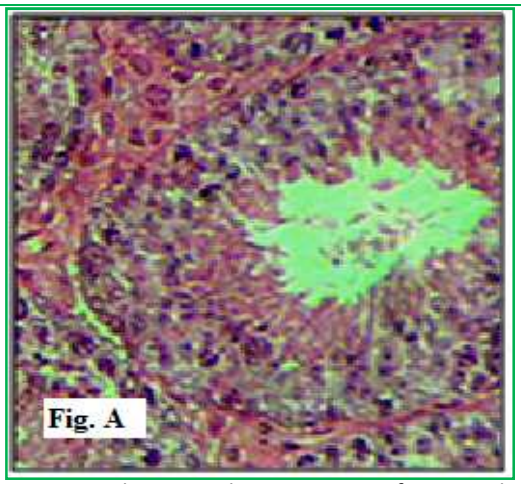

Fig. A: Control group showing, seminiferous tubules with normal spermatogenic cells and mature sperms inside the lumen of the tubules and interstitial tissue in between them.H and E X 400.

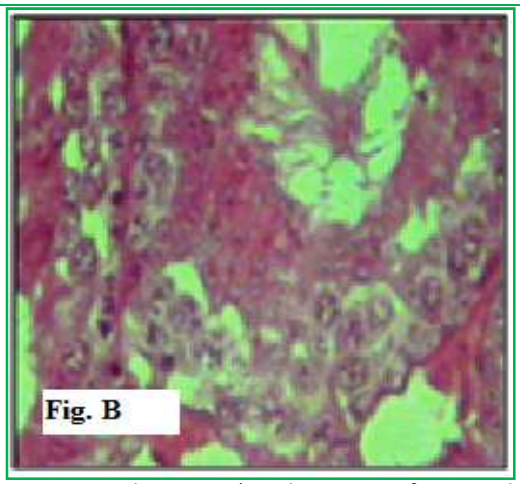

Fig. B: Treated group (Lead acetate for 45 days) showing highly vacuolaied tubules andbunch of abnormal sperms inside the tubules with deeply stained pyknotic nuclei of otherdevelopingspermatogenic cells. H and E X 400.

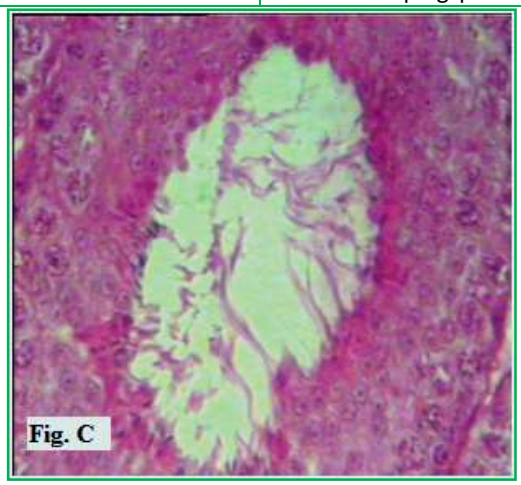

Fig C. Recovery group (LA+Vit.C for 45 days) showing compact appearance of seminiferous tubules with different developing cells of spermatogenesis. H and E X 400.

Oral administration of lead acetate for 45 days to adult male mice resulted decrease in testicular weight and reduce diameter of cellular elements inside the testes and increased lipid peroxidation potential in the testes of the treated mice. Since, in general, elevated LPO is considered as an indicator of oxidative stress (Kappus, 1985 and Janero, 1990), the significantly increased LPO in the lead treated mice compared to the untreated controls demonstrates increased oxidative stress generated in the present experiment. The higher membrane lipid content of testes is presumed to make them more vulnerable to oxidative stress (Georgiou et al., 1987). In addition, Leydig cell, mitochondria and microsomes of testes are known to contribute significantly to an increased production of ROS. Moreover, lead ions are capable of generating and promoting free radical reaction to different extent in different tissues (Acharya and Acharya, 1997).

A significantly reduced sperm count in lead treated mice taken at 45 days after the treatment clearly shows the elimination of germ cells at different stages of development and points to damaging ROS generated through lead catalysis. In fact, oxidative damage to polyunsaturated fatty acids of cell membranes has long been considered to result in the impairment of membrane fluidity and permeability leading to the efficient damage of germ cells, spermatozoa and mature sperm (Roy and Rossman, 1992). Hence, the observed sperm count decreased in the lead treated mice may be due to direct interaction of ROS with the sperm cell membrane. The present finding is also consistent with previous studies (Acharya*et al.,* 2002).

Sperm abnormalities due to chemical mutagens are well documented (Wyrobek and Bruce, 1978). In light of this, the preset chemical, lead acetate has been considered as a potent mutagen causing formation of abnormal male germ cells population at higher doses (Hollarden and Fredrick 1978). However, the observation of the present study with a low dose of lead acetate contradicts this view. It is further stated that various chemical mutagens alter specific gene loci in chromosomes producing morphologically deformed sperm populations (Wyrobek*et al.,* 1983). Recent studies, however, indicate that various species of ROS generated through metal catalysis potentially interact with gene strands causing mutations, thereby inducing changes in sperm morphology (Roy and Rossman, 1992; Hsu *et al.,* 1998; and Bench *et al.,* 1999). Therefore, the present mechanism of abnormal production of sperm and reduced sperm count may be an oxidative stress dependent phenomenon induced by lead catalysis. Testicular histology in this study exhibited severe cellular damage in spermatogenic cell. These observations acts as an indicator that the maturation of spermatogonia through the process of meiosis has been severely distrupted following lead exposure. The observations of the present work are basically in agreement with those of previous investigations conducted on the testes of some mammalian models. The testicular tissue of albino rats exposed to lead acetate through intraperitoneal inoculation presented similar histological changes (Ahmad *et al.,* 2003). In the present study, Vit.C supplemented group associated with a significantly higher sperm count and lower percentage of abnormal sperm population along with a concomitant lower testicular LPO, compared to the lead treated mice group. Vit.C is one of several antioxidative vitamins, which has been postulated to minimize testicular cytotoxic effect in animals treated with pesticides, chemical mutagens, xenobiotics and metals (Hsu *et al.,* 1998; Fraga*et al.,* 1991; Khan and Sinha 1996). The exact mechanism of the protective action of Vit.C against oxidative stress induced cytotoxicity is not understood properly. However, it is believed that it interferes with the intestinal absorption of the trace metals, increases the urinary excretion, and/or creates a synergic effect with the chelator element (Pace andIannucci, 1994). Moreover, vitamins C is a nutritive anti-oxidant, which can neutralize the free radicals generated by heavy metals, and react directly with the peroxides, and executes an important antioxidant function as regenerating the reduced form of GSH (Sies, 1986; Well and Xu 1994). Alternatively, Vit.C as a part of a redox buffer system, can effectively scavenge harmful ROS (Dawson *et al.,* 1990). Such antioxidative action of Vit.C could relieve the male germ cells from oxidative damage, thereby increasing sperm count, decreasing the percentage of abnormal sperm population and prevent the cytotoxic effect of lead acetate.

4.0 Conclusions:

We can conclude that the reactive oxygen species generated by lead is responsible for the testicular dysfunction and affecting the male reproduction,

with decrease in sperm count and abnormal sperm population, resulting in poor fertility outcomes. The beneficial effects of oral supplementation of antioxidant vitamin in testicular dysfunction are also very promising. Future studies on animal models will provide novel information on the safely and effectiveness of antioxidant vitamins in improving the male fertility. Antioxidant vitamin C plays an important role in abating many reproductive hazards of lead. Vitamins are capable of protecting male germ cells at every stage of their development and in the improvement of various factors related to fertility.

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