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Original Research Paper

Effect of Ascorbic Acid on Lead Nitrate Induced Oligospermia in Albino Rats

Medical Science

KEYWORDS : Lead nitrate, Sperm count, Vitamin C.

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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. Higher quantity of ROS in testicular tissue detrimentally affected the developing germ cell, which result in decrease sperm count. Supplementation of vitamin C causes partial recovery from oxidative stress and also causes increase sperm count. Five groups of animals were taken for the study i.e. control and four experimental. After 60 days of experimental period, rats were sacrificed and the testis along with epididymis was taken out for obtaining sperms. Sperm counting was done using Neubauer chamber. Sperm count was found to be decreased in lead nitrate treated groups as compared to control and found improvement in recovery group.

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). Lead is a ubiquitous environmental and industrial pollutant that has a long environmental persistence and never loses 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 spermcount (Bell and Thomas, 1980).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). 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).

Material and methods

The present study was conducted in the Department of Anatomy, King George's Medical University, Uttar Pradesh, Lucknow, India. Thirty male albino rats weighing 150-250 gm were taken. Animals were obtained from animal house of Indian Institute of Toxicology & Research, Lucknow. The rats were maintained under standard laboratory conditions in an air conditioned room and housed in polyethylene cages at temperature 22±3°C and relative humidity 30–70%. They were fed with standard pellet diet and water ad libitum. Animal care was as per Indian National Science Academy (INSA) guidelines for Care and Use of Animals in Scientific Research. The study protocol was approved by the Institutional Animal Ethical Committee (IAEC). After acclimatization for 2 weeks in laboratory conditions, animals were divided into 5 groups of 6 rats each. Group 1 was control, group 2 received low dose lead nitrate (40 mg/kg bodyweight), group 3 received low dose lead nitrate (40 mg/kg body weight) and vitamin C (200mg/kg body weight), group 4 received high dose of lead nitrate (80 mg/kg body weight), group 5 received high dose of lead nitrate (80 mg/kg body weight) and vitamin C (200mg/kg body weight) 6 days a week for 2 months. After 2 months, animals of all the five groups were anaesthetized by intraperitoneal administration of Nembutol (30 mg/kg body weight). Rats were sacrificed and testes were taken out along with epididymis. Epididymis was separated from the testis and Sperms were obtained by mincing the epididymis in normal saline and filtering the suspension through nylon mesh.

Method of sperm count

The epididymal sperm were counted by using Neubauer chamber. Normal saline containing epididymal sperms was drawn to the 0.5 mark of a WBC pipette. Then the semen diluting fluid (soda bi-carb) was drawn to the 11 mark and mixed well. The improved Neubauer chamber was loaded and the sperms were allowed to settle for about 5 minutes. Then sperms were counted in the four corner squares.

$$\text{Sperms/ml of semen} = \frac{\text{Sperms counted in 4 squares} \times 10 \times 20 \times 1000}{4}$$

Result:

Sperm count

It was observed that mean value of sperm count in group 1 (C-control) was (8.15±4.29) which was higher in comparison to the mean values of group 2 (L1-Low dose lead nitrate) (0.92±0.42), group 3(L2-Low dose lead nitrate +Vit C) (1.65±0.93), group 4 (H1-high dose lead nitrate) (0.38±0.16), and group 5 (H2-high dose lead nitrate +Vit C) (1.70±0.76) (Table 1, Fig 1).

Mean sperm count was found to be maximum in Group C ($8.15\pm4.29 \times 10^6$) and minimum in Group H1 ($0.38\pm0.16 \times 10^6$). Minimum sperm count in a specimen was observed to be 0.20×10^6 (Group H1) and maximum as 2.90×10^6 (Group H2). Analysis of variance revealed a significant intergroup difference ($p<0.001$).

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Table 1: Sperm count in different groups (x10^6)

SN	Group	Mean	SD	Min	Max
1.	Group-1 C	8.15	4.29	2.80	14.40
2.	Group-2 L1	0.92	0.42	0.40	1.60
3.	Group-3 L2	1.65	0.93	0.40	2.80
4.	Group-4 H1	0.38	0.16	0.20	0.60
5.	Group-5 H2	1.70	0.76	0.80	2.90
F		7.362			
"p"		<0.001			

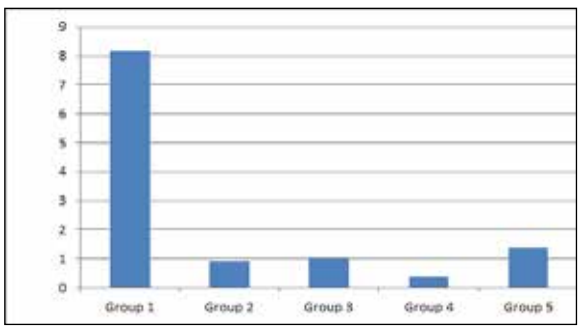

Fig.1

Discussion:

Sperm count

Sperm count is one of the important parameter for testing testicular function. Most of the time histopathology of testis is not feasible, therefore this is a direct method for assessing reproductive capability for an individual. Any factor which is involved in altering the homeostasis of reproduction biology will lead to alteration in final output of number of sperms.

Leiva *et al* (2011) was in opinion that reduction in epididymal sperm number and daily sperm production in male rats treated with lead acetate may be due to that lead acetate administration inhibits spermatogenesis by reducing the length of the stages related to spermiation and onset of mitosis. In our experiment the sperm count was found to be significantly decreased following administration of low and high doses of lead nitrate *i.e.* group II and group IV and we observed improvement in recovery groups *i.e.* group III and in group V. This decline in sperm count is due to the genotoxic activity of lead. Looking in to the histopathology of testis, it was clearly evident that lead nitrate is capable of reducing the number of sperms via their effect on germinal epithelium most likely by disrupting hormonal regulations, mostly via the Hypothalamic- pituitary axis, and then reduces sperm production in seminiferous tubules of the testes. Several tubular sections were also seen devoid of spermatids. **Thomas and Brogan (1983), Lancranjan *et al* (1975), Roy Chowdhury *et al* (1986)** observed that lead has an adverse effect on sperm count and retarded the activity of alive sperms. **Roy Chowdhury (2009)** reported the same finding on lead administration. **Grace *et al* (2004)** observed that there was decrease in sperm count after lead chloride treatment for three days but in the recovery group *i.e.* group treated with lead chloride for three days followed by 32 day recovery period, no difference was observed. **Acharya *et al* (2003)** found significant decrease (P≤0.001) in sperm counts in lead-treated mice during post-treatment phase with respect to control. **Naha *et al* (2005)** worked on persons working in lead acid battery factory and he found significant (P<0.001) reduction in sperm count. **Wadi *et al* (1999)** observed that low dose of lead significantly reduce the number of sperm within the epididymis, while high dose reduce both the sperm count and percentage of motile sperm. **Tohamy *et al* (2010)** who also noticed that inorganic lead impaired the male reproductive function by reducing the sperm which was ameliorated by giving Vitamin C.

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