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RESEARCH ARTICLE

GESTATIONAL AND NEONATAL LEAD EXPOSURE ON SPERMATOGENESIS IN SWISS MICE

[1]Manoj Kumar Panda, [2]Sunita Das and *,[3]Acharya, U. R.

[1]Lecturer in Zoology, U. P. Science College, Sheragada, Ganjam, Odisha
[2]M.Phil. Scholar, Dept. Of Zoology, Berhampur University, Bhanja Bihar, Ganjam, Odisha
[3]Retd. Professor of Zoology, Berhampur University, Bhanja Bihar, Ganjam, Odisha

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ABSTRACT

Lead (Pb) is a potential heavy metal of Group IV B of the periodic table which is soft, malleable metal having bluish white colour. Besides having its useful properties, it causes the reproductive abnormality in male Swiss mice. Testis is the important organ of reproductive failure caused by the accumulation of lead that develops reactive oxygen species that results oxidative stress in the tissues of organism. In the present study, 0.2% of lead acetate in drinking water was given to the mother from the first day of pregnancy and was continued up to lactation phase. At the end of lactation phase, the male pups were separated and after attaining sexual maturity at 9-10 weeks of age, the male pups were sacrificed and the testes were processed for the estimation of various biochemical parameters. Exposed animals showed significantly decrease in the level of antioxidant enzymes like catalase and peroxidase, decreased sperm count and markedly increased rates of sperm abnormality. Oxidative stress was measured in terms of malonicdialdehyde content of lipids. However, antioxidant vitamins like vitamin C, vitamin E and its combined action i.e. Vitamin (E+C) to the lead-induced mice groups could ameliorate the oxidative stress by declining lipid per-oxidation, increasing the level of catalase and peroxidase, increase in sperm count and reduce sperm abnormality.

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INTRODUCTION

Lead is a potential heavy metal of Group IV B of the periodic table. It is a soft, malleable metal bluish white in colour used for the preparation of acid, batteries and in the preparation of medicines to cure some diseases (Gould *et al.,* 1937; Thompson 1967, Vanthoor, 1968 and Moddel, 1977). Occupational mining workers have a high risk of being contaminated with leaded particles and dust mainly through inhalation and intake of food (Skie *et al*., 1972). Specifically, human male reproductive organs have marked capacity to accumulate lead (Quintanilla vega *et al*., 2000). Tremendous deleterious effects have been recorded in the testes as a result of lead intoxication in animals (Ghalberg1981, Wibe *et al*., 1982, Chowdhury *et al*., 1986 and Acharya and Mishra, 1995). The seminal cytology in lead exposed animals normally depicts asthernospermia, hypospermia, teratospermia and remarkable changes in sperm count, morphology and mortality (Bell and Thomas, 1980). Lead induced toxicity in the testes is due to generation of Reactive Oxygen Species (ROS) (Hsu *et al*., 1998; Mariola *et al*., 2004, Wang *et al*., 2006) which are reported to damage the polyunsaturated lipid membrane of cells and sub-cellular particles (Dobroestov *et al*., 1977).

Corresponding author: **Acharya, U.R.**
Retd. Professor of Zoology, Berhampur University, Bhanja Bihar, Ganjam, Odisha

Therefore, lead exposure resulted in increased oxidative stress by increasing lipid peroxidation products. Lipid peroxidation is an index to measure damage caused to biomembranes (Hussain *et al*., 2001). Metal induced ROS are known to degrade the membrane of the spermatogonial cells by causing significant reduction in sperm count that linked to functional impairment of testes and defective spermatogenesis. Abnormal sperm production occurs due to mutation in certain gene loci inducing errors in the morphology of sperm. Experimental evidences demonstrate that lead can cross the placenta where it competes with other ions for transport proteins (Semezuk and Semezuk-Sikora, 2001). During infancy, breast feeding also can be source of lead exposure which is mobilized from bones to milk (Corpas *et al*., 2002). To protect the cell form oxidative injury, aerobic organisms are equipped with both enzymatic antioxidant defences which potentially neutralise ROS and protect the cells from oxidative stress. Among the enzymatic antioxidants, superoxide dismutase (SOD), catalase and peroxidase are important which can neutralise the noxious oxygen radicals and hydrogen peroxide, thus, can protect the cells from oxidative injury. Catalase causes the decomposition of hydrogen peroxide to molecular oxygen and water (Chance *et al*., 1982). Considering the above information, the present study has been undertaken to study the process of spermatogenesis at the spermatogonial stage in the foetus which are exposed to lead from the mother during gestation and during lactation phase. Results of the proposed study will also emphasize the transfer channelization of lead through placenta and also through mother's milk. It will also prove the noxious effect of lead and its ROS in adversely affecting spermatogenesis is Swiss mice.

MATERIALS AND METHODS

Test Animal

For the present study, Swiss albino mice (Mus musculus) with 15 to 25 gm bodyweight were procured from a commercial farm, Ghosh Enterprises, Kolkata and then, acclimatized in animal house and temperature was maintained within 26^{o}C +2^{o}C. The mice were regularly fed with balanced diet and tap water was provided to the animal *ad libitum*. After a week of acclimatization the mice were chosen for the proposed study.

Experiment protocol

The first group of female mice, which served as the experimental groups, 0.2% of lead acetate in drinking water was given to the mother from the first day of pregnancy and was continued up to lactation phase. The total exposure period to lead acetate was approximately six weeks (three weeks during gestation and 3 weeks during lactation). At the end of lactation phase, the male pups were separated and were reared separately with normal water and balanced diet till they attain sexual maturity. At 9-10 weeks of age, the male pups were sacrificed and the testes were removed and processed for biochemical parameters like lipid peroxidation and protein. Enzyme activity of two antioxidant enzymes like catalase and peroxidase were also analysed. For sperm count and sperm abnormality studies from vas deferens were collected and processed following standard procedures (Wyrobeck and Bruce, 1975). The control group of mice i.e. the pregnant females were supplemented with normal water without lead acetate and the whole procedure was followed like that of the experimental. Morphometric indices like body weight of the pups, testes weight of the male mice were recorded in both the control and experiment groups.

Sperm head Morphology and Sperm count assay

Both experimental and control mice were sacrificed by cervical dislocation and the caudal epididymis was dissected out for preparation of sperm sample for the study of sperm count and sperm head abnormalities. The sperm were squeezed out from the vas difference in PBS at room temperature aspirated gently by pasture pipette and left for 5 minutes and was centrifuged for 1 minute at 1000 rpm and the supernatant was discarded. A small drop of sperm suspension was taken on clean grease-free slide smeared gently with a glass rod and left overnight for natural drying. Next day the slides were dipped in distilled water, dried and stained with 10% Giemsa diluted in fresh Sorenson's buffer (pH-6.8) for 1hr, washed in tap water and observed under microscope. About 1000 sperm from each specimen were scanned. Morphologically abnormal sperm were recorded as per (Wyrobeck and Bruce, 1975). For sperm counting, sperm suspension was taken in the haemocytometer and the number of sperm heads counted in R.B.C. counting chamber.

Statistical evaluation

The data generated out of lead acetate treatment in mice for the determination of various biochemical parameters taken for the study, were compared with that of the control values. The significance of the data was verified by student's 't' test as described by Garret (1956). 'P' value at below 0.05 level were considered significant. . The data are reported here as mean ± SEM.

Observation

Lead exposure to female mice during gestation and lactational phase resulted in abnormal spermatogenesis in the male pups indicating the fact that lead ions are channelized through placenta and mother's milk to the foetus during pregnancy and lactation respectively. Lead intoxication in male pups resulted in a significant declines ($p \leq 0.05$) in body weight compared to control (Table - IV & Fig - IV) mice. Similarly in lead intoxicated mice testes weight decreased significantly ($p \leq 0.05$) than controls (Table – IV & Fig - IV). Body weight of the neonatal live babies also declined significantly ($p \leq 0.05$) than the control pups (Table – IV & Fig - IV). Lipid peroxidation potential of the testes increased significantly ($p \leq 0.01$) than the control (Table - I & Fig - I) testes indicating oxidative stress. Testicular protein content also indicated a declined trend than the controls ($p \leq 0.01$), (Table - I & Fig - I). Activity of two antioxidant enzymes like peroxidise and catalase decreased significantly ($p \leq 0.05$), (Table - II & Fig - II) ($p \leq 0.01$) than the untreated controls (Table - II & Fig - II). Sperm count profile of the lead treated mice indicated significant decline ($p \leq 0.001$) than the controls (Table - III & Fig - III). Similarly percentage of abnormal sperm population increased significantly ($p \leq 0.0001$) due to lead treatment over the control mice (Table - III & Fig - III).

Table 1. Lead acetate treatment (0.2%) in drinking water during gestation and lactation and its effects on lipid peroxidation (n moles/gm wet wt. of tissue) and protein content (mg/gm) in the testes of male offsprings of Swiss mice

Value represents ± SEM

Lipid peroxidation		Protein content	
Control	Lead treated	Control	Lead treated
45.62 ± 0.08	72.95 ± 1.52**	552.75 ± 22.18	265.47 ± 26.57**

** P ≤ 0.01

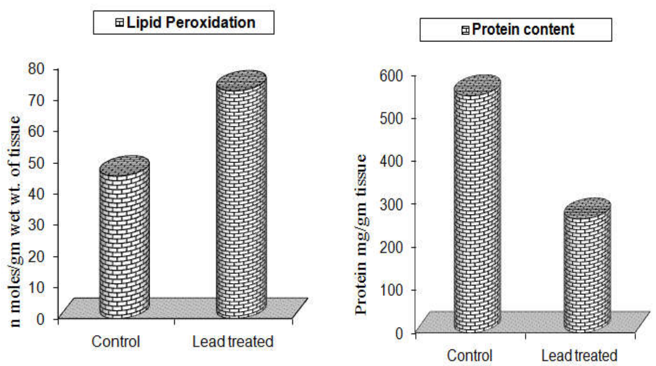

Fig. 1. Lead acetate treatment (0.2%) in drinking water during gestation and lactation and its effects on lipid peroxidation (n moles/gm wet wt. of tissue) and protein content (mg/gm) in the testes of male offsprings of Swiss mice

Table 2. Lead acetate treatment (0.2%) in drinking water during gestation and lactation and its effects on enzymes catalase and peroxidase (Units/mg of protein) in the testes of male offsprings of Swiss mice

Value represents ± SEM

Value represents ± SEM

CATALASE		PEROXIDASE	
Control	Lead treated	Control	Lead treated
44.53 ± 2.38	26.75 ± 2.01**	29.55 ± 1.2	18.68 ± 1.7*

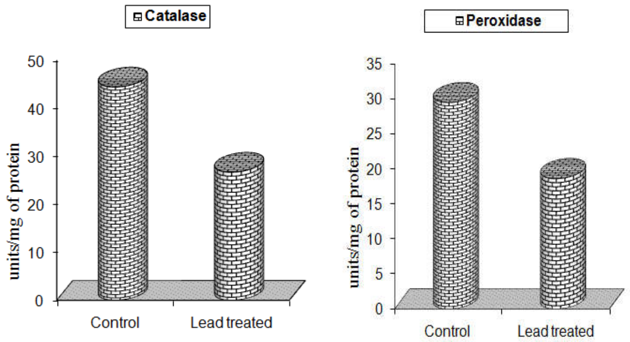

Fig. 2. Lead acetate treatment (0.2%) in drinking water during gestation and lactation and its effects on enzymes catalase and peroxidase (Units/mg of protein) in the testes of male offsprings of Swiss mice

Table 3. Lead acetate treatment (0.2%) in drinking water during gestation and lactation and its effects on sperm count x 10^6 and the percentage (%) of sperm abnormality in the testes of male offsprings of Swiss mice

Value represents ± SEM

SPERM COUNT		SPERM ABNORMALITY	
Control	Lead treated	Control	Lead treated
22.9 ± 0.91	11.2 ± 0.85***	3.7 ± 0.05	46.23 ± 1.51****

*** P ≤ 0.001, **** ≤ 0.0001

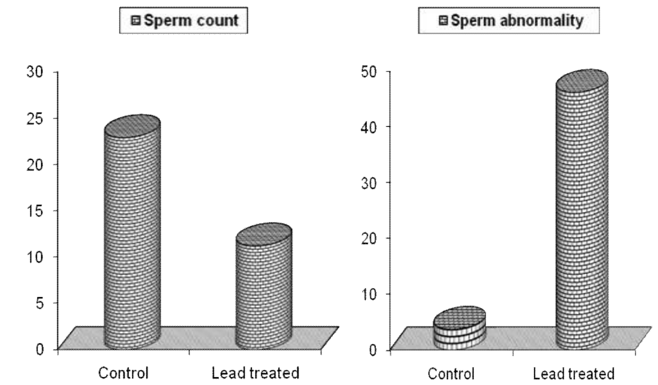

Fig. 3. Lead acetate treatment (0.2%) in drinking water during gestation and lactation and its effects on sperm count x 10^6 and the percentage (%) of sperm abnormality in the testes of male offsprings of Swiss mice

DISCUSSION

Lead has been extensively associated with detrimental effects on the male reproductive system and associated with reduced human semen quality (Telisman *et al*., 2000, Alexender *et al*., 1996). Oxidative stress demonstrated to reduce sperm count significantly and increased population of deformed sperm along with increased lipid peroxidation.

Table 4. Lead acetate treatment (0.2%) in drinking water during gestation and lactation and its effects on body weight (gm) and the testes weight (gm) in the testes of male offsprings of Swiss mice

Value represents ± SEM

BODY WEIGHT		TESTIS WEIGHT	
Control	Lead treated	Control	Lead treated
21.5 ± 0.7	13.2 ± 0.5*	0.252 ± 14.2	0.104 ± 12.7*

* P ≤ 0.05

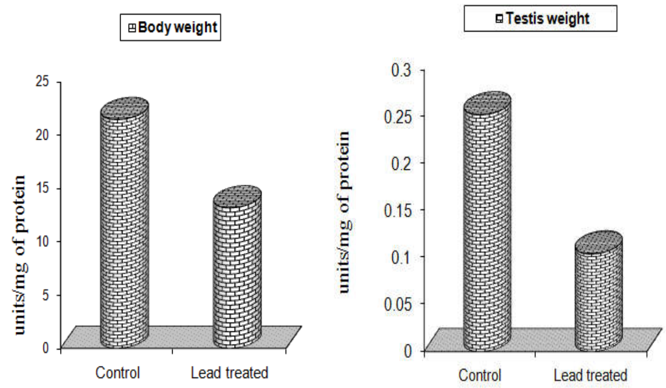

Fig. 4. Lead acetate treatment (0.2%) in drinking water during gestation and lactation and its effects on body weight (gm) and the testes weight (gm) in the testes of male offsprings of Swiss mice

Sperm count decrease possibly is associated with lead induced membrane damage of spermatogonial cells and spermatocytes leading to sperm count decrease (Hsu *et al*., 1998, Mishra and Acharya, 2004). It is due to apoptotic activity induced by lead catalysed ROS which trigger caspase activity in spermatogonial and spermatocyte cells leading to programmed cell death (Wang *et al*., 2006). Increased abnormal sperm population in the present study is linked with chromosomal aberrations due to lead induced ROS that induce structural deformities in spermatogonial stem cells resulting in abnormal sperm population (Wyrobeck and Bruce, 1975). Moreover, lead toxicity depletes cells antioxidant defence system, as indicated in the present study (Gurer and Ercal, 2000). Lead induced free radicals can alter membrane and can inhibit K^+ dephosphorylation, step of Na^+ - K^+ ATPase (Bertoni and Spernkle, 1988). Such protein degradation and/or membrane bound enzymatic activity inhibition are instrumental for membrane degradation resulting in cell damage. Antioxidant enzymes like peroxidase and catalase, in the present study, indicated significant decline in enzyme activity in lead treated mice over the control groups. In the present study an attempt has been taken to investigate the deleterious effects of lead acetate on male reproductive system following prenatal and/or lactational exposure to lead is correlated with a multitude of adverse reproductive effects both in males and females (Mc. Givern *et al*., 1991). Besides male reproductive impairments leading to change in semen qualities due to neonatal lead exposure in mice is reported to be associated with decreased macrophage number in the testes which are crucial in regulating reproductive function in males through regulation of steroidogenesis (Hales, 2002). Recent studies (Sokol *et al*., 2002, Biswas and Ghosh, 2004, Slimani *et al*., 2009) indicate that long term lead exposure alters the level of testosterone, impaired function of Leydig cells of the testes. Our study also

depicts loss of growth in male pups pre-treated with lead compared to control groups. The changes in the nucleo-protamine complex can affect fertility and development of the offspring which results in an increased number of dead pups and tendency for small litter relative to unexposed controls.
From the above study, it is quite evident that lead intoxication in some way or other is very much injurious to the biological systems and hence, its eradication has become a global concern.

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