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EFFECT OF PERINATAL EXPOSURE TO LEAD ACETATE ON TESTICULAR LIPID PEROXIDATION IN ADULT RATS

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ABSTRACT

To asses whether prenatal exposure to lead induces testicular oxidative status at adulthood, pregnant Wistar rats were exposed to an *ad libitum* drinking water containing lead acetate at 0.05% and 0.15% respectively, throughout their pregnancy period. All the animals were allowed to deliver pups. Lead exposure was continued throughout the lactation period. Weaned pups were allowed to grow on normal diet and tap water (free from lead) and on postnatal day 100, animals were sacrificed, testes was isolated and analyzed for lipid peroxidation. Significant increase in the lipid peroxidation levels were observed in the testis of lead exposed rats when compared with controls. Significant decrease in the activity levels of antioxidant enzymes: superoxide dismutase and catalase in the testes were observed in the testis of lead exposed rats. From the results it can be concluded that perinatal exposure to lead induces oxidative stress in the testis of rats at adulthood.

KEY WORDS: **Lead acetate, rat, testis, antioxidant enzymes, lipid peroxidation**

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INTRODUCTION

Lead is a non-essential metal that occurs naturally in the environment. The levels of lead in the environment are constantly increasing due to industrial activities[1]. The contamination of lead is mainly due to its wide usage in mining and smelting of lead ores, gasoline, manufacturing and recycling of batteries, in solders of cans of food or beverages, glazed ceramic[2,3]. The ubiquitous nature and the properties like immutable, non-biodegradable, and bioaccumulation of lead makes it to persist throughout food chain[4] and may pose detrimental effects on human health[5]. Toxicological studies documented that lead can cause adverse health effects on heart, brain, kidney, reproductive tract and also causes chromosomal abnormalities[6,7]. Lead has ability to cross blood-testis barrier and thus the testis and other accessory reproductive organs are vulnerable to lead toxicity[8,9]. The male reproductive consequences of lead include asthenospermia, oligospermia and terratospermia[10] and its accumulation in the testis and/or epididymus affects the germinal cells at different levels of differentiation (spermatogonia, primary spermatocytes, spermatids or spermatozoa)[11]. In recent years, there is a major concern towards the *in utero* exposure to xenoestrogens on developing male reproductive system, since estrogenic compounds have the ability to impair fertility[12]. Lead is also estrogenic compound that may cross blood-placental barrier to affect developing embryo[13,14]. It is also well known that lead crosses the placenta and accumulates in fetal tissues over the period of gestation[15]. Oxidative stress is believed to be one of the major contributors to reproductive disorders[16]. Oxidative stress results from increased lipid peroxidation or decreased intrinsic antioxidant defense in different tissues. It has been shown that lead-induced oxidative stress results in tissue injury via oxidative damage to macromolecules like lipids, proteins and DNA[17]. Recently it has been reported that lead exposed mammals showed the generation of ROS, stimulation of lipid peroxidation and inhibited antioxidant defense system suggesting the oxidative stress is one of the possible mechanisms of action of lead-induced toxicity[18,19]. Reproductive studies demonstrate that low levels of ROS are crucial for sperm functions whereas excessive amounts of ROS may be detrimental[20,21]. It has also been reported that lead induces oxidative stress and affects the testis and epididymis to impair sperm morphology and maturation[22]. Therefore, purpose of the present set of experiments was to evaluate the effect of perinatal (gestational and lactational) exposure to lead acetate on oxidative status in the testis of rats.

MATERIALS AND METHODS

Maintenance of Experimental Animals

Wistar strain rats were purchased from authorized vendor (M/S Raghavendra Enterprises, Bangalore, India). Rats were housed in polypropylene cages (18" X 10" X 8") lined with sterilized paddy husk, and provided filtered tap water and rat food (purchased from HLL Animal Feed, Bangalore, India) *ad libitum* in an air-conditioned environment (25 ± 2°C) with a 12-hour light and 12-hour dark cycle. All the experiments were conducted in accordance with the guidelines for the care and use of laboratory animals[23] and approved by the Institutional Animal Ethical Committee at Sri Venkateswara University, Tirupati, India (Resolution No. 10/(i)/a/CPCSEA/IACE/ SVU/PSR-MRA).

Test Chemicals

Lead acetate was purchased from E-Merck (Bombay, India). All other chemicals used in the study were of the highest purity available and obtained from local commercial sources.

Experimental Design

Pregnant rats were allocated into three groups of ten animals each. Rats in group I served as controls and animals in group II and III were exposed to *ad libitum* drinking water containing lead acetate at 0.05% and

0.15% respectively, throughout their pregnancy period. The lead acetate concentration selected in the present study was similar to the concentrations reported earlier for Wistar rats[24]. All the animals were allowed to deliver the pups. Lead exposure was continued throughout the lactation period. After completion of the weaning period (twenty three days) following birth, the male pups were separated and allocated into colony cages (4 animals per cage). They were maintained on standard rat diet and free access to tap water and maintained up to post natal day 100. Male rats from control and both experimental groups were weighed and sacrificed by cervical dislocation. Testis was immediately isolated and weighed to its nearest milligram by using Shimadzu electronic balance (Model No: BL-220H) and used for biochemical analysis.

Determination of Lipid peroxidation

The levels of lipid peroxidation in the testis was measured in terms of malondialdehyde (MDA; a product of lipid peroxidation) content and determinated by using the thiobarbituric acid (TBA) reagent. The reactivity of TBA is determined with minor modifications of the method adopted by Hiroshi et al[25]. Tissues were homogenized (10% W/V) in 1.15% potassium chloride solution.To 2.5 ml of homogenate, 0.5 ml of saline (0.9% NaCl) 1.0 ml (20%% W/V) trichloroacetic and (TCA) was added. The contents were then centrifuged for 20 minutes on a refrigerated centrifuge at 4000 Xg. To 1.0 ml of supernatant, 0.25 ml of TBA reagent was added and the contents were incubated at 95^{o}C for 1 hr, 1 ml of n-butanol was added to it. After through mixing the contents were centrifuged for 15 min at 4000 Xg in a refrigerated centrifuge. The organic layer was transferred into a clear tube and it absorbance was measured at 532 nm. The rate of lipid peroxidation was expressed as μ moles of malondialdehyde formed /gram tissue.

Assay of Superoxide Dismutase

Superoxide dismutase (EC 1.15.1.1) was assayed in the microsomal fraction according to its ability to inhibit the auto oxidation of epinephrine at alkaline medium Misra and Fridovich[26]. Testes from control and experimental rats were homogenized (10% W/V) in 50 mM ice cold sodium phosphate buffer (pH 7.0) contain 1mM EDTA. The homogenate was centrifuged at 105,000 X g for 60 min. The clear supernatant thus obtained was used to the enzyme activity. The reaction mixture in a final volume of 2.0 ml contained: 0.05 M carbonate buffer (pH 10.2), 30 mM epinephrine (freshly prepared) and the enzyme extract changes in absorbance were recorded at 480 nm, measured at 10 sec internals for 1 min in a spectrophotometer. The enzyme activity was expressed as units/ mg protein /min.

Assay of Catalase

Catalase activity was assayed by the method of Chance and Machly[27]. The reaction mixture in a final volume of 2.5 ml contained: 0.05 M phosphate buffer (pH 7.0) and appropriate amount of enzyme protein. The reaction was initiated by the addition of 19 mM H_2O_2. The decomposition of H_2O_2 was followed directly by measuring the decrease in absorbance at 240 nm in spectrophotometer (Hitachi model, U, 2001). The catalase activity was expressed as μmoles of hydrogen peroxide /mg protein/min.

Protein Content

Protein content in the enzyme source was determined by Lowry et al[28] using bovine serum albumin as standard.

Statistical Analysis

The data were statistically analyzed using one-way analysis of variance (ANOVA) followed by Tukey's test. $P< 0.05$ was considered significant. The data were presented as mean ± S.D. All statistical tests were performed using the Statistical Package for Social Sciences (SPSS) version 16.0.

RESULTS

General Observations

No mortality was observed in rats of control and experimental groups. No clinical toxicity

symptoms and/or behavioral abnormalities were observed in rats of all groups.

Effect of perinatal exposure to lead acetate on malondialdehyde levels in the testis of rats

Table 1 shows the levels malondialdehyde (MDA) in experimental and control testis. The levels of malondialdehyde were markedly increased in the testis of experimental rats when compared with the testis of control rats.

Effect of perinatal exposure to lead acetate on the activity levels of superoxide dismutase (SOD) and catalase in the testis of rats

Significant decrease in the activity levels of SOD was observed in the testis of lead exposed rats over control rats (Table 1). The activity levels of catalase in the testis of lead exposed males were significantly decreased when compared to controls (Table 1).

Table 1

Changes in lipid peroxidation and testicular antioxidant enzymes in adult rats exposed to lead acetate during perinatal period.

Parameters	Controls	0.05% Pb	0.15% Pb
Lipid peroxidation (moles of malondialdehyde formed/g wet wt)	$6.14^{a} \pm 1.32$	$12.71^{b} \pm 2.12$ (107.0)	$14.13^{b} \pm 1.78$ (130.1)
Catalase (μ moles of H_2O_2 metabolized/mg protein/min.)	$0.39^{a} \pm 0.021$	$0.17^{b} \pm 0.03$ (-56.4)	$0.21^{c} \pm 0.02$ (-46.15)
Superoxide dismutase (Units/mg protein/min.)	$0.86^{a} \pm 0.05$	$0.47^{b} \pm 0.03$ (-45.3)	$0.49^{b} \pm 0.07$ (-43.02)

Values are mean ± S.D of ten individuals
Values in the parentheses are percent change from that of control
Mean values that do not share same superscript differ significantly from each other at p< 0.05.

DISCUSSION

The present study clearly demonstrates that gestational and lactational exposure to lead induces testicular oxidative stress as evidenced by increased lipid peroxidation in the testis of rats. It is well known that lead has ability to induce oxidative stress in tissues[29,30]. In the present study, the increase in the lipid peroxidation levels in the testis of lead exposed rats indicates induced oxidative stress. Earlier it was demonstrated that lead induced tissue damage is mediated by oxidative stress[31]. Further, it has been reported that lead affects the testicular functions like steroidogensis and spermatogenesis by inducing oxidative stress (authors' unpublished data) and the results are in consonance with Hamadouche et al[31]. To combat the oxidative stress, testes have developed an array of antioxidant enzymes such as superoxide dismutase and catalase. In general, SOD is the first line of defense against oxidative stress[32] and play a pivotal role in dismutation of superoxide anions to hydrogen peroxide and catalase neutralizes hydrogen peroxides to molecular oxygen and water. In the present study, a significant decrease in the enzymes such as SOD and catalase in the testes of experimental groups indicates a failure of antioxidant defense system. The decrease in these enzymes in lead treated rats clearly postulates improper dismutaion of superoxides and improper decomposition of H_2O_2. Many studies have suggested that in rats, lead-induced oxidative stress results in increased lipid peroxidation products in the testes and epididymis[33]. Earlier studies also demonstrated that lead exposure causes generation of ROS and alterations in the antioxidant defense systems in animals and occupationally exposed workers[34]. In conclusion, our studies indicate that perinatal exposure to lead causes disturbances in the testicular pro- and anti-oxidant metabolism of male rats as evidenced by an elevation in the lipid peroxidation products and decrease in the

activities of superoxide dismutase and catalase.

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