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The Effect of Lead Acetate on the Testes of Male Albino Rats

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ABSTRACT

Lead is the oldest environmental contaminant which is a known abortifacient and spermicidal agent in case of high exposure. The present study was designed to observe the changes in the testes of male albino rat due to excessive and indiscriminate exposure to lead. Lead acetate dissolved in 5 ml of distilled water were administered orally to male albino rat at 0, 25, 50, 75 and 100 mg/Kg body weight of rat in groups A – E, respectively for 28 days with group A rats serving as control. The animals were sacrificed at the beginning and the end of the experiment and testes excised and weighed. Standard histopathological processing, sectioning and mounting of the tissues were done. The histopathological changes that occurred in the testes were mainly; tissue architecture degeneration, necrosis, vacuolization, maturing spermatogenic cells in seminiferous tubules, thickened basement membrane, myoepithelial cell and scanty leydig cells and were dose-dependent. The body weight of the lead exposed rats significantly ($p<0.05$) decreased when compared with the control rats. The average organ ratio of the paired testes of the lead exposed rats decreased significantly ($p<0.05$) with increase in dosages when compared to the control. The testicular sperm count also decreased with increase in dosages throughout the exposure period.

Keyword: Lead toxicity, Albino rats, Testes, Sperm count, Seminiferous tubules

INTRODUCTION

Man is exposed to various types of environmental contaminants at different stages of his life span, of which many of them are harmful. Lead is a heavy soft metal which occurs in nature either as an oxide or as a salt. Lead is a ubiquitous environmental and industrial pollutant that has been detected in every environmental and biological system. It came into use very early in the history of civilization and its poisonous effects were discovered (Imran *et al.* 2003).

Lead is considered as one of the hazardous pollutants and toxins that are found in the environment. Most of the lead in our environment comes from gasoline used in our cars, industrial emission, drinking water, petroleum refining, in construction, bullets of gun etc. The manipulation of lead for these uses has led to contamination of air, dust and soil by lead (Kiran *et al.* 2008). The use of surma, an eye cosmetic, which contains 100 % lead sulphide, had recently been shown to cause lead poisoning (Ali *et al.* 1988). Human exposure to lead continues to be a serious public health problem (Pirkle *et al.* 1998). It has been proven that daily intake of lead above 0.3 mg for a long period is toxic to man (Harvey 1970). Reproduction dysfunction by lead has distinct morphological and biochemical features such as disorganized epithelia, decrease sperm quality, alteration of sperm morphology and low androgen level (Alexander *et al.* 1996; Hsu *et al.* 1997).The testes are the reproductive organ of the male albino rat which are averagely 4.5 cm long, 2.5 cm wide and 3.0 cm thick and are surrounded by three layers; tunicae vaginalis, albuginea and vaculosa (Waugh & Grant, 2001). In each testis, there are about 200 to 300 lobules and within each lobule are 1 to 4 convoluted seminiferous tubules, between which are group of interstitial cells of leydig. Seminiferous tubules are surrounded by myoepithelial cells, and are lined by germ and sertoli cells (Waugh & Grant, 2001).

Lead is a potent nephrotoxic agent causing extensive damage to tubular cells, causes reduced growth impaired reproductive function, spleenomegaly, damage to the haemopoitic, central and peripheral nervous system. Archarya *et al.* (2003) reported that intraperitoneal injection of lead acetate to Swiss mice stimulated testicular weight loss with constant increase in the incidence of abnormal sperm population and decrease in the total sperm count. It has being proven that administration of lead during gestation period causes higher risk for reduced reproductive efficiency in adulthood with possible infertility in man which and might be attributed to the dysfunction of the sertoli cells (McGiven 1991). Rodamilans *et al.* (1988) suggested that prolonged lead exposure initially produced a direct testicular toxicity followed by hypothalamic or pituitary disturbances on long exposures. Exposure of lead increases the stabilization of the sperm chromatin, which in turn affects the decondensation of nucleus, thereby interfering with the fertility of male albino rats. The present study was

carried out to ascertain the toxic effects of lead acetate on sperm count and to determine the extent of damage of lead acetate on the testes of male albino rats which might lead to sterility or interference with reproductive potency of the rat.

MATERIALS AND METHODS

Forty five (45) adult male albino rats (*Rattus norvegicus*) with mean weight of 210 ± 27g used in this study were obtained from the Animal Breeding and Genetics Laboratory, Department of Zoology, University of Nigeria, Nsukka. The animals were maintained at temperature of 28 ± 2 ^{0}C, 75 – 80 % relative humidity, with 12:12 light and dark photoperiod, in clean aluminum wire cages. They were fed (Top Feed Grower's Marsh) and watered *al libitum* and allowed to acclimatize for two weeks before commencement of the study.

A Completely Randomized Block Design of five treatments (groups) replicated thrice was employed for the study. Each treatment had nine (9) male rats (3 rats per replicate). The treatments were labeled A – E. Five sub-lethal concentrations of lead acetate (0, 25, 50, 75g and 100 mg/kg) were prepared from commercial stock to correspond to treatments A – E, respectively. Group A rats (control) were given 5 ml of distilled water and 5 ml of the various doses of lead acetate; 25, 50, 75 and 100 mg/kg were administered orally (once daily at 9.00 am) to rats in group B, C, D and E, respectively for 4 weeks.

Body weight of each animal was recorded at the beginning (W_1) and at the end of the experiment (W_2).

At the end of the experiment all the animal chloroform anaesthetized and the testes dissected out. The average organ ratio was determined according to the formula; Average organ ratio of paired testes = weight of paired testes (g)/ weight of body weight (g). The excised testes were fixed in 10% formol-saline solution and processed for histological examination using standard histological techniques. Sections of organ were cut at 5 µm and differentially stained with Heamatoxylin and Eosin (HE). Permanent sections were observed under light microscope. The epididymes of rats in the control and test groups were dissected and extraneous tissues were trimmed out. The epididymes was put in Bijou bottles containing physiological saline (pH 6.8) and the epididymal sperm reserves were determined using standard haemocytometric method. Experimental data were subjected to Analysis of Variance using SPSS version 16.0. Mean differences at $p < 0.05$ was considered statistical significant.

RESULTS AND DISCUSSION

The effect of lead acetate on the testes showed spermatogonia cells with darkly stained nuclei and destroyed sperm cells, majority of seminiferous tubules were shrunken and had a wavy outline. The basement membranes were thickened and hyalinised. Debris of shredded cells occupied most of the lumen of the seminiferous tubules. The control group had undestroyed testis tissue architecture with prominent seminferous tubules (T) (Fig. 1). After 14 days of exposure of male rats to 25 mg/kg of lead acetate, the testicular architecture showed absence of interstitial space, degeneration of testicular architecture and necrosis (Fig. 2). Interstitial tissues, several maturing spermatogenic cells in seminiferous tubules of testis appeared after 14 days of exposure of male rats to 50 mg/kg of lead acetate(Fig. 3). In some cells the nuclear membrane had been ruptured and was accompanied by fragmentation of nucleus (karyorrhexis). After 28 days of experiment, Necrosis (N) occurred severely in testes treated with 100, 75 and 50 mg/kg of lead acetate (Figures 4, 5 and 6). Dead sperm cells were equally noticed in all treatment groups of the male rat but were severe in group D and E (Fig. 7 and 8) which has the highest concentrations. Congestion of blood was seen in group D and proliferations noticed in group E with the highest number of necrotic cells (Fig. 7 and 8).

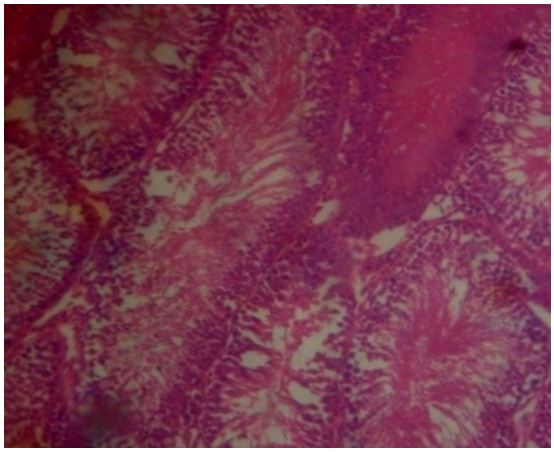

Fig 1. Photomicrograph of showing prominent seminferous tubules (T) in the control group. [5µm, (H&E) Mag. X 40]

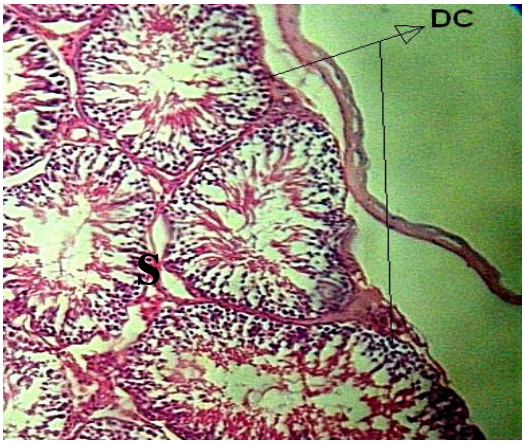

Fig 2. Photomicrograph of lead acetate treated group B rat testis after 14 days showing absence of interstitial space, tissue architecture degeneration and necrosis. [5µm, (H&E) Mag. X 40]

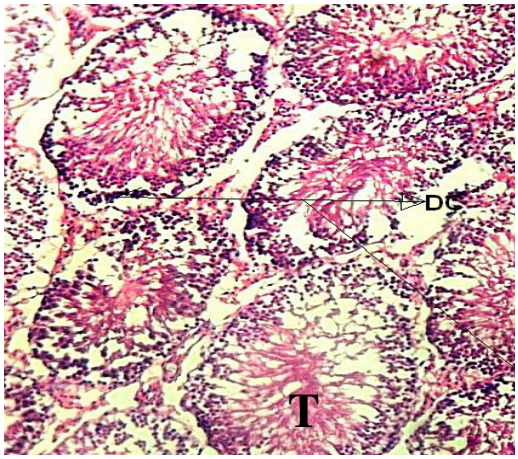

Fig 3. Photomicrograph of group C rat testis after 14 days showing absence of interstitial tissues, several in numbers of maturing spermatogenic cells in seminiferous tubules. [5µm, (H&E) Mag. X 40]

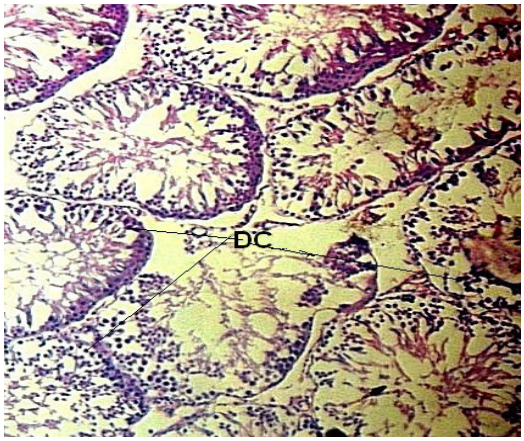

Fig 4. Photomicrograph of group D rat testis showing dead cells, thickened basement membrane. [5µm, (H&E) Mag. X 40]

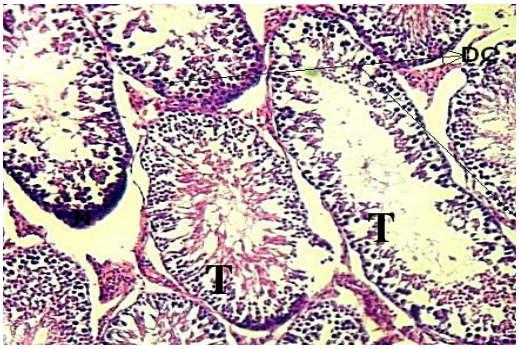

Fig. 5. Photomicrograph of group B rat testis after 28 days showing interstitial tissues, several in numbers of maturing spermatogenic cells in seminiferous tubules. [5µm, (H&E) Mag. X 40]

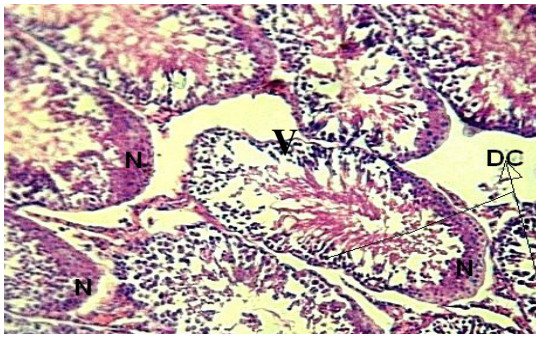

Plate 6: Photomicrograph of group C rat testis after 28 days showing Necrosis (N) and vacuolation [5µm, (H&E) Mag. X 40]

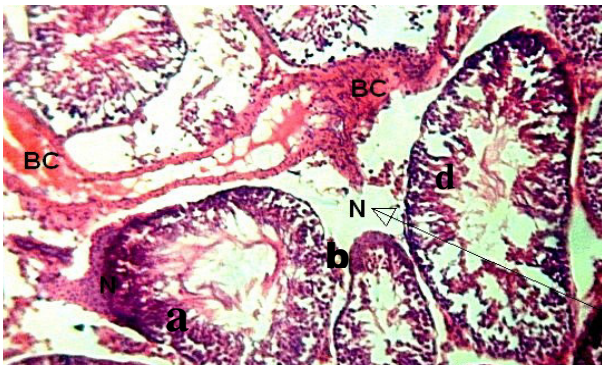

Fig. 7: Photomicrograph of group D rat testis showing narrow lumen seminiferous tubules, thickened basement membrane, scanty leydig cell [5µm, (H&E) Mag. X 40]

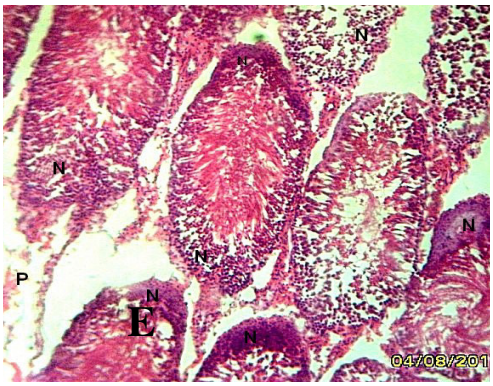

Fig. 8: Photomicrograph group E rat testis showing exudation (E) into the interstitial space and degeneration / necrosis (N) of spermatogenetic cells. [5µm, (H&E) Mag. X 40]

Key: a: lumen of seminiferous tubules, b: Thickened basement membrane, c: myoepithelial cell, d: scanty leydig cells, N: necrosis

There was a significant decrease in the sperm count of male albino rats exposed to varied dosages of lead acetate which decreases with increase in concentration (Table 1). Lead has the potential to cause damage to the reproductive system by prolonged exposure. In the present study, the male rats exposed to lead acetate showed a significant decrease in the body weight (Table 1) and testes (Table 2). This reduction in weight of sex glands was accompanied by an alteration of the normal histological structure. The average tissue ratio, which is a better way to asses the damage to the testes in relation to the body, was significantly reduced (Table 3). All these effects were indicative of atrophic changes that had taken place in the testes which is in consonance with Chowdhurry *et al.* (1986) who reported atrophy of cells in testes of rats treated with lead acetate. Male rats treated with lead acetate for 28 days exhibited disordered arrangement of germ cells, a decrease spermatogenic cells layer in the seminiferous tubules and necrosis. The findings of this study agreed with previous reports which indicated that lead altered testis histology resulting to structural defects in spermatids and sperm of mice, rats and rabbits (McGiven 1991; Archarya *et al.* 2003). Furthermore, toxic effects of lead on reproductive system in male rats were dose-dependent. Plucking and stringing phenomenon were absent, suggesting the degeneration of seminiferous tubules, thickening of basement membrane and condensation of the stroma. The pale colour of testicular tissues was suggestive of reduced vascularity. This indicated reduced demand due to arrest of spermatogenesis as was later proved on microscopy. These findings corresponded with the reports of Thomas & Brogan (1983) that lead acts as a spermicidal agent in case of high exposure. Although unfavourable reproductive effects usually occur at relatively high lead exposure, lower doses for longer periods may also alter the male reproductive system in a manner similar to that previously reported at higher doses for shorter periods (Sokol *et al.* 2002). Heavy metals generally have been observed to cause damages to reproductive organs. This concured with Chinoy & Bhattacharya (1996; 1997) reporting that a single dose of aluminum chloride (400 mg/kg) for 15 days or chronic treatment (200mg/kg) for 60 days administered to male mice caused alterations in the metabolism of testis, epidydimis and vas deferens leading to sperm motility and reduction in fertility rate.

Table 1: Effect of lead acetate on the weight of albino rats for 28 days

Treatment (mg/kg)	Initial weight (kg)	Final weight (kg)
0	290.00±20.80	290.00±20.80
25	291.70±14.20	265.30±18.80
50	330.00±23.90	296.30±21.90
75	360.00±23.10	317.00±24.00
100	373.30±29.10	309.30±28.30

Table 2: Effect of lead acetate on the sperm count of albino rats for 28 days

Treatment (mg/kg)	Mean sperm count
0	1.19x107 ± 1.67x104
25	1.16x107 ± 4.41x104
50	1.39x107±2.89x104
75	1.12x107±7.64x104
100	1.02x107±5.35x104

Table 3: Effect of lead acetate on the average tissue ratio of albino rats for 28 days

Treatment (mg/kg)	Mean weight of paired testis (g)	Mean body weight at the end of experiment (g)	Average tissue ratio
0	1.405	290	0.0048
25	1.145	265	0.0043
50	1.110	296	0.0037
75	1.019	317	0.0032
100	0.817	309	0.00061

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