ORIGINAL ARTICLE

Effects of Toxic Dose of Lead Acetate on Testicular Morphology in Albino Rats

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ABSTRACT

Objective: The objective of this study was to observe the morphometric changes in the testes of albino rats exposed to lead acetate.

Study Design: Experimental animal study.

Place and Duration of Study: This study was conducted from January to April, 2009 at National Institute of Health Islamabad.

Materials and Methods: Animals were obtained from the animal house of N.I.H and were divided into two groups A and B. The animals in group A were used as control, while those of groups B were treated with lead acetate that was given intraperitonially in the dose of 4mg/kg body weight, 5 days a week for 6 weeks. After 6 weeks the animals of group A (control) and group B (experimental) were sacrificed by an overdose of ether anesthesia. The testes were fixed in formalin and then processed for paraffin embedding. Five micrometer thick section were cut, stained with hematoxylin & eosin, and observed microscopically for germinal epithelium thickness and the basement membrane of seminiferous tubules.

Results: The histological comparison of testes of both groups of animals showed that after six weeks, the width of germinal epithelium and the number of spermatogenic cells had decreased in the test group as compared to the control group ($p<0.05$) and in majority of the seminiferous tubules, the basement membrane was disrupted.

Key Words: *Lead, testes, morphometric changes, rats.*

Introduction

In Pakistan, fertility is decreasing day by day. So it is mandatory to rule out the causes which are responsible for this disaster. Human beings are exposed to various types of environmental pollutants, many of which are harmful. One of the most important factors is lead, which is found in air, soil, and water which is one of the oldest environmental toxins. Lead is one of the most serious environmental threats to human health especially in developing countries like Pakistan.

Human beings are exposed to various harmful environmental pollutants and lead is one of them. It is one of the oldest environmental toxins with a specific gravity of 2.55 its molecular weight is 379.33 and is blue in color.[1] For a few months, a daily intake of 3.5 mg lead, results in toxicity.[2] A total amount of 80 mg of lead in whole blood is considered as an absolute example of lead poisoning.[3] Lead is known to cause toxic effects on the reproductive system, central nervous system (CNS) and skin. Some of the researchers have investigated the changes in the testes, secondary to lead poisoning.[4] In clinical and animal studies, lead toxicity caused adverse effects on male reproduction and fertility.[5] This metal induces reduction in the semen volume and density, sperm count and increased morphological abnormalities of the spermatogenic cells and the spermatozoa both in humanand experimental animals.[6,7] Lead toxicity in testis induced rupture of nuclear membrane which was accompanied with fragmentation of nucleus (karyorrhexis).[8] They also showed morphological changes in cells of testes.[9] Their results showed that some of the tubules contained only spermatogonia, which were scanty in number, bigger in size and had eccentrically placed dark nuclei. Some of the tubules contained only spermatogonia, which were scanty in number, bigger in size and had eccentrically placed dark nuclei.[10] The Sertoli cells had gained more prominence due to disappearance of other cells populating their neighbourhood.[11] In the females, lead caused irregular menstrual periods, decreased ovarian weight, decreased corpora leutea.[12] Abortions, decreased fertility, miscarriages and stillbirths.[13]

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Materials and Methods

A total of 30, eight weeks old healthy adult male albino rats of Sprague Dawley strain, weighing 200-±10 gm were used in the study. These animals were randomly divided into two groups; one was labeled

as Control (A), while the other was labeled as Experimental group (B). All animals were kept in the animal house under standard conditions at a room temperature ranging between18° C to 26° C for six weeks. They were maintained on 12 hours light and dark cycle. The rats were fed ad libitum. Day 0 was considered to be the starting day of experiment. The animals in group B were treated with intraperitoneal lead acetate in the dosage of 4 mg / Kg body weight / day, 5 days a week for a period of 6 weeks. These animals were sacrificed at the end of six week. After sacrifice, each animal was taken out of the jar and placed on the dissecting board. The scrotal sac was then opened with the help of forceps and scissors. Testes were examined with the help of hand lens and their colour, consistency and gross appearance were noted. The testes were fixed in formalin and then processed for paraffin embedding. Five micrometer thick section were cut, stained with hematoxylin& eosin, and observed microscopically for germinal epithelium thickness and the basement membrane of seminiferous tubules.

Results

The testes of rats in the control group A were easily pushed out of the scrotal sac, well vascularized and were soft in consistency. They were pink in colour and on cutting, gave little resistance. The mean weight of paired testes was 1.28 gm (SD $\pm$ 0.04) Table I.

The testes of rats exposed to toxic dose of lead (Group B) showed reduction in size; they were pale looking, tough in consistency and showed resistance on cutting. It was difficult to pluck out any tubule from the testes and the stringing out phenomenon was absent. The mean weight of paired testes was 1.16 gm (SD ± 0.029) in Group B.

At the beginning of the study, average weight of animals in group A was 197.67 gm (SD ± 5.85), while in group B it was 199.87 gm (SD ± 6.27). At the end of the study, average weight in group A was 261.40 gm (SD = 8.33), in group B it was 219.73 gm (SD = 7.40), the difference between the groups was insignificant ($p > 0.900$) Table II.

The epithelial height was significantly higher in group A as compared to groups B, ($p < 0.001$). In group A epithelial height was 84.45± 2.34 and in lead toxic group B the epithelial height was 68.54± 2.16 (Figure 1, 3 & 4). The difference between all the groups was significant ($p < 0.001$) Table III.

In lead treated group, in majority of the seminiferous tubules, the basement membrane was disrupted Figure 2.

The average germ cell count in group A was 304.53 cells/cross section of seminiferous tubule (SD $\pm$ 28.46), while in group B. it was 116.91 cells/unit (SD $\pm$ 32.66). The difference between all the groups was significant ($p < 0.001$) Table IV.

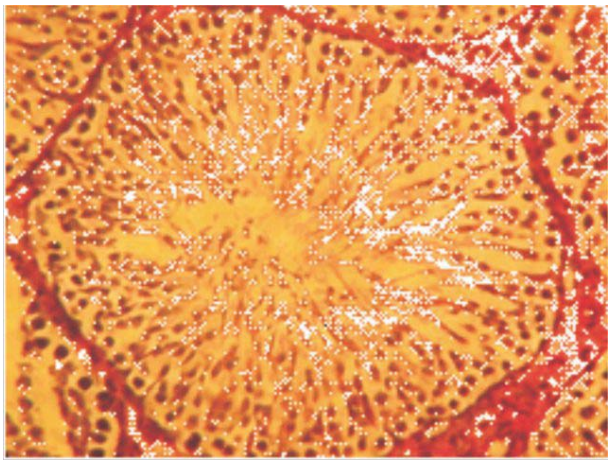

Fig 1: Photomicrograph section of testis showing Normal Germ Cell Count and Height of Germinal Epithelium. Animal Number 5, PAS Stain x 225

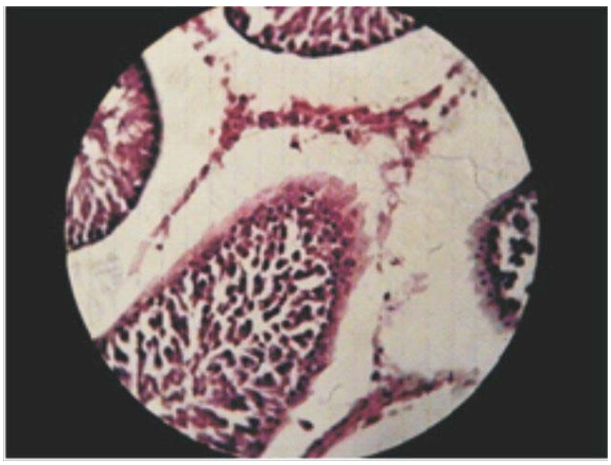

Fig 2: Photomicrograph; section of testis showing Disruption of Basement Membrane (BM) of seminiferous tubule in lead toxic group B, animal number 15, H&E Stain, x 175,

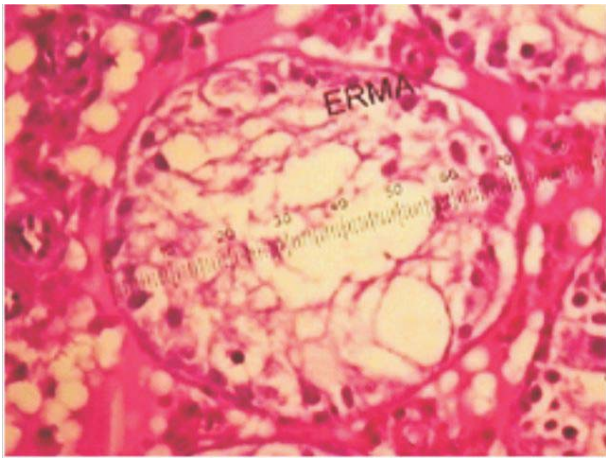

Fig 3: Photomicrograph; cross section of testis; lead toxic seminiferous tubule with reduced number of germ cells, group B, animal number 13, H &E Stain x 175

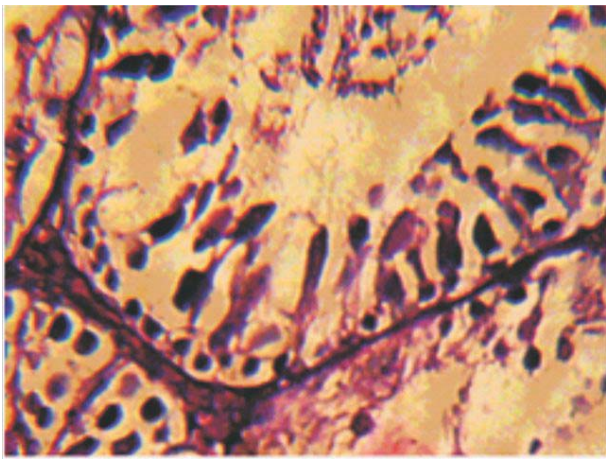

Fig 4: Photomicrograph Section of testis of lead toxic group (Group B) animal number 10, showing reduced germ cell count and reduced height of germinal epithelium. PAS stain. x 400

Table I: Mean absolute weights (G) of testes of control and experimental groups after 6 weeks N= 15

Groups	Absolute Weight of Testes	Statistical significance of difference (p – value)
Control (A)	1.28 ± 0.04	<0.012
Experimental (B)	1.16 ± 0.029	

Table II: Mean body weights (g) of animals of control and experimental groups

Groups	Initial	Final	Statistical significance of difference (p – value)
Control (A)	197.67 ±5.85gm	264.25 ±2.93	< 0.001
Experimental (B)	199.87± 6.2gm	271.95 ±1.83	< 0.001

Table III: Epithelial height (µM) of seminiferous tubules in control and experimental groups

GROUP	Mean ± SE	Statistical significance of difference (p – value)
Control (A)	304.53±28.46	< 0.001
Experimental (B)	116.91±7.377	

Table IV: Mean germ cell count of seminiferous tubules in control and experimental groups

Groups	Mean ± SE	Statistical significance of difference (p – value)
Control (A)	84.45±2.34	< 0.001
Experimental (B)	68.54±2.16	

Discussion

The group B animals (treated with lead) for six weeks lost their body weight by 22%. This significant drop in the weight was due to loss of appetite and gastrointestinal disturbances, which were also evidenced by Harvey in 1970.[14] Most of the animals also developed ulcerative lesions at the sites of injections due to necrotic changes induced in skin by lead, which failed to respond to any conservative treatment. Similar incident had also been reported by Der et al in 1974.[15] In a study conducted by Ahmed et al.,(2003), rats lost 16% of body weight. Lead was administeredintraperitonially for four weeks in the dose of 0.25ml/100g body weight once daily for 30 days. The study findings were in accordance with the present study.[1]The testes were reduced in size and weight and thus the mean weight of paired testes of these animals were significantly less than those of control. All these effects were indicative of atrophic changes that had taken place in the testes. Ahmed et al, (2003) found that the testes were reduced in size

and weight.[1] The pale colour of the testicular tissues was suggestive of reduced vascularity. This indicates reduced demand due to arrest of spermatogenesis as was later proved on microscopy. These findings correspond with the observations made by Thomas & Brogan and Daniel Lerda, who showed that lead becomes a spermicidal agent in case of high exposure.[16] The germ cell count was significantly reduced in group B, as compared to the control group A, which indicated the intensity of harm caused by lead on the cells of spermatogenic series.[17] The sertoli cells were relatively spared because of their resistance to various malicious agents as was documented by Leeson, Leeson. and Paparo.[18] In another study conducted by Chowdhury et al., (1997), on lead-induced rats, weight reduction was observed. Lead was provided in the dose of 1mg, 2mg, 4mg and 6mg/kg body weight. There was a corresponding decrease in body weight with the increase in lead dose which is in accordance with the present study.[19]

In a study conducted by Biswas and Ghosh (2006), lead acetate was administered to male albino rats at a dose of 8mg/kg body weight for 21days, which lowered the weights of testes and accessory sex organs.[20]

All these scientists described variety of toxic changes induced by lead in the testes depending upon dose and duration of the treatment which are in accordance with present study.

Conclusion

This study provides evidence that lead has toxic effects on testis.

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