ORIGINAL ARTICLE

TOXIC EFFECTS OF LEAD ON THE GERMINAL EPITHELIUM IN MALE ALBINO RATS

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ABSTRACT

Objective: To determine the toxic effects of lead on the germinal epithelium of testes of albino rat.

Study Design: Experimental study.

Place and Duration of Study: Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi, from August 2003 to December 2005.

Methods: Forty adult Albino rats selected for the study were divided into two groups; group A, received injection normal saline 1 ml intraperitoneally daily for eight weeks. Group B received lead chloride in a dose of 10 mg / kg body weight intraperitoneally daily. The testes were removed and fixed in Bouin's fluid for 24 hours. They were dehydrated in ascending strength of alcohol and the paraffin blocks were made. Four μm thick tissue sections were obtained, stained with PAS Iron Hematoxylin method and the morphometric study was done. Student's T-test was used for statistical analysis.

Results: Student's T test was used to determinate significance; P value = 0.05 was taken significant. Mean ±SEM diameter of seminiferous tubules was 291.92±1.11706 mm and 198.54 ±1.67282 mm in groups A and B respectively after eight week of treatment. Mean diameter of seminiferous tubule of group B was decreased significantly (P< 0.0001) as compared to groups A. Mean ±SEM thickness of germinal epithelium was 96.19±1.01215 mm and 50.69±1.20064 mm in groups A and B respectively after eight week of treatment. Mean thickness of germinal epithelium of seminiferous tubules of group B was decrease significantly (P< 0.0001) as compare to group A.

Conclusion: Heavy metal lead present in environment had direct toxic effects on male germinal epithelium and produced damaged on male germinal epithelium.

Keywords: Lead chloride, Albino rats, Seminiferous tubules and Germinal epithelium.

INTRODUCTION

Most of the heavy metals are toxic to the human body. The oldest and most important heavy metal which is toxic to the most of organs of body is lead. Lead poisoning may affect body organs for several years even in the absence of continued exposure. Reproductive toxicity is an important feature of lead toxicity. During exposure, lead accumulates in testis tissue dependent upon the dose. Lead toxicity induces a significant increase in apoptoic cell death in the seminiferous tubules of young growing rats. It is also associated with disruption of spermatogenesis, histoarchitecture and lowered enzyme activities in testis.[1]

Accumulated data suggests that there is a close relationship between declining reproductive health and environmental pollutants like fluoride (F) and lead (Pb).[2] Reproductive dysfunction induced by fluoride (F) and lead (Pb) has distinct morphological

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Received: October 23, 2008; accepted: April 20, 2009

and biochemical features such as disorganized germinal epithelia, giant cells in the lumen, decreased sperm quality, and low androgen levels.[3,4] Earlier investigations indicate that high lead exposure can also reduce sperm quality, decrease sperm count and motility, besides sperm morphology.[5,6] The previous altering studies suggest that disturbance of energy metabolism plays an important role in reducing sperm activity and blocking sperm maturation.[7, 8] In recent years, there are increasing reports indicating low sperm quality caused by F and Pb alone. Epid emiological investigations indicate that F pollution is accompanied by enhanced Pb levels in drinking water.[9, 10]

The diminution of semen quality due to occupational exposure of heavy metals is a major health concern around the world.[11-13] Lead exposure and moderate lead absorption produces alteration in fertility with decreased production in spermatozoa in the battery factory workers probably due to the direct toxic effect of lead on germinal epithelium of testis during spermatogenesis.[14-16] Blood lead levels also inversely correlated with sperm count and viability.[17] Reduction in sperm motility, count, density, and low antioxidant profile along with increase incidence of sperm abnormality and sperm membrane lipid per oxidation was prevalent after occupational lead exposure.[18, 19]

Occupational exposure to heavy metal lead, caused toxicity in industrial workers of smelters, acid battery plants, lead production units, and storage battery plants etc.[20-21] Lead exposure and moderate lead absorption produced alteration in fertility with decreased production of spermatozoa, probably due to the direct toxic effect of lead on germinal epithelium of testis during spermatogenesis.[22-25] Among different abnormalities, significant reduction in total motile sperm proportion, count, viability, forward progression, sperm kinetics, teratogenicity (morphological abnormalities) of spermatozoa were reported along with elevated blood lead levels after exposure to lead.[26-28] Recently it has also been reported that lead exerted some deleterious effects on testicular steroid genesis indirectly by decreasing serum level of gonadotropin.[29]
The objective of this study was to determine the toxic effects of lead on the germinal epithelium of albino rats.

MATERIAL AND METHOD

The experimental study was carried out at Basic Medical Sciences Institute (BMSI), Jinnah Postgraduate Medical Centre (JPMC), Karachi, from August 2003 to December 2005. Forty adult male albino rats between the ages of 90 to 120 days were obtained from Animal House, BMSI and JPMS. They were maintained at food and water ad libitum. The animals were divided into two groups A and B, each group consisted of 20 animals. The animals were kept in separate cages.

Group A: Served as Control Group and divided into four subgroups (A1, A2, A3 & A4), based on the period of treatment (1, 3, 5 and 8 weeks), Each subgroup consiststed of five animals. This group received injection Normal Saline 1 ml. intraperitoneally daily for their respective period of treatment.

Group B: Lead Group was also divided into four subgroups (B1, B2, B3 & B4) based on the period of treatment (1, 3, 5 and 8 weeks). Each subgroup consiststed of five animals. This group received Lead Chloride in dose of 10 mg/kg body weight in distilled water, intraperitoneally daily for eight weeks.[30] On the day of completion of treatment, the animals were sacrificed under deep Ether anesthesia. The scrotal sacs were identified and opened. Testes, vas deferens and blood vessels were excised.

TISSUE TREATMENT: The testes were fixed in Bouin's fluid for 24 hours, after that they were cut longitudinally into two equal halves and again post fixed in fresh Bouin's fluid for next 24 hours. The tissues were dehydrated in the ascending strengths of alcohol, cleared in xylene. Infiltrated and embedded in paraffin wax, the tissue blocks were made, and were cut into 4 μm thick sections with the help of rotatory microtome. The sections were mounted on albumenized glass slides and stained with PAS-Iron Hematoxylin. Morphometric study of germinal epithelium was measured with the help of ocular

micrometer scale under light microscope.

The level of significance (P) was calculated by the help of student's t-distribution table and the P value was read against the table degree of freedom (d. f.). The significance level was considered as P = 0.05

All the calculations were done utilizing computer software, "SPSS 15.0" in Window 2000 XP.

RESULTS

The diameters of seminiferous tubules were recorded at different time intervals in different groups as shown in Table-I. The diameter of seminiferous tubules was shown in Figure1. The diameter of germinal epithelium was decreased from 291.92 ±1.17906 mm (group A) to 198.54 ± 1.67282 mm (group B). There was statistically significant decrease in diameter of seminiferous tubules ($P<0.001$) in group B as compared to group A after eight weeks of treatment. The thicknesses of germinal epithelium of seminiferous tubules were recorded at different time intervals in different groups as shown in Table-II. The thickness of germinal epithelium of seminiferous tubules is given in Figure-2. The thickness of germinal epithelium was decreased from 96.19± 1.01215 mm (group A) to 50.69 ± 1.20064 mm in group B. There was statistically significant decrease in thickness of seminiferous tubules ($P<0.001$) in group B as compared to group A after eight weeks of treatment.

DISCUSSION

Many recent studies have indicated an increasing prevalence of various abnormalities of reproductive system in human males. There is growing concern about the considerable decrease in sperm density over the last 50 years in general population worldwide[31,32]

The present study is based on the fact that the trace elements or heavy metals produce male genital system abnormalities and damage the germinal epithelium producing oligospermia,asthenospermia, teratozoospermia and azoospermia, which account for 20–25% of cases. [33]

One of the important trace element, lead was used for the study. Lead intoxication and its effects on male germinal epithelium and male reproductive system has been focussed in many pervious studies.[33, 35] But the morphometric study of seminiferous tubules in respect to diameter of seminiferous tubules and thickness of germinal epithelium is still lacking. The study was designed to observe the morphometric changes that appeared after the lead accumulation in testes of albino rats. Lead was used in a dose of 10 mg/kg body weight intraperitoneally; the same dose was used by Batra and coworkers.[35] The heavy metal was given in injectable form so that accurately calculated doses of solutions can be administered to animals. This was against the study of researchers Antnio & co. workers (2004)[34] and Batra & his colleagues 1998, 2004).[30,35]

The decrease in diameter of seminiferous tubules in lead treated group was due to the degeneration of collagen fibers and germinal epithelium. The degenerated germinal epithelium collected in the lumen of seminiferous tubules as slough. The degeneration of interstitial tissues resulted in widening of interstitial spaces between the seminiferous tubules. This finding can be correlated with finding of Batra et al (1998, 2004).[30,34]

The thickness of germinal epithelium decreased in the lead treated group and this was due to loss of series of cells comprising of germinal epithelium, ranging from spermatogonia to spermatozoa with final reduction in the numbers of mature sperms. This finding correlated with finding of Antnio & colleagues (2004) Batra et al. (1998, 2004).[30, 34]

Reduced width of germinal epithelium, which was seen in this study seems to be due to damage of germinal epithelial cells which was also reported by other researchers, Barta & coworkers (2001, 2004).[30,36] The number of Spermatogonia and spermatocytes was almost intact in lead exposed animals; this reduction may be mainly due to decrease of number of spermatids Batra et al. (2004).[30] Lead induced apoptosis of germinal cells, reported by Adhikari &

his colleagues (2001) may be a possible mechanism for loss of germinal epithelium (Adhikari et al., 2001).[37]Batra and coworkers (2001) observed, dose depended reduction in the activity of two major enzymes in the testis, alkaline phosphatase and Na-K ATPase, in lead exposed animals, which is another probable mechanism of lead induced reproductive toxicity (Batra et al., 2001).[36]

According to the study there was reduction of germinal epithelium width and number of sertoli cells. The number of spermatogonia and primary spermatocytes was lower than control as demonstrated by Han et al., (1997).[38]

CONCLUSION

Based on this study, it is concluded that lead severely damages the germinal epithelium and causes degeneration of collagen fibers which results in shrinkage of seminiferous tubules with decreased diameter of seminiferous tubules. At the same time the direct toxic effects of lead on germinal epithelium reduces thickness of germinal epithelium. The presence of lead in environment has opened the new era for further study in human.

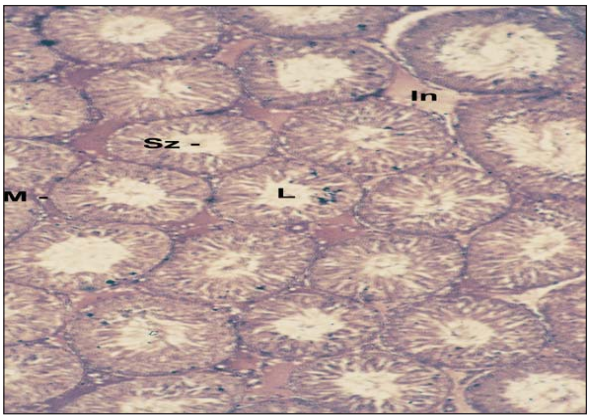

Figure – 1: Photomicrograph of PAS-Iron Hematoxylin 4 mm thick stained section of testis showing regular compact arrangement of seminiferous tubules with intact BM, lumen of tubules contained spermatozoa in control albino rat, low magnification

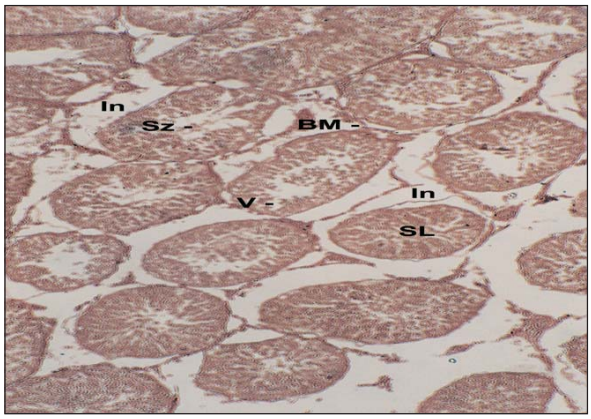

Figure – 2: Photomicrograph of PAS-Iron Haematoxylin stained 4 mm thick section of testis showing shrunken seminiferous tubules with mark widening of interstitial space, distorted BM, and lumen of tubules contained slough, after eight weeks in lead treated albino rat, at low magnification.

Table 1: Mean* diameter (µm) of seminiferous tubules of albino rats in different groups at variable time interval

Group	First week (1)	Third week (2)	Fifth week (3)	Eight week (4)
A	271.48 ± 3.15837	279.11 ± 1.78070	283.6 ± 1.64112	291.92 ± 1.17906
h = 20	h = 5	h = 5	(h = 5)	(h = 5)
B	234.25 ± 3.10299	229.19 ±1.69550	211.46 ± 2.07906	198.54 ± 1.67282
h = 20	h = 5	h = 5	(h = 5)	(h = 5)
P- Value	A1 VS B1 0.020	A2 VS B2 0.008	A3 VS B3 0.002	A4 VS B4 0.000

*** Mean ± SEM (Mean of 5 animals in a subgroup)**
P-Value ≤0.05 is significant

Table 2: Mean* Thickness of Germinal Epithelium (m) of seminiferous tubules of albino rats in different groups at variable time interval

Group	First week (1)	Third week (2)	Fifth week (3)	Eight week (4)
A	82.56 ± 1.00945	88.21 ± 0.97324	90.41 ± 1.22951	96.19 ± 1.01215
h = 20	h = 5	h = 5	(h = 5)	(h = 5)
B	70.99 ± 0.93983	66.89 ± 0.92875	56.79 ± 0.737962	50.69 ± 1.20064
h = 20	h = 5	h = 5	(h = 5)	(h = 5)
P- Value	A1 VS B1 0.020	A2 VS B2 0.008	A3 VS B3 0.002	A4 VS B4 0.000

***Mean±SEM**
P value ≤ 0.05 means statistically significant.

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