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Chronic Exposure to Heavy Metals Declines Sperm Quality and Damages Tissue Architecture

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Abstract

This study aimed to assess the toxic effects of heavy metals in adult male rats after chronic exposure in early life stages. Juvenile male Wistar rats were kept in constant supply of drinking water having heavy metal salts such as Sodium Arsenite, Cadmium Chloride and Lead Acetate of dose 100 times higher than Maximum Contamination Limit (MCL; laid done by US EPA, 2009) for 3 months along with control rats with a supply of heavy metal free water. The result showed decreased sperm count and increase in abnormal sperm in all treated cases. Histopathology showed that testes and liver tissues of treated rats were severely damaged. Thus, this study showed that chronic exposure to heavy metals such as Arsenic, Cadmium and Lead in juvenile period may result in reduced reproductive capacity at later stage of life.

Keywords: Heavy Metal, Live, Male Rats, Reproduction, Testis

1. Introduction

Chronic exposure to different heavy metals such as Arsenic, Cadmium and Lead causes irreversible toxic effects to male reproductive system that leads to reduced fertility or congenital malformation[1–4]. Treatment with Arsenic in experimental rodents has shown to produce steroidogenic dysfunction leading to impairment of spermatogenesis[5]. Acute exposure of Cadmium chloride causes reduced sperm count, degeneration of reproductive tissues, ischemia, testicular edema, hemorrhage and necrotic lesions[6,7]. Studies have also revealed reduced sperm count, decreased sperm motility, increased incidence of teratospermia and testicular degeneration in occupationally lead-exposed male workers[8]. The detrimental effects on reproductive health due to indiscriminate exposure to these heavy metals have become the major health concern in recent times and instigated several studies in this field[9].

However, there are not enough reports regarding effect of heavy metal at juvenile stages which may come out to be the most vulnerable stage in life span. On the other hand, toxic load of heavy metals is much higher in liver, the main detoxifying organ of body. Therefore, this study aims to assess and compare the effects of chronic exposure of heavy metals both in sperm health and testes (as reproductive tissue) and in liver (non-reproductive tissue) tissues in juvenile rats and their result in adulthood.

2. Materials and Methods

2.1 Maintenance of Animals and Treatments

Male Wistar rats bred in our own laboratory were maintained under similar conditions in a temperature and humidity controlled room on a 12-h light/dark cycle having free access to food and water. Triplicate sets (n = 3) of juvenile male rats were selected as treated study subjects for each of the heavy metal treatments. Similarly, triplicate sets of age and size-matched controls were maintained. Juvenile rats of age 15 days and with approximate weight of 20-25 gm were taken as treated and control study subjects. Initially, rat pups were allowed to stay with mother, later on, were separated at day 21 and weaned. Treated sets of rats were kept in constant supply of metal treated drinking water for a period of 3 months. Sodium Arsenite,

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Cadmium Chloride and Lead Acetate (Sigma; St. Louis, MO, USA) of dose 100 times of Maximum Contamination Limit (MCL) (US EPA, 2009) were used in drinking water (Table 1). The doses are selected to match the actual environmental doses found in affected areas[1–3,9,10]. All study subjects had free access to normal food. Triplicate sets of controls were also maintained and given heavy-metal free drinking water for same duration (Table 1). The duration of the experiment was 3 months during that period the juvenile rats were grown to adulthood. All the studies were conducted as per tenets of Institutional animal ethical committee, Calcutta University.

Table 1. Treatment and doses of heavy metal treatments in juvenile male rats

Treatment with Heavy Metals	Doses of treatment (MCLX 100)	
Sodium Arsenite (As)	0.01mg/L	1mg/L
Cadmium Chloride (Cd)	0.005mg/L	0.5mg/L
Lead Acetate (Pb)	0.015mg/L	1.5mg/L
Controls		

MCL: Maximum Contamination Limit by US EPA (2009)

2.2 Sperm Analysis and Histology Studies

After 3 months duration, the treated and control subjects were sacrificed by cervical dislocation. Spermatozoa from cauda epididymis were collected and total sperm as well as, defective spermatozoa were counted using Standard Haemocytometric method[11]. Total number of spermatozoa were calculated in millions (10^6/ml) and comparison of spermatozoa from treated and control rats was done (Table 2). The morphological defects at head, neck or tail of spermatozoa were counted and documented (Supplementary Figure). Out of total spermatozoa, percentage of defective spermatozoa was calculated (Table 2). Testes and liver tissues (for comparison with non-reproductive tissue) were dissected out in normal saline from treated and control rats. The tissues were fixed in Bouin's fixative, paraffinized, micro-sectioned and stained with routine Haematoxylin-eosin stain. The stained slides were documented for comparison of treated and control tissues (Figures 1 and 2).

Table 2. Concentration of spermatozoa and percentage of defective spermatozoa after heavy metal treatments

Treatment Concentration of Spermatozoa (millions/ml)		p value	% of Defective sperm	p value
	Mean ± SD		Mean ± SD	
Control	65±1.53		5.5±1.29	
Arsenic	77±4.04	0.06	15.43±1.98*	0.002
Cadmium	10±2.08*	0.001	22.6±1.95*	0.000
Lead	15±2.65*	0.002	24.15±2.43*	0.001

p values calculated compared to controls; SD: Standard deviation

2.3 Statistical Analyses

Two tailed paired t-test was conducted to compare total sperm count and defective spermatozoa in control and treated rats. For all statistical data analyses Microsoft Excel (Microsoft XP) was used p value <0.05 was considered significant.

3. Results

3.1 Sperm Count and Sperm Morphology

The total sperm count has been significantly reduced ($p<0.05$) in cadmium and lead treatment (Table 2) compared to respective controls. All three heavy metal treatments showed a highly significant percentage of defective spermatozoa ($p<0.05$) compared to control rats (Table 2, Supplementary Figure).

3.2 Histopathology in Testes

Heavy metal treated testes tissues showed significant damage in tissue architecture compared to control tissue (Figures 1 A-D).

3.2.1 Control Testes

Testes in control rats showed that seminiferous tubules and interstitial cells were in integrity. Seminiferous tubules were showing wide lumen into which spermatozoa were projecting.

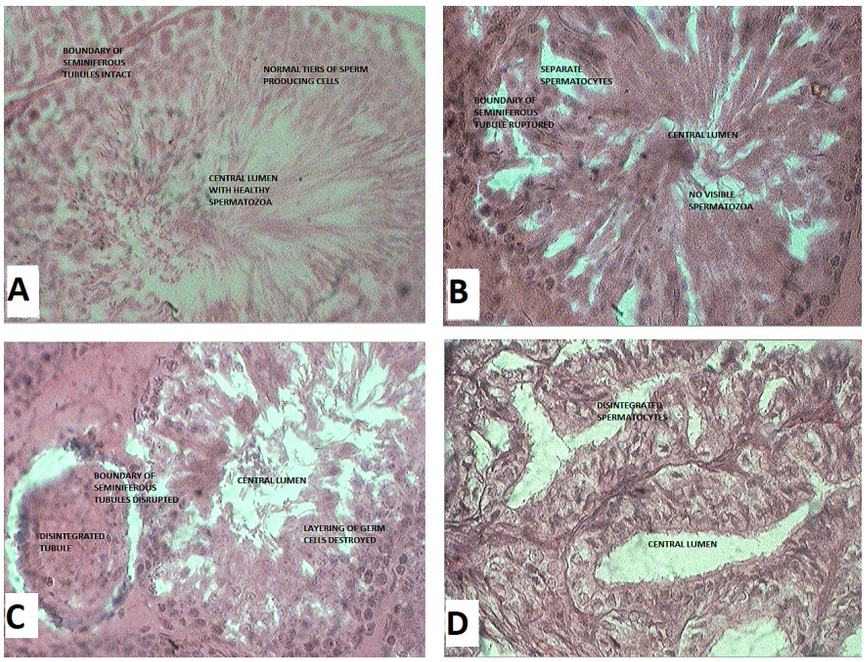

Figure 1. Testicular tissues after treatments A. Control testes showing normal tissue architecture; B. Arsenic treatment showing abnormal vaculation in spermatogonial array; C. Cadmium treated testes showing ruptured seminiferous tubules and disrupted central lumen; D. Lead treated testes showing severe tissue damage. Magnification 400X.

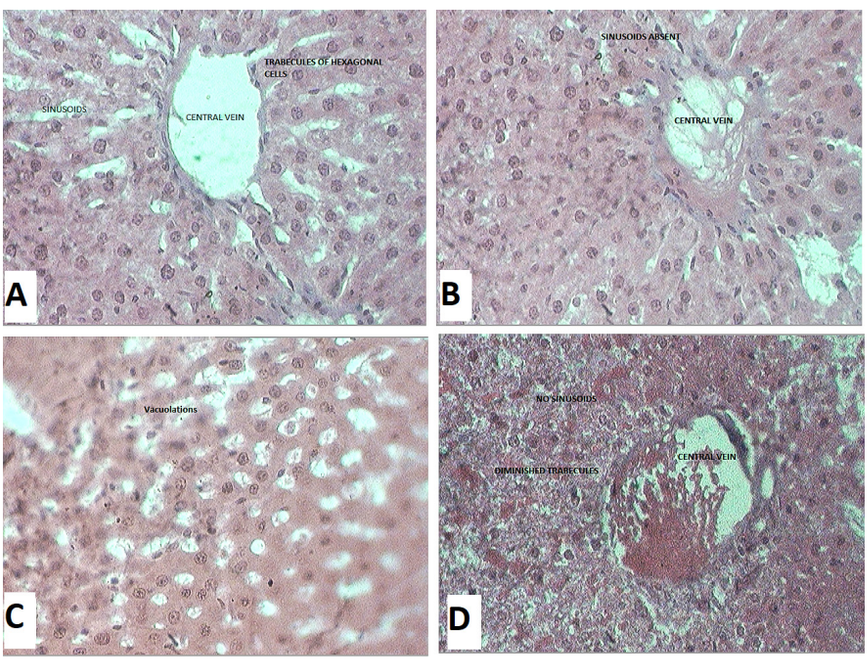

Figure 2. Liver tissues after treatments A. Controlliver showing normal tissue architecture; B. Arsenic treated liver showing not much changes; C. Cadmium treatment showingfatty liverand abnormal vacuolations; D. Lead treated liver showing severely disrupted tissue. Magnification 400.

Array of generations of sperm-producing cells starting from spermatogonia were clearly visible. Connective tissue fibres surrounding the seminiferous tubules were intact separating the tubules from peritubular space (Figure 1A).

3.2.2 Arsenic Treated Testes

Connective tissue fibers covering the Seminiferous tubules were disintegrated and inter-cellular gaps between array of sperm producing cells and Sertoli cells were increased. Spermatozoa projecting into the lumen showed abnormal shape and arrangement. In many cases, lumens were occluded or abnormally vacuolated (Figure 1B).

3.2.3 Cadmium Treated Testes

Germ cells were sloughed off leaving empty spaces in the epithelium and spermatocytes were also found occluding the testicular lumen. The lumen showed abnormal aggregation of germ cells leaving no space for spaermatozoa. In most the cases, no spermatozoa are seen in abnormally vacuolated lumen showing spermatogenesis process in halt. The connective tissue covering was destroyed in many cases resulting in release of spermatogonia in interstitial spaces (Figure 1C).

3.2.4 Lead Treated Testes

Complete disorganization of tissue architecture of seminiferous tubules was observed after exposure to lead. In most of the cases, spermatogonia, spermatocytes, spermatids and spermatozoa were completely absent. Abnormal arrays of disintegrated cells were seen in the epithelium and near lumen. In most cases, atrophied seminiferous tubules were prominent characteristics after exposure of lead (Figure 1D).

3.3 Histopathology of Liver Tissues

3.3.1 Control Liver

Hexagonal or pentagonal liver lobules are demarcated by central vein and terminal portal triads. Hepatocytes are arranged in trabecules that run radiantly from central vein to peripheral portal triads or tetrads. The trabecules are separated from each other by well marked sinusoids. Each hepatocyte is a polygonal cell with large spheroid nucleus (Figure 2A).

3.3.2 Arsenic Treated Liver

Sinusoidal network were degenerated leaving hepatocyte boundaries blurred. Central vein and portal triad showed prominent occlusion, perivascular edema and fibrosis. In some cases, hypertrophied hepatocytes were showing vacuolations and abnormal nuclear shape (Figure 2B).

3.3.3 Cadmium Treated Liver

The trabecular or sinusoidal networks were blurred in some cases, while in others they were dilated. Portal triads were occluded, hepatocytes showed hypertrophy and nuclear degenerations in few places. Irregular nuclear boundaries and vacuolations were frequent (Figure 2C).

3.3.4 Lead Treated Liver

Sinusoids were indistinguishable or in most cases absent showing coagulative necrosis. Boundaries of hepatocytes were blurring and indistinguishable. Portal areas were showing congestion, perivascular edema, fibrosis and apoptotic necrotic cells. Hepatocytes were showing cloudy or foamy swelling in cytoplasm, vacuolation and fatty changes (Figure 2D).

4. Discussion

4.1 Effect of Heavy Metals on Spermatozoa

Severity of reproductive malfunction has also been depicted through percentage of defective spermatozoa, which is quite high in case of metal-exposed conditions. The disruption of spermatogenesis and impaired sperm morphology may be because of the generation of Reactive Oxygen Species (ROS) through oxidative stress by heavy metals like lead and cadmium which can disrupt sperm membrane architecture. Although, arsenic exposure did not show any apparent effect on sperm count or defective sperm percentage, it may have other route of action on reproductive system. Thus, study showed that early stage is much more vulnerable towards exposure to these metals.

4.2 Effect of Heavy Metals on Testes and Liver Tissue

The histological studies showed that cellular architecture of both liver and testes are disrupted after exposure to heavy metals. These results were also confirmed by some earlier studies[12,13] though there were no studies showing early exposure of these heavy metals. This confirms that these heavy metals may have crossed the blood-testes barrier to exert its effects in later stages of life. Although, in all cases the assault of heavy metals were pronounced, the effects the lead treatment were most severe where the identity of the tissues were completely destroyed. All studies combined, we observed that there is a dramatic decline in health parameters both in reproductive tissues as well as non reproductive tissues such as liver in animal model. And in comparison, testicular tissues are showing more severe damage than liver tissues.

5. Conclusion

All the parameters such as decrease in sperm count, increase in defective sperm concentration and increased damage in testicular tissue suggested that this chronic exposure of all the heavy metals in early or juvenile stage affect the reproductive capacity later in adult life.

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