EFFECT OF LEAD ACETATE ON SPERM MORPHOLOGY AND TESTIS OF WISTAR ALBINO RATS

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ABSTRACT

In the present study different doses of lead acetate was used to know the effect on testis, sperm count, sperm morphology in different experimental groups. 108 male wistar rats were divided into three equal groups (I, II &III) i.e. each group contains 36 rats. Group I kept as control. Group II & III rats were orally administered lead acetate @ 60 mgs & 30mgs /kg. bwt / 3 days in a week for 90 days. Every fortnight from each group rats were sacrificed with anesthetic ether and epididymis collected for sperm count, testes for measurement of testicular weight and pieces of testis fixed in Bovin's fixative for histopathological and histochemical studies. The study revealed that lead acetate caused a dose dependant reduction in sperm count, testicular weight, and abnormal sperm count was increased significantly when compared to control. Hisopathologically testis shows necrosis of seminiferous tubules. Complete separation and disappearance of seminiferous tubular epithelium. Lead also caused reduction in testicular alkaline phosphatase activity.

Key words: Lead poisoning, Testis, Sperm morphology, Histopathology, Histochemistry, Albino rats

In farm animals significant pollution is likely to occur from lead mining, painted and metallic lead in storage batteries, licking paints / puttyans from rubbish dumps. Areas near lead industrial establishments may be enriched by aerial deposition as a consequence, soil pollution as well as quality deterioration of edible portions of vegetation can be produced due to metal enrichment. Animals by eating this vegetation can accumulate enough lead to produce clinical signs of lead poisoning. Deterioration of edible portions of vegetation can be produced due to metal enrichment. Animals by eating this vegetation can accumulate enough lead to produce clinical signs of lead poisoning. Young animals are usually poisoned when they lick painted pens, troughs etc because of allatrophagia. Chronic lead poisoning commonly seen in young children from sucking lead paint or lead toys, in families engaged in painting and petroleum industries.

Continuous environmental and occupational lead exposure may contribute to hemopoitic, renal, nervous, gastrointestinal and reproductive disorders in man and animals. The absorbed lead is conjugated in the liver and passed to the kidney where a small quantity is excreted in urine and rest accumulates in various body organs and interferes with their function (Taib et al., 2004). The accumulated heavy metals inhibit reproduction by interfering with the endocrine system affecting spermatogenesis and steriodogenesis (Yamaguchi et al., 2007).Reproductive dysfunction by lead has distinct morphological and bio-chemical features such as disorganized epithelia, decrease sperm quality and alter sperm morphology and low androgen

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levels. Lead has a primary toxic effect on the hypothalamic – pituitary unit, a primary effect on the testes and acts at all levels of the reproductive axis.

Studies were conducted on 108 healthy adult male Wistar rats weighing more than 150 g. All the rats were housed comfortably in standard rat cages at 25° ±1° C and a 12:12 hour interval light / dark cycle throughout the experimental period of 12 weeks and provided *ad libitum* feed and water. The approval of the institutional animal ethics committee was obtained prior to commencement of the experiment. After 10 days of acclimatization the animals were randomly divided into three equal groups (n-36). Lead acetate ($(CCH_3 COO_2)_2$ Pb $3H_2O$, M.w = 379.33) with a laboratory Reagent grade procured from the Qualigens Fine chemicals, Bombay) was given orally after mixing in double distilled water to rats at the dose rate of 1/10 LD_{50} (60mg/ Kg b.wt/3 days in a week), 1/20 LD_{50} (30mg/ Kg b.wt/3 days in a week) respectively to the groups II and III. Group I was kept as control. Rats from each group were randomly sacrificed at fortnight intervals after starting the experiment *i.e.*, 2nd, 4th, 6th, 9th, 10th and 12 weeks. Necropsy was done and organs were collected.

Sperm count were made from the sperm suspension, prepared by mincing both the cauda epididymis in 4ml of PBS with few drops of 10% formalin, with the aid of Neubauer hemocytometer at 400 X (Rastogi and Levin, 1987).

At each sacrifice the epididymis was carefully separated from the testis of both control and experimental animals. Both epididymis was minced in 1ml phosphate buffered solution (PBS, pH 7.4) and filtered through 80 m nylon mesh to obtain a filtrate. From the filtrate, smears were made and stained with 1% eosin yellow and were visualized under 40x or oil immersion objective and abnormalities of either head or tails were noticed. 200 sperms were screened for each animal and total abnormalities were expressed as evidence / 200 sperms / animal (Nayanatara *et al.*, 2008).

A detailed postmortem examination was conducted on all the sacrificed rats in the experimental groups, testes were carefully separated and weighed by using digital weighing balance and the gross lesions were recorded and representative tissue pieces from testis were collected and preserved in 10% Bovin's fixative for histopathological studies. Fixed tissues were processed by routine paraffin embedding technique. Sections of 5-6 microns thickness were cut and were stained with routine Haematoxylin and Eosin method (H&E) (Culling 1974).

For histochemical studies pieces of testis from both experimental and control groups were collected in to chilled neutral buffered formalin. Frozen sections were taken and the alkaline phosphatase activity in testis was demonstrated by the method described by Bancroft and Cook (1994).

The results were analysed statistically by performing oneway ANOVA (Snedecor and Cochron, 1967).

In present study there was significant decrease in sperm count values in lead treated groups (Group II and III) when compared to control animals (Group I). No significant difference was noticed between lead treated groups. These results were in accordance with the findings of Sokol and Berman (1991) and Imran Ahmad *et al.* (2003). The decreased sperm count might be due to spermicidal effect of lead in case of high exposure, lead is injurious to spermatogenic and leydig cells (Imran Ahmed *et al.*, 2003). Most of the testicular germ cells might have been destroyed either due to membrane damage and macromolecular degradation incurred by ROS leading to a significant decline in sperm count and testicular weight loss.

A significant increase in abnormal sperm count values were observed in lead treated groups (Group II & III) when compared to group I (Control). The abnormal forms like immature sperms, head and tail abnormalities (Fig. 1) were observed. These results were in accordance Khan and Sinha (1996) and Wadi and Ahmad (1999). The increased abnormal sperm count in the present study might be due to gene mutations that were induced by ROS generated by metals (Reid *et al.*, 1994). Lead act as germ cell mutagens affecting specific gene loci

in spermatogonia cells by increasing the percentage of sperm abnormality (Soares *et al.*, 1979).

Statistically, significant decrease on testicular wet weight in Group II (Higher dose) was noticed when compared to control (Group I). These results were in accordance with the findings of Ait Hamadouche *et al.* (2009). In contrary, no significant change in testicular wet weight was observed by Wadi and Ahmad (1999). Decreased wet weight of the testis might be due to decreased diameter of seminiferous tubules as this contributes to the weight of the testis (Imran Ahmad *et al.*, 2003), or due to reduced protein content, as the growth rate of any organ is proportional to its protein contents (Vijaya Kumar and Saraswathi, 2007), or might be due to loss of tubular epithelium as observed histopathologically.

In the present study gross lesions were prominently observed in testis. During 2nd and 4th week of experimental period no specific changes were noticed in testis of lead treated groups. In 6th week, slight reduction in size of testis was conspicuous in lead treated animals. By the end of 12th week, severe reduction in size of testis was noticed in lead treated group of higher dose, where as in other lead treated group, reduction in testis size was observed as dose dependent manner. Histopathologically testis of lead treated animals (Group II&III) revealed a dose dependant changes like mild interstitial edema, in focal areas disruption of basement membrane were noticed. In focal areas of separation of epithelium on basement membrane of seminiferous tubules were observed by the end of 2nd week. Shrunken tubules and at places basement membrane of tubules lined by only spermatogonia were observed during 4th week after feeding lead. Desquamated seminiferous tubular epithelium and presence of cellular debris in the centre of tubules were conspicuous in majority of animals. Disappearance of leydig cells in interstitium, widening of interstitial spaces and intertubular edema (Fig.2) were noticed by the end of 6th week. Shrinkage and necrosis of seminiferous tubules and thickened basement membrane was conspicuous in majority animals. Prominent spermatogonia, complete absence of germinal epithelium and leaving few spermatogonia on basement membrane of seminiferous tubules (Fig.3) were observed by the end of 10th week. Moderate edema, complete separation and disappearance of seminiferous tubular epithelium from basement membrane in majority areas, necrosis of seminiferous epithelium, mild infiltration of mononuclear cells in interstitium, and presence of multinucleated cells in seminiferous tubules were observed by the end of 12th week.

The lesions in the present study was in agreement with Almansour (2008) The tubular changes might be due to susceptibility of the seminiferous epithelium to lead toxicity with a possible effect on the intracellular junction between the strata of the germinal cells. The appearance of intratubular spermatid giant cells in the tissues indicates spermatocyte degeneration and spermatid giant cells were known to develop as a result of the failure of cytokinesis during meiotic division or due to the disruption of cytoplasmic bridges connecting germ cell clones.

In the present study Alkaline phosphatase activity was detected in testis by using Gomari method. In sections where brownish to black color develops indicate enzyme activity in this organ. In presented study, intense reaction was found in interstitial tissue and basement membrane of seminiferous tubules and less reaction in spermatogonia cells of lead fed groups (Fig.4) as dose dependent manner (Group II & III). In contrary to the present findings decreased activity of enzymes was noticed by Seema Rani *et al.* (1988). The observations made in this investigation might be due to damage of cell membrane of cells due to accumulation of lead or crossing of lead in the basement membrane of tubules.

In conclusion, it was observed that lead acetate toxicity produced adverse effect on the testicular weights, sperm morphology and histological structures of seminiferous tubules of lead acetate treated rats in dose dependant manner and indicating the reproductive toxic nature of the lead.

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TABLE I. MEAN TESTICULAR WEIGHT AND SPERM COUNT IN LEAD INDUCED TOXICITY OF RATS.

Parameters studied (n=36)	Group I (n=36)	Group II (n=36)	Group III (n=36)
Sperm count (10^7 / ml)	$14.50^a \pm 3.02$	$9.17^{ef} \pm 3.37$	$11.67^{bcd} \pm 3.56$
Abnormal sperm count (%)	$4.00^d \pm 0.52$	$17.00^a \pm 2.84$	$15.00^a \pm 1.15$
Testicular wet weight (gm)	$14.50^a \pm 3.02$	$9.17^{ef} \pm 3.37$	$11.67^{bcd} \pm 3.56$

Mean with different superscripts differ significantly ($p < 0.05$)

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