# TERATOGENIC EFFECTS OF LEAD ACETATE ON KIDNEY

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**Background:** Lead remains a considerable occupational and public health problem, which is known to cause a number of adverse effects in both men and women. Conflicting reports have appeared on lead induced nephrotoxicity in experimental studies in the past. There is hardly any work on its teratogenic effects on kidney. Present study was therefore designed to investigate the effects of lead acetate on developing kidney. **Methods:** Twelve mice were used as experimental model and were divided into two groups of six animals each; group A served as control group and B was used as an experimental group. Lead acetate (10 mg/kg) dissolved in 0.02 ml of distilled water was administered as a single daily dose orally to group B whereas weight related amount of distilled water was given to group A for the entire period of experiment. On 18$^{th}$ day of gestation foetuses were dissected free of uterine wall under the dissecting microscope and were sacrificed; kidneys were removed and fixed in 10% formalin, dehydrated in ascending grades of alcohol, cleared in xylene and infiltrated with filtered paraffin. The paraffin blocks were made and five micron thin sections were obtained using a rotary microtome. The sections were stained with Hematoxylin and eosin and, PAS; these were examined under light microscope. **Results:** Significant decrease in cortical thickness was observed which varied from 578.6±1.4 µm in group A to 515.6±5 µm in group B ($p$<0.001). Diameter of renal corpuscles varied from 57.7±0.07 µm in group A to 50.5±0.07 µm in group B ($p$<0.001). Moderate cortical tubular atrophy showing thickening of endothelial basement membrane in glomeruli, desquamated epithelium with degenerated nuclei in proximal and distal tubules were observed in group B in contrast to group A. **Conclusion:** The results of the investigation indicated that lead acetate administration to the dams produced deleterious effects on the developing kidney in mice.

**Keywords:** Lead acetate, nephrotoxicity

## INTRODUCTION

Lead (Pb) is a poisonous metal, which is ubiquitous in both organic (Tetraethyl lead) and inorganic (lead acetate, lead chloride) forms in environment[1] and is emitted from automobile fuels, industrial discharge and paints.[2] Workers employed in these industries are more exposed to lead than general public. Numerous studies have shown that 15–30% of lead exposure in humans occurs through inhalation and 70–85% with food and drinks from gastrointestinal tract.[3] Children are particularly susceptible to the toxic effects of lead; but mostly such exposed children have no symptoms. The vast majority of cases, therefore, go undiagnosed and untreated.

Lead remains a considerable occupational and public health problem, which is known to cause a number of adverse effects in both men and women. Rubio J *et al*[4] reported effects in rats include low number of testicular spermatids, low daily sperm production, low epididymal sperm count, abnormal prostatic function and changes in serum level of testosterone.[5] Most of these effects have been reported in workers exposed to lead even at an acceptable level (10 µg/dl) recommended by Occupational Safety and Health Administration (OSHA) in 1993.[3] Lowering of seminal plasma protein with concomitant rise of free amino acid in blood in lead exposed workers indicated the alteration in protein metabolism, suggesting disturbance in cellular nutritional status, necessary for cell survival, and its proper function.[6]

The toxicity of high-dose lead exposure affects the reproductive function in both female and male. Female workers at high dose lead exposure had reported in an increase in the frequency of miscarriages and stillbirths. Increased prevalence of menstrual disturbances, spontaneous abortion and threatened abortion were reported to occur among lead exposed women.[7] lead acetate is, however, reported to arrest growth and maturation of the ovarian follicles upon oral administration to mice.[8] The levels of lead acetate in blood during pregnancy in Karachi had been reported to range between 2.28–36.35 g/dl.[9]

The present study was designed to investigate teratogenicity of lead acetate on kidney of foetuses when their dams were treated with lead.

## MATERIAL AND METHODS

Twelve female and four male albino mice of BALB/c strain, ten weeks old and weighing 30–34 g, were procured from the National Institute of Health, Islamabad. The animals were allowed free access to food and water and were allowed to acclimatize for 7 days in Experimental Research Laboratory of University of Health Sciences, Lahore.

Three females and one male mice were housed in a single cage for mating purpose. The female mice were separated from the male and housed in separate cages after confirmation of pregnancy; appearance of

vaginal plug was considered as day zero of pregnancy. Subsequently, the mice were divided randomly into two groups.

Lead acetate trihydrate of E-Merck were procured through University of Health Sciences, Lahore. Lead acetate (10 mg/kg) dissolved in 0.02 ml of distilled water was administered as a single daily dose orally to experimental group B whereas comparable weight related volume of distilled water was given to group A for the entire period of experiment.

On 18th day of gestation, pregnant mice were euthanized by chloroform. The animals were laid supine on the dissecting board and pinned through the fore and hind paws. A midline incision through skin and muscle was given from the xiphisternum to the pubic symphysis with a knife and extended laterally at its lower end to achieve maximum exposure of abdominal cavity.

Uterine horns were incised along the side opposite to the placentae; fetuses with their membranes were dissected free of the uterine wall under the dissecting microscope, these were then euthanized using chloroform and decapitated. A mid line abdominal incision was given to expose and remove the kidney; these were inspected for their color, consistence and any visible deformity. Each kidney was washed with distilled water, weighed and fixed in a 10% formalin solution for 72 hours. The specimens were dehydrated in ascending series of ethanol, cleared in xylene and infiltrated with filtered paraffin with melting point of 56–58 ºC. The paraffin blocks were made and five micron thin sections were obtained using a rotary microtome (Leica RM 2125).

Hematoxylin & Eosin, and PAS stains were used for histological study using light microscope (Leica DM 1000).

Measurement of cortical thickness and diameter of renal corpuscles was made after calibrating eyepiece graticule with stage micrometer at various magnifications using ×10 and ×40 objectives.

The diameter of renal corpuscles in corticomedullary area was measured at magnification ×400; three renal corpuscles with the vascular or urinary pole were selected in each PAS stained sections. The line connecting the vascular pole or the urinary pole to the opposite end was taken as the vertical axis and the widest distance at a right angle to the vertical axis was regarded as the diameter of the renal corpuscle; it was measured between the inner edges of the thin parietal layer of the cells forming Bowman's capsule, regardless of its size.[10]

Cortical width (from capsule to arcuate arteries) was measured by selecting at least five different points, perpendicular to the cortex while going around the circumference of the kidney from one pole to another at magnification of ×100.

The statistical analysis was carried out using SPSS version 15.0. Student's *t*-test was applied to observe quantitative differences between two groups. Chi-square was applied to observe associations in qualitative variables. A *p*-value <0.05 was considered statistically significant.

## RESULTS

Histological features of the kidneys showed clearly defined cortex and medulla. The cortex was distinguishable into two zones, an outer nephrogenic zone and inner deep part of cortex. The deep part of cortex contained convoluted tubules, renal corpuscles, straight tubules and medullary rays (Figure-1). The cortical thickness of kidneys was 578.6±1.4 µm and 515.6 ±5 µm in groups A and B respectively (Figure-1, 2). The foetal kidneys from lead exposed group B exhibited thin cortex as compared to group A. The *t*-test showed significant difference between mean cortical thickness of two groups ($p<0.001$, Table-1).

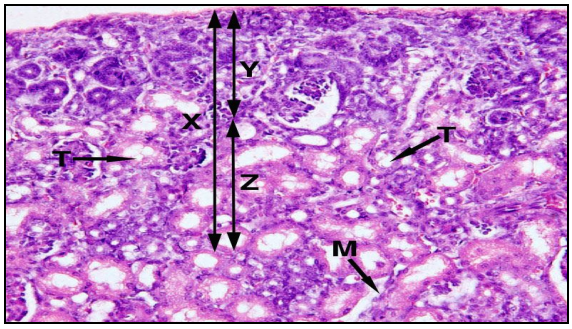


**Figure-1: Photomicrograph of foetal kidney (group A)**
Cortex (X) double head arrow, nephrogenic zone (Y) double head arrow, deep part of cortex (Z) double head arrow, medullary ray (M), and cortical tubules (T). H&E stain ×200.

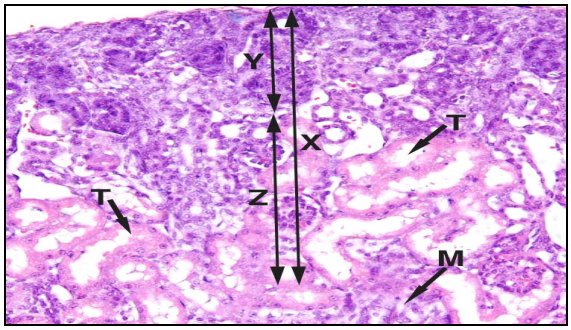


**Figure-2: Photomicrograph of foetal kidney (group B)**
Decreased thickness of cortex (X) double head arrow, nephrogenic zone (Y) double head arrow, deep part of cortex (Z) double head arrow, medullary ray (M) and cortical tubules (T). H&E stain ×200.

**Table-1: Comparison of Cortical thickness of kidney between the groups (Mean±SD)**

| | Group A (n=42) | Group B (n=42) | *p* |
|---|---|---|---|
| **Cortical thickness (µm)** | 578.6 ± 1.4 | 515± 5 | <0.001* |

*significant

Study of the cortical portion of the kidneys showed many renal corpuscles consisting of glomeruli having loops of capillaries surrounded by Bowman's capsule. (Figure-3,4). Diameter of renal corpuscles was 57.7±0.07 µm and 50.5±0.07 µm in groups A and B respectively. Renal corpuscles from group B showed decrease in diameter (atrophy) and thickening of endothelial basement membrane (Figure-4); there was a statistically significant difference when mean diameter of renal corpuscles among two groups was compared with each other ($p$<0.0001, Table-2).

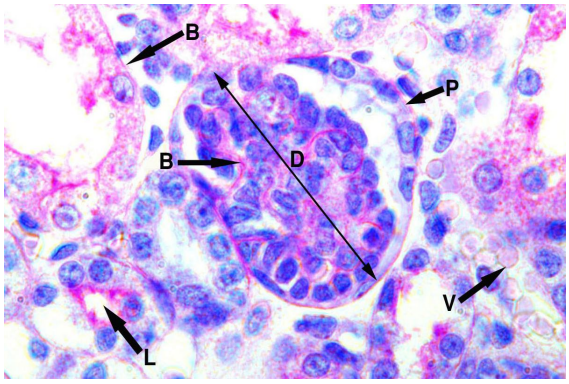


**Figure 3: Photomicrograph of kidney**
(Group A) showing parietal layer (P) of Bowman's capsule, diameter of renal corpuscle(D) double head arrow, tubular (B) and endothelial (B) basement membrane, interlobular vessel (V) and lumen of tubules (L). PAS stain ×630.

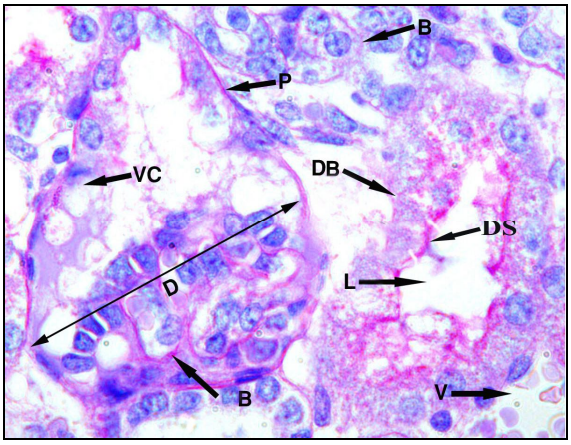


**Figure-4: Photomicrograph of kidney**
(Group B) showing parietal layer (P) of Bowman's capsule, decreased diameter of renal corpuscle (D) double head arrow, tubular (B) and endothelial (B) basement membrane, Disrupted basement membrane (DB), Interlobular vessel (V), Vacoulation (VC),lumen of tubules (L) and desquamated epithelium(DS). PAS stain ×630.

**Table-2: Comparison of diameter of renal corpuscles of kidney between two groups**

| | Group A (n=42) Mean ±S.D | GroupB (n=42) Mean ±S.D | *p* |
|---|---|---|---|
| **Diameter(µm)** | 57.7± 0.07 | 50.5± 0.07 | <0.001* |

*significant

Deep cortical area consisted mainly of the Proximal and distal tubules. However, proximal convoluted tubules were more numerous and were clearly distinguished from the distal convoluted tubules, since the former had small and irregular lumina, lined with single layer of cuboidal cells (Figure-3, 4). Whereas the latter had large lumen lined by low cuboidal cells. In lead exposed group B, tubular lumen was dilated and cell size was smaller indicating tubular atrophy. The basement membrane of epithelial cells of the tubules was thickened (Figure-4) as observed in PAS treated preparations which stained the basement membrane in addition to the luminal surface of the lining epithelium (Figure-3, 4). In some areas, the boundaries of the cells were not well defined. Many of the epithelial cells were anucleated, vacuolated and showed desquamation into lumen, all signs indicating degeneration (Figure-4). Thirty two percent of foetuses in group B showed tubular atrophy and degenerative changes and these changes were not evident in group A (Table-3). Chi square test observed significant association between Groups and tubular atrophy and degenerative changes of the epithelial cells ($p$<0.0001, Table-3).

**Table-3: Comparison of renal tubular atrophy and degeneration in the tubules of kidneys of two groups**

| Groups | Renal tubular atrophy and degeneration | | |
|---|---|---|---|
| | Absent (n%) | Present (n%) | Total |
| **Group A** | 42 (33.3) | 0 (0.0) | 42 |
| **Group B** | 10 (7.9) | 32 (25.4) | 42 |

Chi square value= 77.66, $p$<0.0001

## DISCUSSION

In present work, group B of the experimental animals showed a decrease in cortical thickness and diameter of renal corpuscles , the differences between the two groups on both the scores, when compared with each other, were statistically significant ($p$<0.0001). Moreover, histological sections of kidney from group B demonstrated renal tubular atrophy and degenerating cortical tubules in contrast to control group A, when the two groups were compared the difference was statistically significant ($p$<0.0001). Our findings were in accordance with those from the previous studies.[11,12] Vyskocil A[11] reported signs of functional impairment of tubules in developing kidney of both in male and female rats when 0.5% lead acetate in drinking water was given to them for 5 months; this increased beta 2-microglobulin and lactate dehydrogenase excretion in urine and an increase of lysozyme in tubular cells.[11]

Cortical tubular atrophy and degeneration, observed in our investigation, implied retarded growth of kidney. Yan MH[12] fed 0.025% lead acetate in drinking water to female rats during pregnancy and reported the growth retardation similar to our findings; they also observed that it was produced on account of

toxic effect of lead on placenta indicated by decidual necrosis, increased number of trophoblastic giant cells and fibrin deposition around the villi under light microscopic examination, interfering with nutrition and oxygen exchange between mother and pups.

Chowdhury AR performed biochemical and histopathological studies on the testes of rats after feeding lead acetate at a concentration of 1.0 g/l over a period of 60 days. Testicular atrophy along with cellular degeneration was conspicuous.[13] Swiss mice 10 weeks old were treated by intraperitoneal injection of 10 mg/kg of lead acetate and reported that it stimulated lipid peroxidation in testicular tissue and was associated with an increase generation of noxious reactive oxygen species (ROS) produced indirectly by lead acetate causing decrease in sperm count.[14]

An elevation of mean blood pressure was found in rats treated with low lead (0.01% lead acetate) for 3 months which was considered to be related due to an increase in endothelin-3 and reactive oxygen species in kidney.[15] The kidney is an organ highly vulnerable to damage caused by reactive oxygen species (ROS), likely due to the abundance of polyunsaturated fatty acids in the composition of renal lipids. ROS are involved in the pathogenic mechanism of conditions such as glomerulosclerosis and tubulointerstitial fibrosis.[16] Lead deposited predominantly in the proximal tubule was considered to be the main reason for its deleterious effects on the cortex of the kidney.[17] As in evident, lead acetate exerts its toxic effects through generation of reactive oxygen species on different organ system. We believe that the teratogenic and toxic effects on the developing kidney may have the same mechanism in our investigation also.

## CONCLUSION

Present work has shown that lead acetate has toxic effects on the developing kidney of the mouse.

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