Original Article

Chronic Lead Acetate Nephrotoxicity: A Histological Study on Albino Rats

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Abstract

Introduction: The human beings are constantly exposed to heavy metals in our environment, all of these are capable of combining with wide variety of organic molecules. They are potent enzyme inhibitors, which inactivate the enzyme system of cell. The lead hazards in the environment are identified as a serious Public Health problem particularly in children less than 7 years of age.

Aims: In the present study our aim was to observe histological changes induced in kidney to high light the extent of damage done by chronic lead acetate toxicity.

Material and Methods: The Albino rats were exposed to lead acetate in a dose of 8 mg/kg body weight and sacrificed after, 7, 14 and 21 days. Their kidneys were studied.

Results: Time scaled treatment with lead acetate results in renal damage. The Proximal convoluted tubules showed a damage ranging from cloudy swelling to complete cell death.

Key words: Lead acetate, Toxicity, kidney.

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Introduction

The human beings are constantly exposed to heavy metals in our environment; most of them exert toxic effects in high concentration. There are about 40 heavy metals which are capable of combining with wide variety of organic molecules and are potent enzyme inhibitors because of their interaction with ligands present in proteins and they inactivates the enzymes system of cell.[1]

Lead is used extensively in the production of tetra ethyl lead, used to increase the efficacy of petrol and act as anti-knocking agent. It is also used in car batteries, paints, soft solder for welding, and also lead compounds are used in the glazed tins of fruit juices and other food stuffs. It is found in nature as lead sulphides, carbonates and sulphates.

Lead acetate is used in clinical dental practice as material for reconstruction of teeth,[2] and as protective

shield for x-ray machine because of its radiation absorbing property.[3]

The average daily intake of lead is 0.3 mg. Lethal dose in human beings is 20 gm and toxicity occurs if 0.5 mg is absorbed per day. It affects vital organs like liver, kidney and central nervous system.[4] It might act as reproductive toxicant, higher levels of lead acetate for short period of time induce a change in hypothalamic gonadotrophic releasing hormone at molecular level.[5] Nutritional deficiency of Zn, Cu, and Iron increase its absorption.

Lead poisoning occurs from ingestion of acid soluble lead compounds, or inhalation of lead vapours. It produces local as well as systemic symptoms, such as astringency, thirst, metallic taste, oliguria, abdominal pain, haematemesis and malena are frequently seen.[6] In children chronic lead poisoning produces encephalopathies with seizures, intellectual deficiency, and mental retardation.[7]

Lead after absorption is carried to various tissues of the body. In blood it causes basophilic stippling due to its inhibitory effect on α-Amino levulinate dehydratase enzyme which affects hemsynthesis.[8]

Renal toxicity occurs in two forms, reversible usually seen after acute exposure of children to lead acetate and irreversible interstitial nephropathy is more commonly observed in long-tem industrial lead exposure.[8]

Lead toxicity produces histopathological changes in the renal proximal tubular epithelium which causes interstitial nephritis and often associated with hypertension.[9] It accumulates in proximal convoluted tubules of renal cortex produces both morphological and biochemical evidences of toxicity.[10] Although a lot of work has been undertaken to observe the effect of acute exposure of kidney to lead. The present study was designed to evaluate morphological and histological changes in kidneys of albino rats after chronic lead acetate exposure.

Materials and Methods

Adult albino rats weighing 150 – 200gms were taken from National institute of Health Islamabad. They were housed at animal house of Postgraduate Medical institute, Lahore and fed on standard commercial rat diet, care was taken regarding maintenance of optimal light and temperature.

The animals were divided at random into two groups A&B comprising 15 albino rats each. They were given respective identification marks and kept in a separate wire screened cage. Group 'A' was control group, randomly divided into three subgroups A_1, A_2 and A_3 each having 5 rats, the animal was given one ml/kg body weight of distilled water intraperitonially daily A_1, A_2 and A_3 for 7, 14, and 21 days respectively and sacrificed 24 hrs after giving the last dose.

Group B, was experimental and randomly divided into three subgroups B_1B_2 and B_3 each having five rats. The animals were treated by giving lead acetate 8 mg / kg body weight intraperitonially daily for 7, 14 and 21 days respectively and sacrificed 24 hrs after giving the last dose.

Both kidneys were removed, weighed and examined for gross features. The net weight of paired kidneys of each group was recorded and relative tissue weight index was calculated by the following formula:

$$\text{R.T.W.I} = \frac{\text{Mean weight of paired kidneys}}{\text{Mean body weight in grams}} \times 100$$

Renal tissue was fixed in 10% formalin, processed and paraffin blocks were prepared for light microscopy, the tissues were cut at 4 µmm thickness with

Table 1: Dose Plan.

Group	Sub-groups	N: of Rats	Treatment	Direction of Treatment	Sacrificed
Control Group A	A_1	5	Normal diet and distilled water/ml/kg body weight	7	24 hrs after the last dose
	A_2	5		14	Do
	A_3	5		21	Do
Experimental Group B	B_1	5	Normal diet and lead acetate 8 mg/kg body wt / day	7	24 hrs after the last dose
	B_2	5		14	Do
	B_3	5		21	Do

Table 2:

Groups	Sub Groups	Duration of Experiment	Mean body weight at the start of experiment (in gm)	Mean body weight at the end of experiment (in gm)	Paired kidneys weight (gm)	Relative tissue weight index % R.T.W.I	Means body weight gain/ loss gm/day
A (Control	A_1	7 days	170.00	177.60	2.65	1.40	1.08
	A_2	14 days	175.8	191.00	2.73	1.42	1.08
	A_3	21 days	176.00	198.89	2.77	1.39	1.09
B (lead Experi-mental group)	B_1	7 days	190.33	192.66	2.67	1.38	0.33
	B_2	14 days	170.3	145.50	2.63	1.80	-1.77
	B_3	21 days	200.00	163.33	2.80	1.81	-1.74

Table 3:

Groups	Sub Groups	Duration of Experiment	Mean body weight gain/ loss (gm)	Mean body weight of paired kidneys (gm)	Mean no of necrosed PCT / HPF	Mean no of hemorrhages/ HPF	Histological grading
A (Control)	A_1	7 days	177.60±1.25	2.65 ± 0.20	0	0	0
	A_2	14 days	191.00±1.55	2.73 ± 0.20	0	0	0
	A_3	21 days	198.89±1.8	2.77 ± 0.20	0	0	0
B (Lead Experimental group)	B_1	7 days	192.66±1.43	2.67 ± 1.43	0	0	+0
	B_2	14 days	145.50±0.60	2.63 ± 0.75	16.60 ± 1.52	10.12 ± 1.02	++
	B_3	21 days	163.33±0.70	2.80 ± 0.81	18.64 ± 0.72	13.12 ± 1.08	+++

* Significant (P<0.05) ** Highly significant (P<0.01) *** Very highly significant (P<0.001)

0 Normal architecture.
\+ 0 Mild to moderate congestion of the glomerular capillaries.
\+ Mild congestion of the glomeruli and minute areas of focal necroses.
++ Mild to moderate congestion, fairly large areas of focal necrosis.
+++ Marked congestion, fairly large areas of focal necrosis and inflammatory cells in necrosed areas.

rotary microtome. The slides were stained with haematoxyline and eosin by standard procedure in histology laboratory of King Edward Medical University. The stained sections of the tissues were examined under light microscope with following parameter.

The numbers of hemorrhagic foci in each of 4 slides in the interstitium 1 mm^2 of the kidney of each animal were counted under high power field at random. The number of necrosed proximal convoluted tubules in known area were counted using a grid. Necrosis was categorized as:

O Normal tubules.
\+ Mild to moderate glomerular congestion with few hemorrhagic foci.
++ Mild to moderate congestion with minimal area of focal necrosis of proximal convoluted tubules.
+++ Marked congestion, having large area of focal hemorrhages and necrosis.

Bio-statistical analysis of the recorded data was carried out applying student's 't' test. P value was calculated to depict significance.

Results

General Physical Conditions and Behavior

All the rats of control group (A) and lead treated subgroup (B_1) remained healthy throughout the course of experiment, no morbidity or mortality was observed. Lead treated subgroup B_2 and B_3 for longer duration were lethargic, physically week and showed weight loss (table 2). The diet and water intake was reduced. Relative tissue weight index of animals in control group (A), remained constant while there was slight increase in lead treated group (B) at the beginning which later become static of mean body weight (gm), paired kidney weight (gm) mean no of hemorrhages and necrosed PCT / HPF in control group and experimental group.

Appearance of Kidneys

There was no change in gross appearance of kidneys in control groups (A_1, A_2 and A_3) as well as in experimental group (B_1). The kidneys were dark brown in colour, smooth and shiny. Capsule was easily stripped off no pathological lesion seen on cut surface, the cortex was lighter in colour than medulla while kidneys of subgroup B_2 and B_3 were edematous and purplish red in colour. The cut surface was dark in colour and hyperemic.

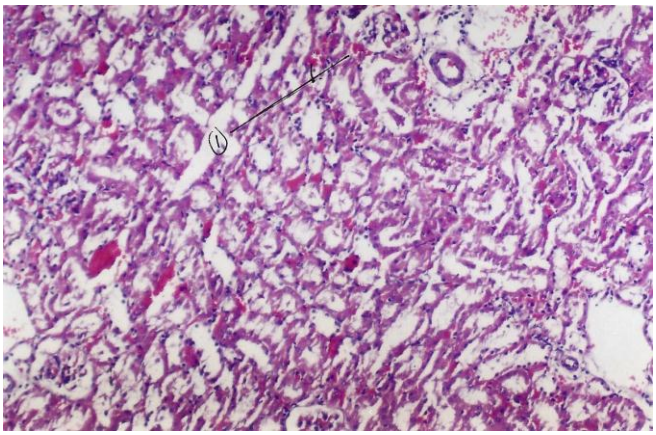
Microscopic appearance of kidneys

Control groups A_1 A_2 and A_3

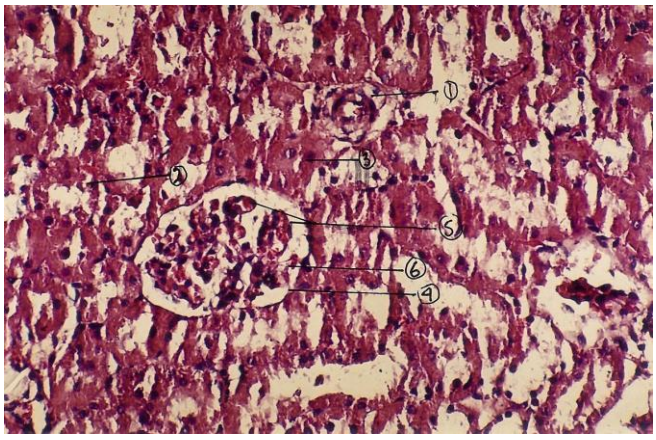
The kidneys of control groups (A_1, A_2) and subgroup (B_1), treated with lead for 7 days showed no abnormality fig (1).

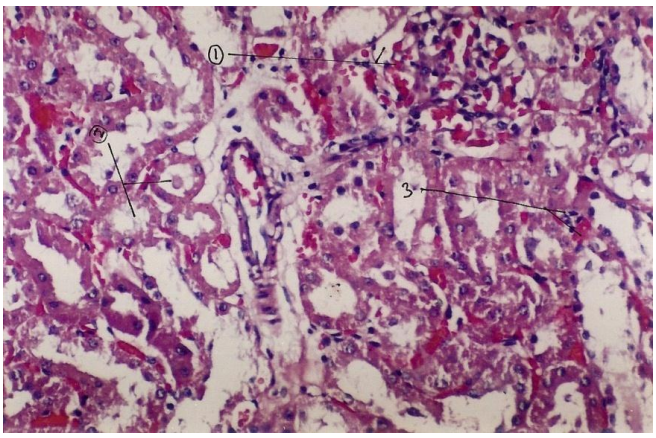
Photomicrograph showing normal kidney of albino rat "control group" showing.
(1) Artery (2) Distal convoluted tubule (3) Proximal Convoluted Tubule (4) Parietal Layer of Bowman's capsule (5) Tuft of capillaries of glomerulus
(6) Visceral layer of Bowman's capsule Staining H&E.
Magnification x 700.

Magnification: × 700 (eye piece x objective lens x numeral aperture × negative enlargement 10 × 20 × 1 × 3.5).
The subgroup B_2 treated for 14 days showed minor changes like extravasation of RBCs in stroma Fig. 2 of renal cortex and medulla appeared normal.



Photomicrograph of rat kidney exposed to lead for 14 days, sub-group (B2) Showing: (1) Extravasations of RBCS in the stroma Staining H&E. Magnification x 350.





Photomicrograph of a kidney exposed to lead for 21 days. Sub-group (B_3) Showing. (1) Glomerulus congested, and capillaries full of RBCs decreased urinary space. (2) proximal convoluted tubule filled with degenerative material, vacuolated appearance of cells and necrosis. (3) Hemorrhagic foci and inflammatory cells in the Interstitium. Staining H&E. Magnification × 700.

In subgroup B_3 the changes appeared more marked. The glomeruli appeared larger in size and more vascular with increased congestion in glomerular capillaries. The proximal convoluted tubules exhibited variable changes, the lumen of few proximal convoluted tubules contained degenerated material. The degeneration was pronounced as shown by marked vacuoled appearance of cells named cloudy swelling. The mean number of necrosed proximal convoluted tubules were highly significant ($P < 0.001$) as compared to (A_3). Nuclear changes varied from condensation to disruption, lastly to complete lysis and disappearances of cells. The interstitium appeared congested with extravasation of RBCs. Fig (3). The haemorrhagic foci in interstitium between Parenchymal cells were 10-11/ HPF. ($R < 0.001$) which was highly significant (Table 3). All these changes were Prominent in cortex, distal convoluted tubules and medulla appeared normal.

Discussion

The present study was designed to asses the effects of lead acetate on kidneys. There was significant decrease in intake of drinking water, aggressive and irritable behavioral changes, black stools in experiment group (B_2) & (B_3).

The control animals of subgroups (A_1, A_2 and A_3) and experimental sub group (B_1) showed normal increase in their body weight. While reduction in body weight in sub group B_2 and B_3 was seen. The mean weight of paired kidneys did not increase in control groups A_1, A_2 and A_3 and experimental group B_1. However there was increase in mean weight of paired kidney in subgroup B_2 and B_3. It was due to edema and hypertrophy of proximal convoluted tubules and congestion of glomerular capillaries these findings were in accordance with those observed by Manish.[12,13]

The increase in congestion of renal tissue in group B_3 indicated by hemorrhages and dark colouration on cut surface is in accordance with the observation made by Parikh [14]. It was caused by spasm and hyaline sclerosis of arterioles and capillaries followed by decreased blood flow to the tissues which leads to further tissue damage.

The renal injuries mainly involved cortical nephrons especially the proximal convoluted tubules, which are the principle site of lead accumulation and retention, such damages result due to inability of cells of proximal convoluted tubules to transport glucose, animoacids, and phosphates producing clinical picture named fancony's syndrome.[9]

The decrease in number of microvilli in cells lining proximal convoluted tubules in the experimental group B_3 and biochemical changes produced by lead also support the histological observation made in present study and are in accordance with the work of Manish.[12]

The changes in proximal convoluted tubules were variable with nuclear changes like pyknosis, margination of chromatin material to cell membranes which is indication of an early necrosis seen in group B_3. The hydropic degeneration changes in the kidney which were consistent with the finding of Robin.[15]

The focal mesengial cells proliferation of glomerular nephritis which is consistent with the finding of Boyd.[16] The presence of inflammatory cells between tubules, and hemorrhagic foci seen in interstium in experimental group B_2 and B_3 are in accordance with other studies and selected morphological parameter, the present study confirm the lead nephrotoxicity in adult male albino rats.

Conclusion

The result of this study adds concern to the use of lead. There is urgent need for further studies on the effects of environmental lead pollution on kidneys and other body systems.

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