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Long-Term Exposure of Lead Acetate on Rabbit Renal Tissue

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Abstract

Background: Lead has been widely used in different industries for ages. It is one of the heavy metals, highly poisonous even at low doses, and has biochemical, physiological and behavioral side effects on human and animals. It has been shown that lead has toxic effects on different tissues such as neural and genitourinary tissues, cardiovascular systems and blood. Therefore, high attention has been paid to its environmental pollutions.

Objectives: Although many histological and biochemical studies have reported about the effects of lead on the renal tissue, there are a few studies about the ultrastructure and morphometric effects of lead on the kidney. Hence, the aim of this study was the evaluation of morphology and morphometrics of rabbit renal urinary barrier ultrastructure following long-term exposure to lead acetate.

Materials and Methods: In this experimental study, 20 male New Zealand rabbits were divided into control and test groups (10 in each). The test group was injected intraperitoneally with chronic dose (8.5 mg/kg of body weight) of lead acetate and for the control group the same volume of normal saline was used, every other day for 10 weeks. After anesthetizing, the biopsies of renal tissues were taken for light and electron microscopic morphometric and morphologic analyses.

Results: Long-term exposure to lead acetate caused histopathology effects including dilatation, congestion, nuclei heterochromatic effects, increase in diameter of renal tubules and urinary barrier thickness in rabbit renal tissue.

Conclusions: Quantitative and qualitative results of long-term lead acetate exposure showed many histopathology side-effects, especially in the urinary barrier.

Keywords: Kidney, Lead Acetate, Morphologic, Morphometric, Urinary Barrier, Glomeruli

1. Background

Lead has been extensively used in different industries for thousands of years. Relevance between lead poisoning and renal disease in humans has been recognized for more than a century. Lead is one of the most important air pollutants and poisonous even at low concentration. It has many side-effects including physiological, mental and histological disorders (1). Exposure to environmental hazardous doses of lead is one of the important health risk factors, particularly in at-risk human and animal populations. Although adults are vulnerable to lead poisoning, children and infants are more at risk due to their lower tolerance and immature immune systems (2). Some of the common lead pollution sources are water pipes made of lead, soldering wire, lead-based paintings, ceramic screens, food packages, pastry powder, leads painting sheets, agriculture products enriched by fertilizers, fungicides, herbicides and so on. Furthermore, lead may contaminate the body through inhaling dust or consuming plant products in polluted farms (3-5). Chronic exposure to heavy metals has adverse effects on humans, animals and plants. Moreover, lead poisoning can affect different systems including neural, cardiovascular and genitourinary (6). Therefore, a lot of attention has been paid to its environmental pollutions (7-9). Recent studies have indicated that it could cause many histochemistry and histopathology changes in renal tissue, including eosinophilic intranuclear inclusions in proximal tubular cells, consisting of lead-protein complexes, mitochondria swelling, and disturbances in proximal tubular function (10). These may lead to severe side-effects such as focal-glomerular sclerosis, glomerular hyalinization, vacuolization, tubular hyperplasia, tubular adenoma, necrosis, tubular dilation and nuclear picnosis (11). However, the severity of adverse effects depends on its plasma concentration and duration of exposure. Clinical signs emerging from renal lead poisoning have been well described

in animal and human models, including glycosuria, aminoaciduria, phosphaturia and Fanconi syndrome (10).

2. Objectives

There are few ultrastructural studies about the lead effects on the kidney on laboratory models. Hence, the aim of this study was the evaluation of histopathological effects of long-term exposure of lead acetate on the renal tissue in rabbit.

3. Materials and Methods

In this experimental study, 20 male New Zealand rabbits weighing 1400 - 1500 g were exposed to the chronic dose of lead acetate. All the experiments were approved by the Ethics Committee of Bushehr University of Medical Sciences. The rabbits were divided into control and test groups (10 in each) and housed in cages separately and fed on pellet and water with no restrictions. The animals were kept in standard laboratory conditions at 22 - 24°C and relative humidity of 40-60%, with 12-hour light/dark cycles. The test group was injected with 8.5 mg/kg of body weight (BW) of lead acetate intraperitoneal; but, for the control group, the same volume of the sterile normal saline was used every other day for 10 weeks of intervention (12, 13). For taking kidney biopsies, the animals were anesthetized by intraperitoneal injection of Ketamine (50 mg/kg of BW) and sacrificed. The kidneys were taken after dissection and fixed in formaldehyde 10%, dehydrated by alcohol, impregnated, paraffin embedded, and 3 μm sections were prepared. The haematoxylin and eosin H & E and periodic acid-schiff (PAS) staining were performed for light microscopy (14). For electron microscopy, after fixation in glutaraldehyde 2.5% and dehydration in acetone, the samples were embedded in araldite resin medium (Proscitech, Australia) and 50 nm sections were prepared and stained with uranyl acetate and lead nitrate (15). Then, the morphology and morphometrics of renal tubules and urinary filtration barrier were assayed using light and electron micrographs [transmission electron microscopy (TEM)]. The morphometrics of light micrographs such as means of small and large diameters of renal corpuscles, distal and proximal convoluted tubules, collecting duct, thick and thin segments of Henle loop, and changes in the thickness of urinary filtration barrier were analyzed using Image Tool (Ver, 3) software.

3.1. Statistical Analysis

The morphometric data and the thickness of different parts of the blood-urinary barrier as well as the diameters of renal tubules were measured. The data of the test groups were statistically compared with the control by t-test and f-test methods using statistical package for social sciences (SPSS) software. The normality of data was analyzed by Shapiro-Wilk test at $P \leq 0.05$. Mean and standard deviation (mean ± SD) was calculated and $P \leq 0.05$ was considered as the significant level.

4. Results

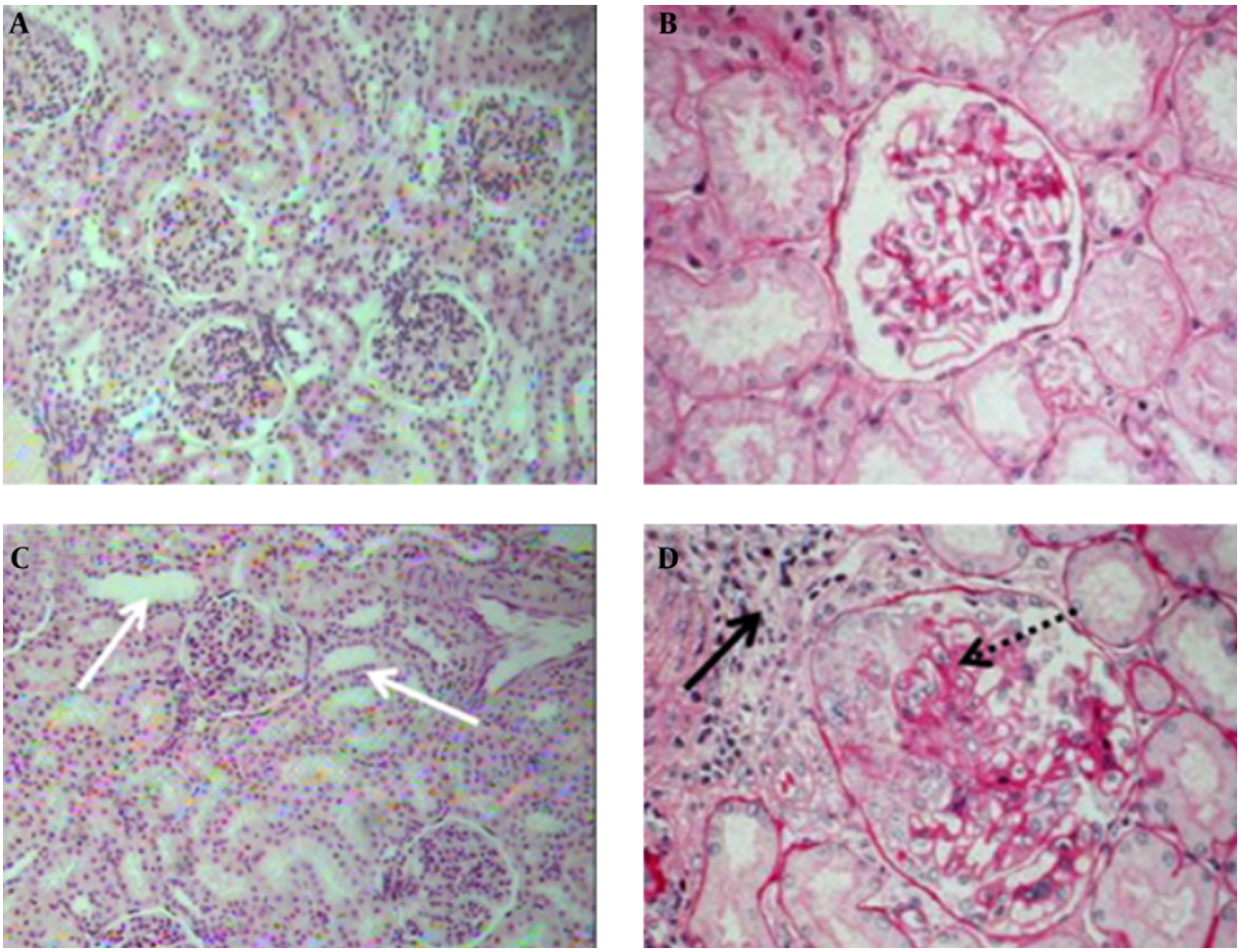
4.1. Morphology

The light photomicrographs of the renal cortex sections of the control group showed that the arrangement of tissue structures including distal convoluted tubules, proximal convoluted tubules, renal corpuscles and the interstitial tissue of tubules were intact and no cellular and tissue damages were found (Figure 1). In addition, other cellular or tissue changes such as inflammation (lymphocyte infiltration), increased fibrosis and interstitial tissue space were not found in the control group. However, distal and proximal convoluted tubules in the test group were dilated and destruction of the cellular structure, congestion, lymphocyte infiltration and fibrosis were observed (Figure 1). It was shown that lead acetate has many effects on the renal tissue following long-term exposure. Electro-micrographs in Figure 2 showed that the ultrastructure of urinary filtration barrier or basal membranes of glomerular podocytes were significantly thicker in the test group compared with the control.

4.2. Morphometry

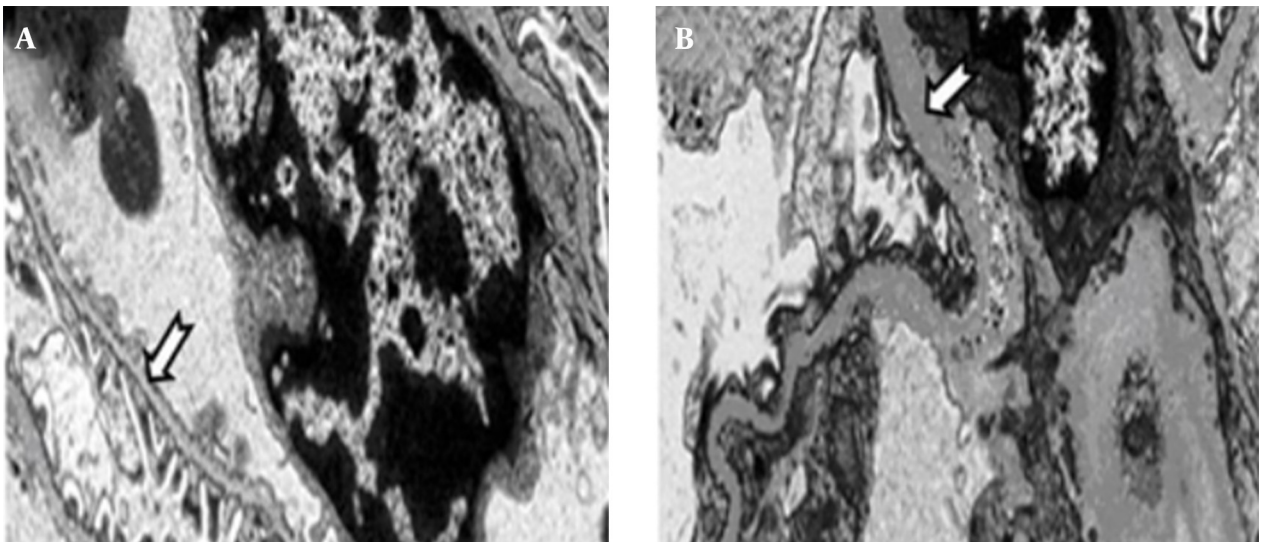
For the assessment of the toxicity of lead on renal tubule cells, leading to impaired urine reabsorption, accumulation of liquid and dilation of tubules, which have important roles in the production of hypertonic urine, the diameter of kidney tubules were measured (Table 1). As shown, the mean diameter of proximal convoluted tubules in the control and test groups were 69.71 ± 1.35 and 85.72 ± 2.12, respectively. Also, the mean diameter of distal convoluted tubules in the control and test groups (71.56 ± 0 .78 and 87.45 ± 1.75, respectively) were increased significantly ($P \leq 0.05$). The mean diameters of renal corpuscle in the control and test groups were 150.21 ± 3.34 and 145.22 ± 2.58, respectively and the mean diameter of renal corpuscles were more decreased in the test group ($P \leq 0.05$), indicating the hazardous effects of long-term exposure of lead acetate on renal corpuscle. Furthermore, the diameter of renal collecting duct has an important role in renal function. As shown in Table 1, the mean diameters of collecting duct in the control and test groups were 135.02 ± 0.53 and 156.02 ± 1.52, respectively. The related data showed that the mean diameter of collecting duct was more increased in the test group compared with the control. Unlike the above results, the diameters of Henle loops (both thin and thick segments) had not changed. The mean thicknesses of thick segments in the control and test groups were 65.14 ± 0.29 and 67.27 ± 1.23, respectively. In addition, the means of thicknesses of thin segments in the control and test groups were 25.16 ± 1.28 and 22.25 ± 1.85, respectively, indicating that the diameters of thin and thick segments of Henle loops were not affected by lead acetate in the control and test groups significantly.

Figure 1. Light Photomicrographs of Rabbit Renal Cortex Exposed to Lead Acetate in the Chronic Phase



A and B, Control group; C and D, test group. Dilation of tubules (white arrow), fibrosis (dashed-black arrow) and lymphocyte infiltration (solid-black arrow); A and C, H & E staining, 100x magnification; B and D, PAS staining, 400× magnification.

Figure 2. Electro-Photomicrograph of Rabbit Renal Glomerular Basement Membrane Exposed to Lead Acetate in the Chronic Phase



Thickness of renal glomerular basement membrane (white arrows) in A, the control and B, test groups (10000× magnification).

Table 1. Morphometric Effect of Lead Acetate Exposure on Rabbit Renal Tubule and Corpuscle in Chronic Phase[a,b]

Tubules and Corpuscle	Diameter, μm	
	Control Group	Test Group
Proximal Convoluted Tubule	69.71 ± 1.35	85.72 ± 2.12[b]
Distal Convoluted Tubule	71.56 ± 0.78	87.45 ± 1.75[b]
Renal Corpuscle	150.21 ± 3.34	145. 22 ± 2.58[b]
Collecting Duct	135.02 ± 0.53	156.02 ± 1.52[b]
Loop of Henle, Thick Segment	65.14 ± 0.29	67.27 ± 1.23
Loop of Henle, Thin Segment	25.16 ± 1.28	22.25 ± 1.85

[a]Data were analyzed with t-test and f-test methods and were expressed as mean ± SD.
[b]Significant difference with the control group; P < 0.05; n = 10.

Table 2. Morphometric Effect of Lead Acetate Exposure on Rabbit Urinary Barrier in Chronic Phase[a,b]

Renal Glomerular Basement Membrane	Thickness, μm	
	Control Group	Test Group
Total Membrane	289.17 ± 0.352	459.15 ± 0.15[b]
Clear Zone, One-Sided	98.27 ± 0.172	140.41 ± 0.91[b]
Dark Zone	190.22 ± 0.62	298.31 ± 0.29[b]

[a]Data were analyzed with t-test and f-test methods and were expressed as mean ± SD.
[b]Significant difference with the control group; P < 0.05; n = 10.

The electro-micrograph of the glomerular barrier including total thickness of membrane, clear zone (one-sided) and dark zone in the test and control groups were shown in Table 2. The thickness of renal glomerular filtration barrier in the test group was significantly affected by chronic dose of lead acetate when compared with the control group at $P \leq 0.05$ significance value.

5. Discussion

Hazardous effects of lead gradually appear in long term. It means that the tolerance to this metal is variable in different animal tissues and emerges by time. However, when the tissue is exposed to high doses of lead for a long term, it can cause immediate and irreversible damages. The toxic effects of lead on different body organs have been widely studied (16). Different tissues such as liver, kidney, brain, bone, bone marrow, fetus, muscle, ovary and especially the testicular tissue could be affected by lead. Kidneys have been reported to be one of the first targets of lead pollutions (17). So far, the reports have shown many side-effects of lead on the function of kidney tissue; while in the present study, the morphologic and morphometric results were evaluated by long-term exposure to the chronic dose of lead acetate. Here, by evaluations of the qualitative results from light photomicrographs, were showed that lead exposure caused dilation in distal convoluted tubules, proximal convoluted tubules and collecting ducts. In addition, the thickness of epithelial cells of tubules decreased and the nuclei of epithelial cells were found to be more heterochromatic. Damage to the renal corpuscles indicated the destruction of mesangial tissue in the vascular pole of corpuscles. The report from Jabeen et al. (18) showed that lead acetate decreased the renal cortical thickness and the diameter of corpuscles significantly and induced the moderate cortical tubular atrophy, indicating the thickening of glomeruli basement membrane; morphometric results of this study are in line with the above findings. The results from other studies have shown that lead can cause increase in peroxidation of fats by decreasing the activity of antioxidant enzymes such as glutathione peroxidase, superoxide dismutase, catalase, glutathione reductase, glucose-6-phosphate dehydrogenase and glutathione S-transferase, leading to oxidative damage to the target tissues following long-term exposure (19, 20). Moreover, in a similar study it was found that lead, even at low doses, had adverse effects on renal tissue (21). Some studies have confirmed the effects of lead on dilation of renal tubules (22); our results are in agreement with these findings. Nephropathy and Fanconi syndrome, in which proximal tubules are functionally damaged, are other toxic effects of lead (23, 24). Another study on environmental effects of lead pollution showed that the exposure to this metal can cause extensive damage to other tissues, particularly the renal filtration barrier (25, 26). In our study, in agreement with the above results, by using light photomicrographs, the dilation of both distal and proximal convoluted tubules, destruction of cellular structure, congestion and lymphocyte infiltration were observed. Moreover, the

mean diameters of distal and proximal convoluted tubules and collecting duct significantly increased in the animals exposed to the lead. In the present study, since glomerular filtration barrier was important in renal tissue and by having the primary function of blood filtration, the ultrastructure of basement membrane of renal glomeruli was also evaluated. In here, the kidney electromicrographs showed that lead acetate could cause an increase in the thickness of urinary barrier. Moreover, morphometric results indicated that the thickness of total basal membrane, clear and dark zones in the test groups were significantly different from the control. In addition, morphological results of the present study showed the congestion of blood stream in renal tissue and it is in agreement with the finding saying that blood flow directly depends on structural health and integrity of urinary filtration barrier (27, 28).

Lead is rapidly absorbed into the bloodstream and deposits in organs like kidneys with more blood and metabolic flows (29). Recent researches indicated that exposure to chronic doses of lead caused several renal histopathology changes such as decrease in microvillus density and disintegration of luminal epithelium of proximal tubules (30). Acute nephritis affects the renal tubules, blood vessels, interstitial tissues and the corpuscle, especially podocytes and capillary loop of the glomerulus (31). Furthermore, changes in ultrastructure of renal tissues such as vacillation of matrix, condensing of mitochondria, dilatation of endoplasmic reticulum and thickening of basement membrane of urinary filtration barrier were observed following long-term exposure to led acetate (32). In agreement with the above findings, our morphometric and morphologic results showed an increase in the dilatation of renal tubules and disarrangement of interstitial tissues following long-term exposure to lead acetate. Moreover, the ultrastructural findings showed an increase in the thickness of basement membrane of urinary filtration barrier. In conclusion, the morphologic and morphometric findings of this study showed that long-term exposure to lead acetate caused renal tubules dilatation, congestion, nuclei heterochromatic changes, decreased tubules epithelial thickness, and increase in the thickness of urinary barrier, which finally lead to renal dysfunctions.

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Footnotes

Authors' Contribution: All the authors contributed to this research article and agreed with the article submission. This was an original research and no part of the work had been published before.

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