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*Full Length Research Paper*

# Toxic effect of lead on kidney function in rat Wistar

**F. Missoun[1*], M. Slimani[2] and A. Aoues[2]**

[1]Departement of Biotechnology, Faculty of Sciences, University of Mostaganem 27000, Algeria.
[2]Departement of Biology, Faculty of Sciences, University of Oran 31000, Algeria.

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**Lead is one of the environmental pollutants that can threat the life of living creatures in many ways. This study was carried out to investigate the effect of lead exposure on kidney function. Fourteen male Wistar rats were divided into 2 groups; group 1 was given a tap water diet and group 2 was given 1000 ppm lead acetate in drinking water for 8 weeks. Lead concentration in blood was determined by atomic absorption. The results showed an increase of calcium in blood. The same happened for phosphaturia and calcium in rats administered with Pb compared to control group. The increase of these parameters would indicate a renal deficiency which is confirmed by a decrease of creatinine and urea in urine samples and presence of calcium oxalate dihydrate crystals observed in samples of urine of exposed rats. All lead-treated rats in the present study have shown intranuclear inclusion bodies in kidney proximal tubular. The determination of the concentration of lead in the blood shows that this factor increases among treated rats. In conclusion, lead administered by oral route causes a renal deficiency to the rats.**

**Key words:** Lead toxicity, nephrotoxicity, chronic lead nephropathy, urine.

## INTRODUCTION

Impaired Kidney functions have been reported as one of the most silent feature of lead toxicity (Chang et al., 1980). Lead nephrotoxicity is characterized by proximal tubular nephropathy, glomerular sclerosis and interstitial fibrosis (Diamond, 2005; Goyer, 1989; Loghman-Adham, 1997). Functional deficits in humans that have been associated with excessive lead exposure include enzymuria, low- and high-molecular weight proteinuria, impaired transport of organic anions and glucose and depressed glomerular filtration rate. A few studies have revealed histopathological features of renal injury in humans, including intranuclear inclusion bodies and cellular necrosis in the proximal tubule and interstitial fibrosis (Biagini et al., 1977; Cramer et al., 1974).

In rats, proximal tubular injury involves the convoluted and straight portions of the tubule (Aviv et al., 1980; Dieter et al., 1993; Khalil-Manesh et al., 1992a, 1992b) with greater severity, at least initially, in the straight (S3) segment (Murakami et al., 1983). Typical histological features include, in the acute phase, the formation of intranuclear inclusion bodies in proximal tubule cells, abnormal morphology (e.g., swelling and budding) of proximal tubular mitochondria (Fowler et al., 1980; Goyer and Krall, 1969), karyomegaly and cytomegaly; and cellular necrosis, at sufficiently high dosage. These changes appear to progress, in the chronic phase of toxicity and with sufficient dosage, to tubular atrophy and interstitial fibrosis (Goyer, 1971; Khalil-Manesh et al., 1992a, 1992b). Glomerular sclerosis has also been reported (Khalil-Manesh et al., 1992a). Adenocarinomas of the kidney have been observed in long-term studies in rodents in which animals also developed proximal tubular nephropathy (Goyer, 1993; Koller, 1985; Moore and Meredith, 1979; Van Esch and Kroes, 1969).

Therefore, the purpose of the present study is to investigate the effect of biochemical alterations of the kidneys following experimental lead poisoning of Wistar rats by chronic exposure to lead acetate as one of the initial events responsible for impairment of renal function.

*Corresponding author. E-mail: missoun_fatiha@yahoo.fr. Tel: 0021375044282. Fax: 0021345265452.

**Abbreviation: Pb,** plomb; **Ca/Ox,** calcium/oxalate; **PbB\PBS,** concentration of lead in the blood; **GFR,** glomerular filtration rate.

## MATERIALS AND METHODS

### Chemicals

All chemical materials that were used in this study including lead acetate were produced by Merck (Germany).

### Animals and treatments

The experiment was conducted in accordance to internationally accepted standard guidelines for use of animals. Fourteen male Wistar rats (90 days of age, 220 - 230 g of body weight) from our breeding stock (Faculty of Sciences, University of Mostaganem, Algeria) were given 1000 ppm lead acetate or tap water as the sole drinking fluid. Rats were maintained on a natural light cycle in an air-conditioned, constant-temperature colony room. Each cage contained one rat which had free access to lab chow and water or lead acetate solution. Urine samples from each rat were collected weekly in presence of sodium azide as antibacterial agent during 8 weeks to analyze some urinary components. Several parameters were followed weekly during the eight week of experimentation including water intake, urinary volume, body weight and crystalluria by polarizing microscopy. Urinalysis reagent Strips (urine) were used for the detection of the following analyzes in urine: Glucose, pH, Specific gravity and Protein.

At the end of the experimental period, urine samples were collected from rats housed in metabolic cages for the estimation of calcium (Mustafa and Mederios, 1985), phosphorus (Fiske and Subbarow, 1925). Urinary creatinine and urea were determined by the alkaline picrate, urease, respectively. Rats were scarified and blood samples were collected. The serum was prepared by centrifuging the blood samples at 3000 rpm for 5 min (Ogbu and Okechukwu, 2001) and serum collected with a Pasteur pipette for the estimation of creatinine, glycemia, iron calcium, and lead concentration.

Serum urea concentration was estimated by the diacetylmonoxime assay (Veniamin and Varkirtzi-Lemonias, 1970). Serum creatinine concentration was determined using Jaffe's reaction as described by Cook (1975). Serum inorganic calcium concentrations were estimated by the method of Ray-Sarkar and Chanhan (1967).

A Perkin-Elmer 3030-B atomic absorption spectrometer equipped with an HGA-400 graphite furnace and an AS-10 autosampler was used to determine lead at the 283.3 nm resonance line (spectral bandwidth 0.7 nm) under gas stop and maximum power conditions. A hollow lead cathode lamp operating at 10 mA was used as the light source. A standard continuous background corrector (deuterium arc bulb) was used. Pyrolytic graphite tubes with L'vov platforms were also used. The ashing and atomization temperatures used were 900 and 1800, respectively. A solution of palladium and magnesium was used as a chemical modifier. Measurements were made using an integrated area mode (Yeager DW, Cholak and henderson (1975).

### Histopathological studies

Slices of left kidney (from seven animals of each group) were fixed in 10% formalin for 24 h and were embedded in paraffin; 5 - 6 µm sections were routinely stained with haematoxylin and eosin (H&E) and assessed in a light microscope (Nikon Eclipse E400). All alterations from the normal structure were registered. The following criteria were used for scoring kidney histology: ++++, a change was very often found in all animals of a group; +++, a change was relatively common in all animals of a group; ++, a change was rare in all animals of a group; +, a change was found in a few animals of a group; ±, a change was sporadic in a group.

### Statistical analysis

The mean ± SEM values were calculated for each group to determine the significance of intergroup difference. Each parameter was analyzed separately using one way analysis of variance (ANOVA). To find the difference between the groups Student't' test was used. P values <0.05 were considered to be significant.

## RESULTS

### Macroscopic observations

No mortality or clinical signs of lead intoxication were observed for any individual of the dose groups over the entire period of the study.

Lead exposed rats showed slower growth in body weight compared to the controlled rats during 8 weeks. The gain of weight decreased gradually from one week to another during the whole period of the lead acetate administration (Table 1). The water intake decreased in rats treated with lead during the experiment, there was significant difference between the two groups in weeks (3, 4, 5, 6,7 and 8) (Table 1).

### Analysis of urinary excretion

The urinary volume of the control rats was 2.5 ± 0.25 ml/24 h, there was a significant increase in the urine output of lead treated rats. Urinary pH and specific gravity remained stable for the two groups through out experiment (Table 2).

Qualitative crystalluria analysis demonstrated the presence of few crystals in the end of experiment (week 7) identified as weddellite (calcium oxalate dihydrate) of four exposed rats, the size of crystals was small and had a pyramidal and envelope shape and apatite (calcium phosphate) of two exposed rats. Cylinders and leucocytes are observed in urine samples of exposed rats by light Microscopy. 0.3 g/l of protein were observed in exposed rats.

### Serum analyses concentration

The serum analyzed concentration in the two groups are shown in Table 3. No significant difference was observed for creatinine concentration between the two groups after 8 weeks of the experiment, however uremia was higher in lead treated rats. Mean serum calcium, iron concentrations were significantly higher in rats treated with lead compared to the control rats. Blood glucose level was significantly lower in the exposed rats. The determination of the concentration of lead in the blood showed that this factor increases among treated subjects.

### Histopathological studies

Macroscopically kidneys appear contracted and have a

**Table 1.** Effect of lead acetate on water intake and body weight in Wistar rats after 8 weeks of experiment.

| Groups | Water intake (ml) | | Body weight (g) | |
|---|---|---|---|---|
| | Control group | Lead group | Control group | Lead group |
| Week 0 | 100. 34 ± 12 | 99.52 ± 17 | 230.23 ± 20 | 224.85 ± 17 |
| Week 1 | 109.42 ± 17 | 93.42 ± 17 | 235.28 ± 12 | 230 ± 23 |
| Week 2 | 106.34 ± 10 | 97.28 ± 8.57 | 240.57 ± 25 | 229 ± 21 |
| Week 3 | 167.14 ± 11* | 106 ± 26* | 244.42 ± 34 | 232.7± 1 28 |
| Week 4 | 146 ± 17* | 113.14 ± 15* | 251.28 ± 28 | 237.7 ± 24 |
| Week 5 | 150.85 ± 20* | 107.42 ± 20* | 254.85 ± 23 | 240.14 ± 21 |
| Week 6 | 158.87 ± 12* | 74.57 ± 23* | 256.5 ± 26 | 232.14 ± 23 |
| Week 7 | 141.34 ± 25* | 87.42 ± 12* | 258.7 ± 20 | 227.24 ± 29 |
| Week 8 | 142.50 ± 23* | 85.44 ± 23* | 259.8 ± 30 | 235.85 ± 35 |

Values are mean ± SEM; n = 7 rats in each group, comparison was between control group vs lead group. With $p < 0.05$; values marked * are significant, g = gram; ml = mililitre.

**Table 2.** Effects of lead on biochemical parameters in urines of Wistar rats after 8 weeks of experiment.

| | Control group | Lead group |
|---|---|---|
| Total volume urine ml /24 h | 2.5 ± 0.25* | 5.74 ± 0.42* |
| pH | 6.24 ± 0.15 | 6.34 ± 0.5 |
| Specific gravity | 1.024 ± 0.005 | 1.023±0.005 |
| Glucose level g/l | ND | ND |
| Protein g/l | ND | 0.3+ |
| Leucocytes | ND | 25+ |
| Cylinders | ND | ++ |
| Créatinine (mg/24 h) | 12.24 ± 0.915 | 7.59 ± 0.438* |
| Urea (g/24 h) | 20 ± 2.22 | 13 ± 5.45* |
| Calcium (mg/24 h) | 3.96 ± 0.178 | 5.08 ± 2.569* |
| Phosphorus (mg/24 h) | 506.4 ± 5.52 | 1076 ± 8.62* |

Values are mean ± SEM; n = 7 rats in each group, comparison was between control group Vs lead group .With $p < 0.05$;values marked * are significant .g = gram; mg = milligram, Keys: ++ = Highly present, + = present, ND = Not detected.

**Table 3.** Effects of lead on biochemical parameters in serum of Wistar rats after 8 weeks of experiment.

| | Control group | Lead group |
|---|---|---|
| Glucose g/l | 0.772 ± 0.073 | 0,458 ± 0.252* |
| Creatinine mg/l | 9.1 ± 0.059 | 10.01± 0.034 |
| Urea g/l | 0.223 ± 0,03 | 0,312 ± 0.034 |
| Calcium (mg/l) | 105 ± 1.915 | 125 ± 4.082* |
| PBS (lead) (µg/l) | 50.6 ± 3.50 | 355 ± 6.55* |

Values are mean ± SEM; n = 7 rats in each group, comparison was between control group Vs lead group .With $p < 0.05$;values marked * are significant. g = gram; ml = milligram; PBS lead = concentration in plasma.

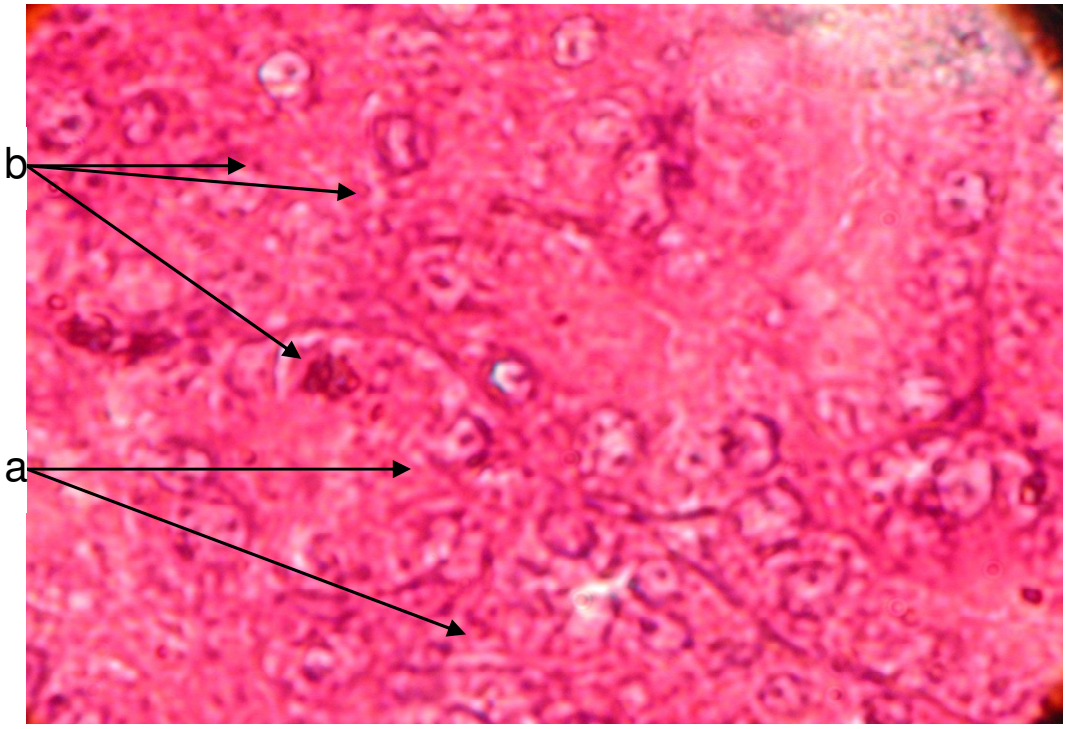


**Figure 1.** Kidney biopsy results in lead treated rats for 8 weeks show: (a) Nonspecific tubular atrophy and interstitial fibrosis. Note the absence of an interstitial infiltrate. (b) Nuclear inclusion bodies (++++) (GR100).

granular surface. The cut surface shows general loss of cortical tissue, corticomedullary demarcation and vascular markings. The pyramids are small but intact. Upon histopathologic analysis, in comparison with respective control rats, lead produced varying degrees of relatively cellular interstitial nephritis. Areas of dilatedtubules alternate with atrophic tubules, rendering a granular appearance to the kidney surface. A large proportion of glomeruli are lost without leaving a trace, which is a characteristic feature. The remaining glomeruli are irregularly distributed, some with periglomerular fibrosis. Glomerular cells had nonspecific abnormalities, such as occasional swelling and distortion of organelles in the cytoplasm, but had normal basement membranes. The glomeruli also showed adhesive glomerulitis,with damage varying from single adhesions to complete obliteration of the capsular space (Figure 1) with development of intranuclear and cytoplasmic inclusions bodies together these inclusions were seen in all lead treated rats (Figure 2).

## DISCUSSION

Kidneys are particularly susceptible to the effect of toxic agents that can cause renal damage and even renal failure. Moreover, many studies show a strong association between lead exposure and renal effects. Acute, high dose lead-induced impairment of proximal tubular function manifests in aminoaciduria, glycosuria and hyperphosphaturia (a Fanconi-like syndrome); these effects appear to be reversible. However, continued or repetitive exposures can cause a toxic stress on the kidney that, if unrelieved, may develop into chronic and often irreversible lead nephropathy (that is interstitial nephritis), confirming the view of a deleterious role for lead. However, developmental studies without concomitant undernutrition are still lacking in the literature (Staessen et al., 1990). The aim of this study was to present the effect of chronic lead intoxication, initiated before conception, on biochemical parameters and renal failure in rats.

This study showed that adult male Wistar rats treated with lead acetate for 8 weeks cause decreases in body weights. This result is in agreement with several studies (Pentenusci, 1988; Hamilton, 1994; Djebli et al., 2004) which suggested that the reduced growth was due to reduced food consumption via lead effects on the satiety set-up. The anorectic effect exercised by this toxic metal used to justify its involvement in the nerve transmission system (catécholaminergic, glutamatergic and serotonin) (Fromentin, 2005). In addition to the anorectic effect in rats treated, water consumption decreases significantly ($P < 0.05$) compared to the control group. This decrease in water intake would be related to dose, which explains the fall of weight in the treaties.

Urinary chemistry is one of the important factors to

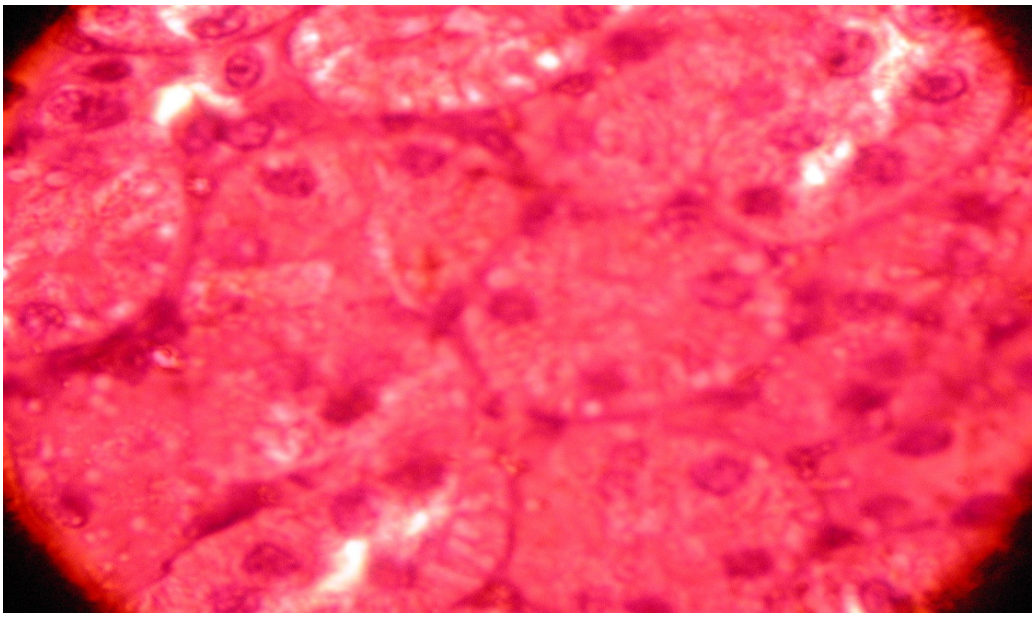

**Figure 2.** Kidney proximal tubular of control rat. Renal glomeruli show normal structure (GR:100).

provide a good indication of the kidneys function. In our results urinary volume is markedly increased in lead group, the increase of urine might be due to diuretic effect of lead or the high concentration of calcium in plasma. In this study no variation in urinary pH and specific gravity was observed. The work of Ghorbe (2001) showed that the lead induces a decrease of urinary pH observed mainly on and after the $45^{th}$ day of the experiment.

The presence of crystals is a risk factor in the pathogenesis of hypercalciuria than renal failure, as has been reported by several authors (Lemann et al., 1991). The amount of crystalluria depends on the level of super saturation above the formation product but also is influenced by the Ca/Ox ratio. An increased urinary calcium concentration is a factor favouring the nucleation and precipitation of calcium oxalate or apatite from urine and subsequent crystal growth. The presence of protein in urine induced the formation of crystals. Super saturation of urinary colloids results in precipitation as a crystal initiation particle which when trapped, acts as a nidus, leading to subsequent crystals growth (Grover and Ryall, 1997). The presence of leucocytes and cylinders in urines of treated rats may be due to the inflammation in urinary tract caused by lead (Cantarow et al., 1962).

The significant increase of calcium and phosphorus ion in urine and the significant increase of calcium in the blood induced by lead acetate might be as a result of impairment of renal function or inhibitory action of lead on cation transport in tissues of the rats (Staessen et al., 1990). In addition, lead also had an inhibitory effect on depolarization induced $ca^{2+}$ uptake and the inhibition was found to be a competitive one. The results sugest that lead calcium messenger system which would have serious consequences on the concentration of calcium in blood, this high concentration may induced hypercalciuria and polyurie which is the beginning of dehydration of rats and the alteration of glomerular filtration rate (GFR) (Staessen et al., 1990). This gradual increase in urinary phosphorus and calcium may lead to the formation of calcium phosphate crystals (Lemman et al., 1991).

The observed decrease in urine and the elevation in the serum of creatinine caused by lead suggest that renal function impairment which might result from intrinsic renal lesions, decreased perfusion of the kidney obstruction of lower urinary tract or due to deranged metabolic process caused by this metal (Cameron and Greger, 1998). Rats treated with lead had significantly decreased urine urea and slight increase in serum urea, serum urea has been reported to increase in acute and chronic intrinsic renal disease and also when there is decreased effective circulating blood volume with decreased ranal perfusion (Cameron and Greger, 1998). Ghorbe et al. (2001) illustrated that oral administration of lead acetate caused significant increase in the blood urea and serum creatinine.

The reduction in serum glucose concentration following the administration of lead may be due to inhibition of the uptake and transport of glucose by lead (Fowler et al., 1980).

The formation of intranuclear inclusion bodies is a pathognomic sign of chronic lead intoxication (Muntner et al., 2003) their accumulation is an early hall mark of lead intoxication nephropathy. The findings of the present study might suggest that lead inclusion bodies may represent accumulation of both extra cellular and intracellular materials as a result of cellular metabolic disturbance and alterations in nuclear membrane

permeability (Bashir et al., 1968).

In the present study, significant increase in the serum lead levels were found in the lead group compared to the control group (Zook, 1972; Falke et al., 1972). The above observations suggest that significant decrements in glomerular filtration rate (GFR) may occur in association with PbB below 355 µg/l and possibly below 100 µg/l. This range is used as the basis for estimates of lead intake that would place individuals at risk for renal functional deficits (Kim et al., 1996b). Experimental studies in laboratory animals have shown that exposures to lead that result in blood lead concentrations exceeding 50 µg/dl can depress glomerular filtration rate and renal blood flow and produce glomerular sclerosis (Aviv et al., 1980; Dieter et al., 1993; Khalil-Manesh et al., 1992a, 1992b).

Increased fractional excretion of calcium and phosphorus, the increase of uremia and creatinemia, the increase of serum calcium concentration and the decrease of creatinuria and glycemia, clinically signifies renal damage.

In comparison with the results of previous studies on male rats in the nephrotoxicity of lead, we conclude that the administration of lead acetate to rats for over 8 weeks induced nephropathy and other associated disorders.

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