

PHYSIOLOGICAL AND HISTOLOGICAL EFFECT OF LEAD ACETATE IN KIDNEY OF MALE MICE

Mus musculus.

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ABSTRACT

Human exposure to lead continues to be a serious public health problem, because lead can cause renal disease and the kidney has the highest concentrations among the soft tissues. Exposure to lead is associated with adverse effects on renal function in laboratory animals and man. An experiment was conducted to study the effect of oral feeding of lead acetate on kidney by estimation the levels of uric acid, urea and creatinine in the serum of mice and the histological parameters of kidney in three different durations. Mice were treated with 0.4 mg/100 ml lead acetate (LA) for 10 days (group A) and for 20 days (group B) and for 30 days (group C).levels of urea in the serum of group A, B, and C increased significantly comparing with the control group, also there was significant differences between group A and group B and between group A and group C, but there was no significant differences between Group B and group C. Level of serum uric acid in groups A, B, C increased significantly comparing with the control group but there was no significant differences among treated group.Serum creatinine levels in group A increased none significantly comparing with the control group. While increased significantly in both of group B and group C, there was significant differences between group A and group C, but there was no significant differences between group A and group B.Kidney sections in group A characterized by foci of congestion and heamorrhage.Glomerular swelling revealed in the kidneys of group B, but the kidney of group C revealed vasculitis, heamorrhage and early hyalinization, normal gromeruli appear in all treated groups.

Introduction:

Heavy metals such as lead are a major environmental and occupational hazard. These non-essential elements are toxic at very low doses and non-biodegradable with a very long biological half life. Thus, expuser to heavy metals is potentially harmful (1).

Human exposure to lead continues to be a serious public health problem (2). The exposure sources include lead in the workplace, in dust and soil, in folk remedies, in crystal or ceramic containers, and in hobby-related materials (2, 3).

Lead exposure is associated with neurologic (4, 5), growth (6), and reproductive defects (6-14). Lead exposure in men has been associated with abnormalities of spermatogenesis (8). An inverse relationship between blood lead and sperm concentration has been reported (10, 11).

Environmental lead poisoning is an increasing health burden and chronic exposure to high levels of lead leads to adverse effect on renal function in both animals and humans(15).lead induced renal damage also occurs in the absence of acute intoxication so that occult lead nephropathy may not be recognized as such restek-Samarzija et al (16).Chronic accumulation of lead in the body eventually leads to impairment in renal function

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(17) .Urea and creatinine are a waste product of amino acid metabolism they removed by kidney(18) biochemical tests such as serum creatinine, urea and uric acid can help diagnose kidney diseases creatinine is an end-product of muscle metabolism it is produced continuously in the body and is excreted in the urine, plasma creatinine can be used as an index of glomerular filtration rate(GFR),GFR and serum creatinine are inversely related. The same relationship is observed for several other substances whose excretion depends on GFR for example when GFR falls, the plasma urea or blood urea rises in similar fashion. (19)The hypothesis advanced by some workers suggesting that a high dose of lead stimulates renal cortical hypertrophy and then result of tubule interstitial changes characterized by interstitial fibrosis, tubular atrophy, and dilatation (20). Recent epidemiological studies have suggested that even low blood levels of lead can be associated with chronic kidney diseases (21, 22, 23, and 24) in the general population, while in most animal studies, acute low-dose lead-treatment caused no significant pathological changes in rats (20),so In present study we examined the effect of high dose of lead acetate 0.4 (gm/100ml) on laboratory mice because the disparity in the onset of renal function abnormalities reported in laboratory animals appear to be related to the duration and dose of lead administration (20).

Materials and methods

Animals:

Twenty four (weight 18-21g) mature male mice of 6 weeks age were housed in plastic cages measuring about (29×15×12) cm, with about four mice per cage. Floors of cages were covered with soft crushed wood shaving; all cages were washed two times per week with 70% alcohol throughout the period of the study.

The animals were kept in the animal house of Suleimani university thus in an air conditioned room with an optimum temperature of 24 ± 2 °C and exposed to about 12-14 hours/day light program, these conditions represent the optimum environmental conditions which are required by the mice. During the period of the experiment abnormal and sick rats were excluded. Water and food was locally prepared and consists of available constituents which fulfill the mice dietary requirements.

Experimental design:

Twenty four mature male mice at the age of six weeks were included in this study. The present work was first divided the 24 mice into four groups as the following:

- 1-Control group: this group was orally administrated normal saline during the four weeks period of the experiment.
- 2- Treated Group (A): this group was orally administrated of 0.4(gm/100ml) lead acetate (CH₃COO) 2Pb during the 10 days period of the experiment.
- 3- Treated group (B): this group was orally administrated 0.4(gm/100ml)of lead acetate during the 20 days period of the experiment.
- 4-Treated group(C): This group was orally administrated 0.4(gm/100ml) lead acetate during the 30 days period of the experiment.

At the end of the period of treatment, blood was collected by heart puncture and serum was separated to estimate serum urea, creatinine and uric acid levels.

Histology

Kidney was isolated and fixed in 10% neutral buffered formalin for 72 hours, and dehydrated through increasing concentrations of ethanol and embedded in paraffin. Serial sections were cut, and stained with hematoxylin and eosin.

STATISTICAL ANALYSIS

We analyzed mean data by analysis of variance and performed post –hoc significant level was $P < 0.05$. Values was mean \pm SEM. We analyzed data using the commercially available software package SPSS (SPSS.Inc, Chicago, IL).

RESULTS

Table (1) showed that Compared to control mice, lead acetate treated mice had significant increasing of blood urea ($P < 0.05$), in treated group (T1) for 10 days duration was 54.7 ± 0.4 (mg/dL), in Treated group (T2) for 20 days duration was 57.2 ± 0.47 (mg/dL) and in treated group (T3) for 30 days duration was 58.9 ± 0.37 (mg/dL), while in control group was 51.82 ± 0.92 (mg/dL). Also the table showed that significant

difference between level of blood urea of groups (T1 and T2) and between (T1 and T3), while there is no significant differences between ($P>0.05$) (T2) and group (T3).

Table (2) showed that compared to control mice lead acetate treated mice has significant increasing ($P<0.05$) in level of serum uric acid, in group (T1) for 10 days duration serum uric acid was 8.9 ± 0.04 (mg/dL), in group (T2) for 20 days duration, serum uric acid was 9.3 ± 0.0 (mg/dL) in group (T3) for 30 days duration serum uric acid was 9.6 ± 0.07 (mg/dL), but in control group serum uric acid was 6.7 ± 0.86 (mg/dL). But there were no significant differences among treated groups.

Table (3) showed that compared to control mice, lead acetate treated mice group (T2) for 20 days duration had significant increasing of serum creatinine ($P<0.05$), was 0.68 ± 0.07 (mg/dL), and also increased significantly in group (T3) for 30 days duration was 0.76 ± 0.01 (mg/dL), while in control group serum creatinine was 0.55 ± 0.04 (mg/dL). but there was no significant differences ($P>0.05$) between level of serum creatinine of control and T1 groups, and there was no significant differences among different treated groups T1, T2 and T3.

Renal histological section of control

mice revealed normal glomerulus with lobular organization and flat capsular epithelium. The tubules are regular lined by cubic epithelium, as appear in figure (1).

Renal histological section of group (A)

(10 days treated mice) revealed some normal glomeruli and some foci of congestion and hemorrhage, as appear in figure (2). Renal histological section of group B (20 days treated mice) revealed areas of congestion, hemorrhage with glomerular swelling as appear in figure (3) Renal histological section of group C (30 days treated mice) revealed some area of hemorrhage, glomerular hypertrophy and swelling and in other area early hyalinization appeared as in figure (4).

DISCUSSION

The present study was undertaken to evaluate the

function and structure of kidney after exposition to 0.4 (gm/100ml) of lead acetate in three different duration. The lead acetate is nephrotoxic (15) so the structure of kidney was assessed on the basis of histopathological analyses and we evaluated kidney function by measuring serum creatinine, urea and uric acid. Kidney of mice exposed to this metal resulted in serious changes in the histology and function of this organ. Histological section of kidney of 10 days treated mice revealed some normal glomeruli and some foci of congestion and hemorrhage whereas some glomerular swelling in the group which treated for 20 days and areas of normal glomeruli with hyalinization, congestion and hemorrhage appeared in the group which treated for 30 days with lead acetate. Similar or more advanced changes in kidney histology and function under lead influence, have been reported by others, administration of lead in animals causes progressive nephropathy the severity of which is related to the dose and duration of exposure, but, acute low-dose lead-treatment caused no significant pathological changes in rats with increase in serum creatinine and urea nitrogen (20). Khalil-Manesh and coworkers (20, 25) have shown that exposure to high doses of lead acetate in the drinking water causes tubule interstitial inflammation followed by fibrosis, glomerulosclerosis, and azotemia, whereas long duration of exposure to low-dose of lead are needed to induce only mild to moderate renal fibrosis and tubular atrophy (26). Lead acetate treated mice had significant increasing of blood urea, uric acid and serum creatinine with increasing duration time. Similar changes in kidney function have been reported by others, Khalil-Manesh and coworkers' acute low-dose lead-treatment caused increase in serum creatinine and urea nitrogen (20). Alterations in renal parameters upon lead exposure were also addressed others. Increased levels of blood urea were encountered beginning with week 14 in calves which fed from the 4th to 20th week of age on a milk powder diet containing 40 mg lead acetate per kg dry substance (27). Oral administration of lead acetate in the diet of mice at concentration 0.5% (W/W) for 1 month induced a significant increase in serum urea and creatinine in comparison with the control group (28). The effect of chronic lead exposure on kidney function in male and female rats was investigated

.Lead acetate was administered orally at the rate of 0.3 and 0.6%. The treatment continued for 15, 30, 45, 60 and 90 days, and the results showed an increase of creatinemia and uremia on the 30th day of the experiment in both sexes (29). Oral administration of 1000 or 2000 ppm lead acetate in albino rats caused significantly increasing in serum urea, uric acid and creatinine (30).

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Table (1): Means of blood urea in lead acetate treated 0.4(gm/100ml) for three durations) and control mice.

Treatment type group	Means±SE.(mg/dL)
Control(zero time)	51.82±.90 ^a
T1(10 days duration)	54.75±0.4 ^b
T2(20 days duration)	57.25±0.47 ^c
T3(30days duration)	58.97±0.37 ^c

Table (2) means of serum uric acid in lead acetate treated 0.4(gm/100ml) for three durations) and control mice

Treatment type groups	Means±SE.(mg/dL)
Control(zero time)	6.7±0.86 ^a
T1(10 days duration)	8.9±0.04 ^b
T2(20 days duration)	9.3±0.1 ^b
T3(30 days duration)	9.6±0.07 ^b

Table (3) means of serum creatinine in lead acetate treated 0.4(gm/100ml) for three durations) and control mice.

Treatment type groups	Means±SE.(mg/dL)
control(zero time)	.55±0.04 ^a
T1(10 days duration)	.63±0.01 ^{ab}
T2(20 days duration)	.68±0.07 ^{b c}
T3(30 days duration)	.76±0.01 ^c

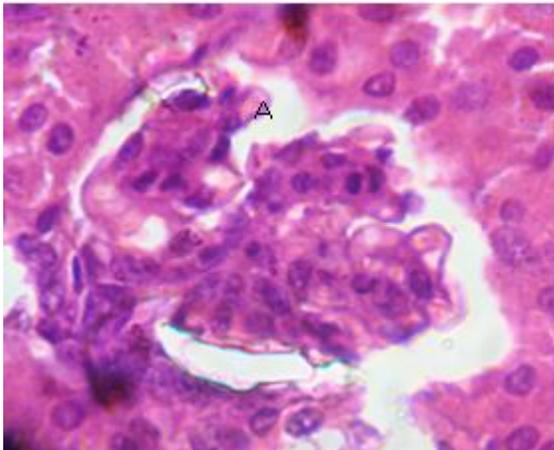


Figure (1) Renal section of control A: normal glomerulus.

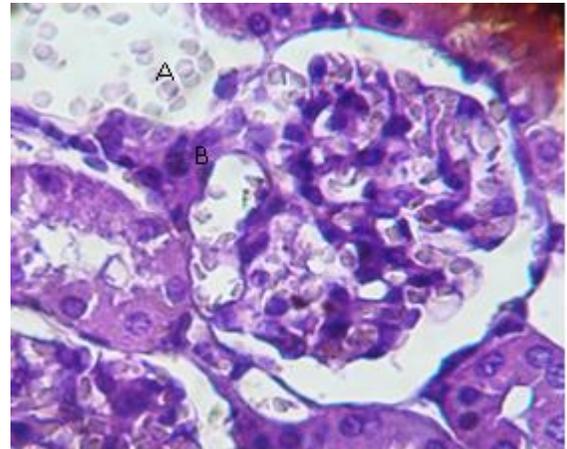


Figure (2) Renal section of group A A: hemorrhaged B: congested area

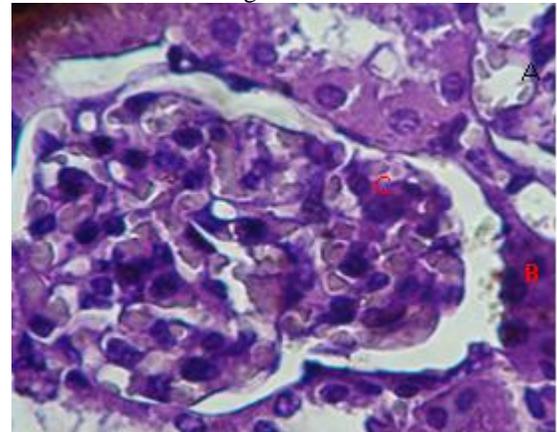


Figure (3): Renal section of group C A: hemorrhaged area B: congested area C: Swelling glomerulus A: haylinized area

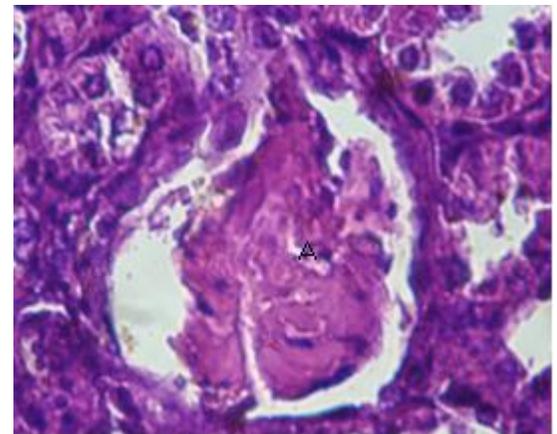


Figure (4): Renal section of group D

التأثيرات الفسيولوجية والنسجية لخلات الرصاص على وظائف كلية ذكور الفئران المختبرية

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الخلاصة:

ان تعرض الانسان المستمر للرصاص اصبح مشكله صحيه عالميه فالرصاص يسبب امراض للكليه والكليه من الانسجه الرخوه التي تحتوي اعلى نسبه للرصاص والتعرض المستمر للرصاص يؤدي الى تأثيرات مضاده لوظائف الكليه سواء للانسان او للحيوانات المختبريه.اجريت الدراسه الحاليه لمعرفة تاثير تناول الفموي لخلات الرصاص على الكليه من خلال دراسة المقاطع النسيجية للكليه مع دراسة مستويات اليوريا وحامض اليوريك والكرياتينين في المصل في ثلاثة فترات زمنية مختلفه للتعرض. عوملت الفئران ب ٠.٤ غرام/١٠٠مليتر لمدة عشرة أيام (المجموعه A) ولمدة عشرين يوماً (المجموعه B) ولمدة ثلاثين يوماً (المجموعه C). المقاطع النسيجية لكلية فئران المجموعه A أتصفت بمناطق نزيفيه وأحتقان أما انتفاخ الأوعيه الدمويه فقد ظهرت في كلية المجموعه B اما كلية المجموعه C أتصفت بمناطق التهابيه في الاوعيه الدمويه مع نزيف و تليف في الانسجه نتيجة القصور الدموي مع تواجد نفرونات طبيعيه في المجاميع الثلاثه. ارتفعت مستويات اليوريا في مصل دم المجاميع الثلاثه (A وB وC) بشكل معنوي مقارنة مع مجموعه السيطرة وكانت الفروقات معنويه بين المجموعتين (A وB) وبين المجموعتين (A وC) ولكن الفروقات لم تكن معنويه بين المجموعتين (B وC)، وانخفض مستوى اليوريا في مصل المجموعتين (A وC) معنويا بالمقارنه مع المجموعه B. ارتفعت مستويات حامض اليوريك في مصل المجموعات المعامله الثلاثه (A وB وC) بشكل معنوي مقارنة بمجموعه السيطرة في حين الفروقات لم تكن معنويه بين المجاميع الثلاثه، كما اظهرت النتائج ارتفاعا غيرمعنويا في مستويات الكرياتينين في المصل المجموعه (A) ومعنويا في المجموعتين (B وC) مقارنة مع مجموعه السيطرة وكانت الفروقات معنويه بين المجموعتين (A وC) في حين لم تكن معنويه بين المجموعتين (B وA). المقاطع النسيجية لكلا المجموعه (A) اتصفت بمناطق نزيف واحتقان، بينما انتفاخ النيبب المجموعه ظهرت في كلا المجموعه (B)، اما كلا المجموعه (C) فقد اظهرت مناطق التهاب في الاوعيه الدمويه مع نزف وتليف في نسيج الكليه، النبيبات الطبيعيه ظهرت في كل المجاميع المعامله