

Study Some of the Histopathological Changes of Acute, Subacute and Chronic Lead Acetate Toxicity related to Catalase Activity in Blood of Adult Male Wistar Rats

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Abstract

The present work was aimed to study the histopathological changes due to lead acetate intoxication and measure the activity of catalase in the serum of adult male rats. Sixty male rats aged (8) weeks and weight (45-60) gm were divided equally into (4) groups as follows: Group I: Rats served as control (C) and received distilled water for 3 months. Group II: Rats served as experimental and received by gavage lead acetate diluted in distilled water at 100 mg/kg B.W. /day for 1 month. Group III: Rats served as experimental and received by gavage lead acetate diluted in distilled water at 100 mg/kg B.W. /day for 2 month. Group IV: Rats served as experimental and received by gavage lead acetate diluted in distilled water at 100 mg/kg B.W. /day for 3 month. Blood samples were collected by cardiac puncture to estimate serum catalase activity. Specimens from brain, liver, kidneys, urinary bladder and spleen, were dissected out for histopathological examination. Results should time dependent significant decrease in serum catalase activity with toxicopathological changes in the targeted organs, the severity of the changes depend on time of exposure, with hyperplasia of urinary bladder. From the above results, it was concluded that lead acetate intoxication lead to significant decrease in serum catalase activity with toxicopathological changes in the targeted organs, these changes were time dependent.

Keywords: Lead Toxicity, Lead Acetate, Catalase Activity, Heavy Metals Toxicity, Rat.

دراسة بعض التغيرات المرضية النسجية للتسمم الحاد تحت الحاد والمزمن لخلات الرصاص

وفاعلية انزيم الكاتالاز في الدم في ذكور الجرذان البالغة

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الخلاصة:

هدف البحث الحالي الى دراسة التغيرات المرضية النسجية الناجمة عن التسمم الحاد، تحت الحاد والمزمن بخلات الرصاص وقياس فاعلية الكاتالاز في مصل ذكور الجرذان البالغة. استخدم في الدراسة (60) جرذ ويعمر (8) اسابيع واوزان تراوحت بين (45-60) غم حيث قسمت الى اربع مجاميع متساوية وكما يأتي: المجموعة الاولى اعطيت ماء مقطر لمدة ثلاثة اشهر وعدت مجموعة سيطرة، المجموعة الثانية جرعت محلول خلات الرصاص وبتريز 100 ملغم/كغم/يوم ولمدة شهر، المجموعة الثالثة جرعت محلول خلات الرصاص وبتريز 100 ملغم/كغم/يوم ولمدة شهرين، المجموعة الرابعة جرعت محلول خلات الرصاص وبتريز 100 ملغم/كغم/يوم ولمدة 3 اشهر. تم جمع عينات الدم من مباشرة وذلك لقياس مقدار الكاتالاز في المصل، كما تم اخذ عينات من الدماغ، الكبد، الكلية، المثانة، الطحال، لغرض اجراء الفحوصات المرضية النسجية. اوضحت النتائج ان هنالك قلة معنوية في فعالية الكاتالاز في المصل اعتمدت على مدة التجريع. اما التغيرات المرضية النسجية فقد ادى التجريع لخلات الرصاص الى تغييرات مرضية سمية وخيمة في الاعضاء المستهدفة تتناسب شدتها طرديا مع مدة التجريع حيث اتسمت ايضا بحصول فرط التنسج في المثانة. يمكن ان يستنتج من النتائج الذكورة اعلاه ان التسمم بخلات الرصاص ادى الى نقصان معنوي في فعالية الكاتالاز في المصل وتغيرات مرضية سمية في الاعضاء الداخلية المستهدفة ازدادت شدتها بزيادة مدة التجريع.

Introduction

Environmental pollution is the presence of a pollutant in environment such as air, water, soil and consequently in food which may be poisonous or toxic and will cause harm to living things in the polluted environment (1). The excessive amount of pollutants such as heavy metals in animals feed and feed stuffs are often due to human actions, resulting from either agricultural or industrial production or accidental or deliberate misuse (2,3,4). Many heavy metals, including lead, are known to induce over production of reactive oxygen species (ROS) and consequently enhance lipid peroxidation, decrease the saturated fatty acids and increase the unsaturated fatty acids contents of membranes (5). In addition, ROS are highly reactive to membrane lipids, protein and DNA. They believed to be the major contributing factors to stress injuries and to cause rapid cellular damaged (6,7,8,3). Target organs affected by lead are bones, brain, blood, kidneys and thyroid glands (9). Biochemical and molecular mechanisms of lead toxicity is poorly understood (10). Therefore, the aim of the present study was to make knowledge on the toxicopathological

effect of lead acetate in adult rats and measure the activity of catalase the blood.

Materials and Methods**- Experimental Animals**

Sixty male rats were used for the present study. The animals were housed in metal cages in the animal house of the Veterinary Medicine College- University of Baghdad and were fed on standard rat pellets, with water provided *ad libitum*, they were allowed to acclimatize for 10-14 days at room temperature.

Dose calculation:

The lead acetate was calculated according to (11).

Experimental Protocol

Sixty male rats aged (8 weeks) and weights (45- 60 gm) were divided equally into 4 groups: **Group I:** Rats served as control (C) and received distilled water for 3 months. **Group II:** Rats served as experimental and received by gavage lead acetate diluted in distilled water at 100 mg/kg/ B.W. /day for one month. **Group III:** Rats served as experimental and received by gavage lead acetate diluted in distilled water at 100 mg/kg/ B.W. /day for two months. **Group IV:** Rats served as experimental and received by gavage

lead acetate diluted in distilled water at 100 mg/kg/ B.W. /day for three months.

Catalase Assay

Serum catalase assay was done according to (12).

Blood Collection

Blood collection were done every month of experiment via cardiac puncture technique.

The blood were collected in plain tubes and used to separate serum which stored at (-20 C), then for measurement of catalase.

Histopathological Study

At the end of each period of experiment. Five animals of each group were sacrificed by intramuscular injection of high dose of ketamin hydrochloride. Specimens were taken from brain, liver, kidneys, urinary

bladder, spleen, the tissues were fixed in 10 % formaldehyde solution then processed routinely by using the histokinette. Tissue sections were embedded in paraffin, sectioned by microtome and stained with hematoxylin and eosin (13).

Statistical Analysis

Least significant difference (L.S.D.) was used to compare the significant difference between means. Data were analyzed using statistical analysis system (SAS) (2001) program.

Results and Discussion

Catalase Activity:

The results of catalase activity in serum (KU/L) are shown in table (1). The values 1st, 2nd, 3rd months of lead groups (48.12± 5.36; 35.06± 2.16; 21.04± 1.48) compared with control.

Table 1: Values of catalase (mg/ gm) in serum of experimental groups rats at different exposure.

| Groups | MEAN ± SE |
|--------------|----------------|
| Control | 61.2 ± 4.83 A |
| First Month | 48.12 ± 5.36 B |
| Second Month | 35.06 ± 2.16 C |
| Third month | 21.04 ± 1.48 D |

L.S.D. 12.18

Catalase is a universally present oxido-reductase that decomposes H₂O₂ to water and molecular oxygen and it is one of the key enzymes involved in removal of toxic peroxides (3). A decline in catalase activity in serum in lead acetate toxicity was observed in the present study which suggests a possible delay in removal of H₂O₂ and toxic peroxides mediated by catalase and in turn an enhancement in the free radical mediated lipid peroxidation under lead toxicity. This results agreed with (14,15,16).

Histopathological Findings

*** Brain**

First Month: there is mild perineuronal and perivascular edema, shrinkage of neurons appeared dark in color (Fig 1), with slight demyelization of white matter.**Second Month:** Increase in the amount of edemation fluid with severe congestion of cerebral and menigeal blood vessels.**Third Month:** In addition to previous pathological lesions there is severe demyelination of the brain stem and white matter (Fig 2),vacuolation of neurons and disappearance of Nissl granules (fig3).

*** Kidneys**

First Month: Severe congestion of glomerular tufts, others showed atrophy of their tufts with degeneration and necrosis of cortical renal tubules with cystic dilation of cortical and medullary renal tubules containing eosinophilic hyaline casts (Fig 4). Also intranuclear inclusion bodies are numerous in the epithelial cells of renal tubules (Fig 5).**Second Month:**In addition to the histopathological lesions noticed in the previous period perivascular cuffings with moderate infiltrate of mononuclear cells were seen . Hemosiderin pigment are deposited in the epithelial cells lining the cortical renal tubules .Many glomeruli underage hyperplasia of epithelial cells lining the parietal layer of the Bowman`s capsule with periglomerular infiltration of inflammatory cells (Fig 6).

***Urinary bladder:**

First and second month: Mild hyperplasia of the transitional epithelium.**Third month:** Marked papillary hyperplasia of the epithelial lining with increase in secretions (fig7)

***Liver**

First Month: Extensive areas of coagulative necrosis and hemorrhage with increase in the apoptotic cells (Fig8). Infiltrate of inflammatory cells in the walls and around central veins more also noticed. **Second and Third Month:** There is severe congestion of sinusoids and central veins, In addition to hyperplasia and slight peribiliary fibrosis with infiltration of inflammatory cells (fig9) with deposition of hemosiderin pigment in the portal and inflammatory cells mainly neutrophils in their Lumina. Other sections showed mononuclear infiltration in the dilated blood sinusoids.

***Spleen**

First Month: Severe congestion of blood sinuses with moderate depletion of white pulp lymphoid tissue (Fig 10).**Second Month:** Slight thickening of the capsule and trabeculae with infiltrate of inflammatory cells within the blood sinuses.**Third Month:** Marked depletion of lymphoid follicles of white pulp, with hypertrophy of muscular layer of the arterioles and thickening of their walls and vacuolation of the their endothelial linings and muscular layer (Fig 11). Deposition of hemosiderin pigment was also seen (Fig12).

Discussion

The results showing that the animals exposed to lead acetate exhibited edema of the brain and that may be attributed to the disruption of the blood brain barrier (BBB) function leading to disturbance in blood dynamics and escape of the fluid to the nervous tissue and that agree with the previous studies which explained that the first step in the neurotoxic effects of lead might be primarily related to damage to the permeability of BBB through up regulation and activation of the transient receptor potential canonical channel TRPC1/TRPC4 channels in rat brain endothelial cells (17,18,19,20,21) suggested that (BBB) dysfunction is a contributing mechanism in lead neurotoxicity in an *in Vitro* model of the (BBB).

The degenerative changes of the neurons of brain represent ischemic changes resulting from injury of blood vessels (22) . Furthermore lead accumulates in and damages mitochondria (23). Biosynthesis and function of neuronal mitochondria activity is affected by lead with disruptive effects on synaptic transmission in the brain ,lead also affecting brain cells via excitotoxicity and apoptosis(24).Similar results

showed by (25) using different toxic doses of lead acetate on the central nervous system of male mice.

Demyelination of the brain stem was noticed in chronic stages due to toxic effects of lead on Schwann cells, this agreed with (26,25), who predicted that if Schwann cell damage were to be implicated in lead neuropathy, demyelinated internodes would be distributed randomly among myelinated fibers in the affected nerves, whereas segments demyelinated in a process secondary to axonal degeneration would tend to be clustered within certain fibers. The present result agrees with (27) who noted demyelination of the brain stem and spinal cord in rats treated with 75mg/kg B.W /day of lead acetate for 60 days.

The cystic dilatation of cortical and medullary renal tubules with formation of hyaline cast may be related to the toxic effect of lead acetate leading to renal insufficiency resulting in decreased excretion and lead to the accumulation of lead acetate metabolites within the cortical tubules and also sloughing of cells lining these tubules leading to the formation of hyaline cast. The eosinophilic intranuclear inclusion bodies were seen in the kidneys of toxic groups. This result was agreed with (27) and (28) who described the presence of these inclusion bodies in both liver and kidney tissues. These bodies were seen in the 1st and 2nd periods of experiment than at the 3rd period and that because of severe necrosis of epithelial cells at the later stages of the experiment and according to (29) these inclusions are intranuclear protein matrices upon which metallic ions are deposited and are considered a pathognomonic lesion for lead poisoning.

Toxic groups showed papillary hyperplasia of mucosa of urinary bladder forming micropapillae covered by a hyperplastic urothelium of varying thickness in a time dependent manner. According to (30) preneoplastic changes have been identified following exposure of lab animals to toxic chemical agents. The most important one is reparative hyperplasia which involved disruption of homeostasis, its severity increases with dose and time, and it is not seen in controls but it is still reversible during the recovery segment after exposure to a toxic substance. When reparative hyperplasia continues beyond a certain threshold of time and dose it progresses to preneoplastic hyperplasia, which further progresses with continued stimulation to frank neoplasia.

The significant pathological changes in liver section were the extensive areas of coagulative necrosis and hemorrhagic with increase in the apoptotic cells. Necrosis may result from the accumulation of lead in the mitochondria and lysosomes progressive hepatocyte organelles damage, cellular degeneration and necrosis or it may result from hypoxia in the perivenular region with increase in hepatic oxygen demand without an appropriate increase in hepatic blood flow similar results were obtained by (31,32,33). Previous study carried by (34) indicated that necrotic lesions can be a cause of oxidative stress induced by lead. Tissue sections show apoptosis of hepatocytes. Apoptosis is an active and highly regulated form of cell death responsible for the cellular demise of the hepatocytes which occur due to the toxic effect of lead and that agreed with (35). (36) identified macrophage as a critical regulator of fibrosis. Like

myofibroblasts these cells are derived from either resident tissue populations like kupffer's cells or from bone marrow immigrants (37,38,39)

The histopathological changes of spleen tissues showing depletion of lymphoid tissue which become more severe at the end of the experiment and that can be related to the effect of the compound which cause depression in immune system .Similar results were obtained by (32).Deposition of hemosiderin pigment in large amount was due to liberation and deposition of

iron from extravasated RBCs. The experimental findings of the present research demonstrate the ability of lead acetate to cause vascular changes in the splenic arterioles and that agreed with the previous studies which concluded that lead con causes endothelial injury /dysfunction ,impede endothelial repair ,reduce endothelial cells growth ,stimulate vascular smooth cell proliferation and there is a positive association between lead exposure and peripheral arterial diseases (40).

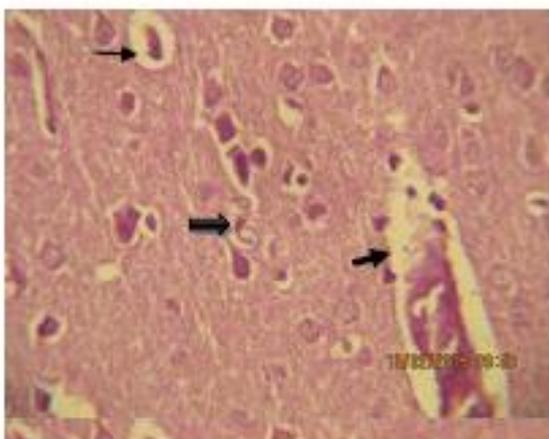


Figure (1): Brain of rat treated with 100mg /Kg BW/day of lead acetate for one month showing neuronal and perivascular edema (→) with shrinkage of neurons (→) (H&E100 X)

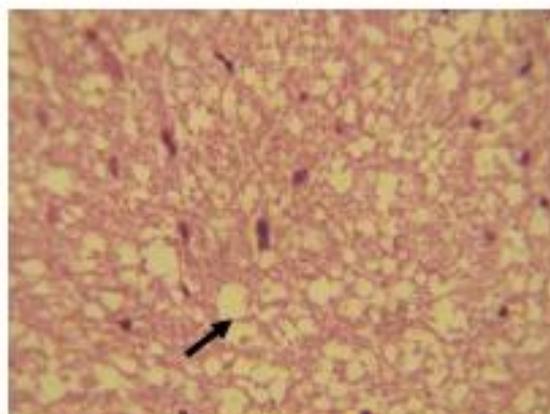


Figure (2): Brain of rat treated with 100mg /Kg BW/day of lead acetate for three month showing severe demyelination of white matter (→)

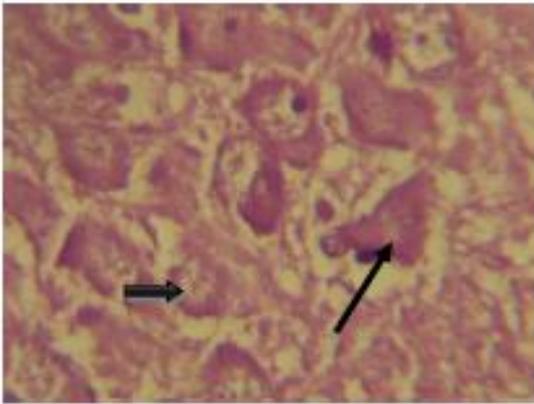


Figure (3): Brain of rat treated with 100mg /Kg BW/day of lead acetate for three month showing vacuolation of neurons(→) and disappearance of Nissl granules (→) (H& E 200X).

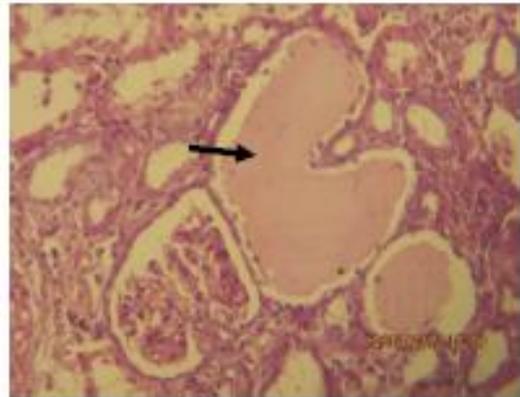


Figure (4): Kidney of rat treated with 100mg /Kg BW/day of lead acetate for one month showing cystic dilation of renal tubules containing eosinophilic hyaline cast (→) (H& E 200X).



Figure (5): Kidney of rat treated with 100mg /Kg BW/day of lead acetate for one month showing intranuclear inclusion bodies in the epithelial cells lining the renal tubules (→) (H& E 200X)

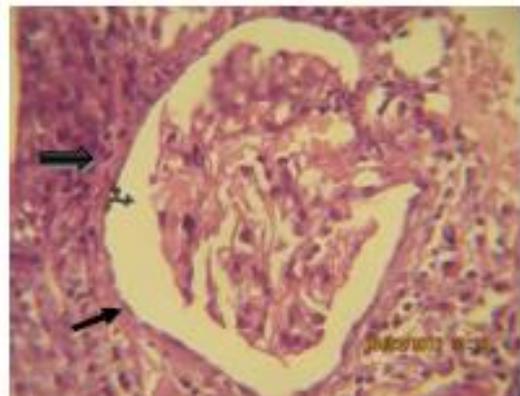


Figure (6): Kidney of rat treated with 100mg /Kg BW/day of lead acetate for two month showing hyperplasia of epithelial cells lining the parietal layer of the Bowman's capsule (→) with periglomerular inflammatory cells infiltration (→) (H& E 200X)

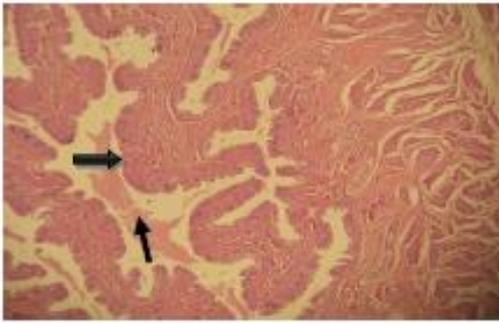


Figure (7): Urinary bladder of rat treated with 100mg /Kg BW/day of lead acetate for three month showing papillary hyperplasia of the epithelial lining (➡) with urine secretions (➡) (H& E 200X).

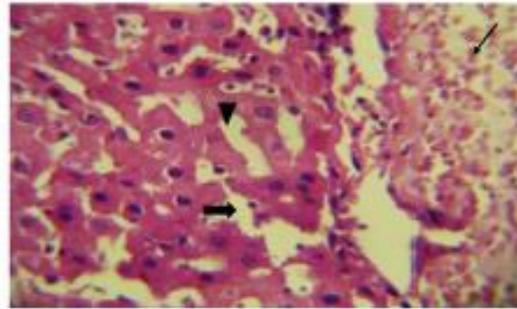


Figure (8): Liver of rat treated with 100mg /Kg BW/day of lead acetate for one month showing extensive area of necrosis (➡) and hemorrhage (➡) with increase in apoptotic cells (➡) (H& E 100X).

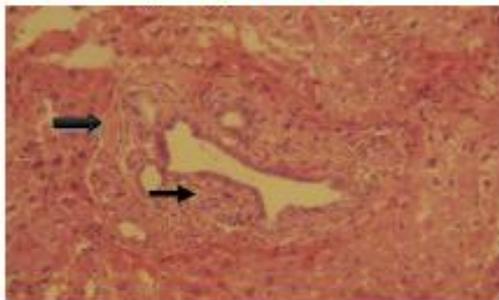


Figure (9): Liver of rat treated with 100mg /Kg BW/day of lead acetate for three month showing peribiliary fibrosis (➡) and inflammatory cells infiltration(➡) (H& E 100X)

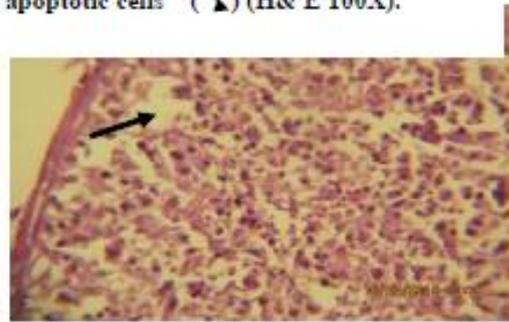


Figure (10): Spleen of rat treated with 100mg /Kg BW/day of lead acetate for one month showing moderate depletion of white pulp lymphoid tissue (➡) (H& E 100X)

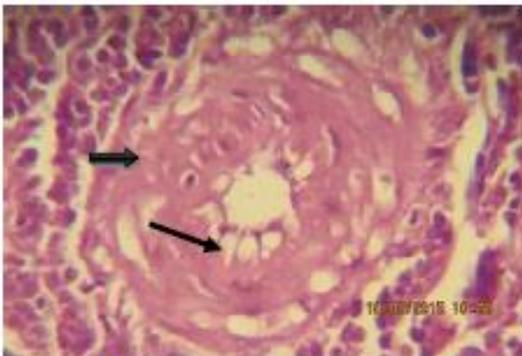


Figure (11): Spleen of rat treated with 100mg /Kg BW/day of lead acetate for three month showing hypertrophy of tunica media of the arterioles and thickening of their walls (➡) and vacuolation of their endothelial linings and muscular layer of the arterioles (➡) (H& E 200X).

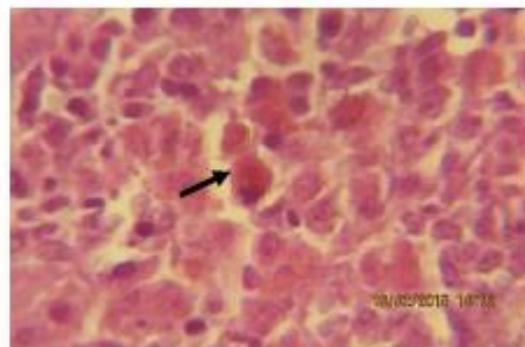


Figure (12): Spleen of rat treated with 100 mg /Kg BW/day of lead acetate for three month showing deposition of hemosiderin pigment(➡) (H& E 200X).

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