Toxicopathological Study of Lead Acetate Poisoning in Growing Rats and the Protective Effect of Cysteine or Calcium

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Summary

The study was designed mainly to investigate the toxicopathological changes in rats ingested toxic dose of lead acetate and the protective effects of cysteine and calcium in case of lead poisoning. The study was done on 84 rats of approximately same age 6-7 weeks, and body weight 45-60 gms divided equally into (6) groups as follows:- The first group was given lead acetate solution orally at a dose of 75 mg/Kg. BW./ day for 2 months. The second group was given lead acetate solution orally at a dose of 75 mg/Kg. BW./ day and cysteine solution at a dose of 200 mg/Kg. BW./ day for 2 months. The third group was given lead acetate solution orally at a dose of 75 mg/Kg. BW./ day and calcium gluconate solution at a dose of 500 mg/Kg. BW./ day for 2 months. The fourth group was given cysteine solution orally at a dose of 200 mg/Kg. BW./ day for 2 months. The fifth group was given Calcium gluconate solution orally at a dose of 500 mg/Kg. BW./ day for 2 months. The sixth group was given orally distilled water/day for 2 months and considered as control group. Toxicopathological changes showed the following: Hepatomegaly, nephromegaly with pale yellow color, and stretched capsule, spleen atrophied or enlarged in size with severe congestion. The brain showed congestion and edema. Liver showed necrosis with formation of hyperplastic nodules and presence of intranuclear inclusion bodies within hepatocytes but less conspicuous than in kidney. Kidney showed degeneration and necrosis of the epithelial lining of proximal and distal convoluted tubules, with presence of large numbers of inclusion bodies especially at the first and second scarification, with atrophy of glomerular tuft and dilation of collecting tubules containing hyline cast. The brain sections showed perineuronal and perivascular edema with malacia and hyperatrophy and hyperplasia of endothelial cells lining the blood capillaries, and severe demyelination of cerebrum and spinal cord. Degeneration and necrosis with edema of myocardium with moderate fibrosis, and atrophy of islets of langerhan’s and fibrosis of pancreas. Spleen showed severe depletion of white pulp lymphoid tissue with extramedullary hemopoiesis characterized by the presence of large numbers of megakaryocytes. Treated groups with cysteine and calcium showed increase in the cellular response and hyperplasia of the organs lymphoid tissue. We concluded that lead acetate poisoning in rats causes toxicopathological changes in the internal organs with precancerous lesions. Using of cysteine and calcium as a protective agent can reduce the toxic effect of lead acetate and improve the histopathological lesions.
دراسة مرضية سمية حول التسمم بخلايا الرصاص في الجردان النامي والتأثير الافتقاني

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الخلاصة

صممت هذه الدراسة لمعرفة تأثير الجرعة السمية لحول حلول خلايا الرصاص وذلك من خلال دراسة التغيرات المرضية في الجردان المجردة تجربة والتأثيرات الانتقائية للمستتين والكالسيوم في حالة التسمم بخلايا الرصاص.

استخدم في الدراسة 84 من الجردان تراوحت أوزارها بين 45-60 و60-70 كيلوغرام، حيث قسمت إلى 6 أعداد baja أعداد مساوية من الحيوانات، وكما يأتي: الزمرة الأولى: جرعة 75 ملغم من محلول خلات الرصاص لكل كغم من وزن الجسم يوميًا ولعدة شهرين. الزمرة الثانية: جرعة 75 ملغم من محلول خلات الرصاص و200 ملغم من كغم من الوزن الجسم يوميًا ولعدة شهرين. الزمرة الثالثة: جرعة 75 ملغم من محلول خلات الرصاص و500 ملغم من محلول الكالسيوم (Calcium) لكل كغم من وزن الجسم يوميًا ولعدة شهرين. الزمرة الرابعة: جرعة 200 ملغم من محلول الرصاص لكل كغم من وزن الجسم يوميًا ولعدة شهرين. الزمرة الخامسة: جرعة 500 ملغم من محلول الرصاص وكل كغم من وزن الجسم يوميًا ولعدة شهرين. الزمرة السادسة: جرعة 500 ملغم من محلول الرصاص وكل كغم من وزن الجسم يوميًا ولعدة شهرين.

وقد تبين من خلال الفحص المجهرى أن شدة الآفات المرضية تزداد مع المدة التي ي تعرض لها الجسم للجرعة السمية كذلك وجد أن الخيار هو الصفة الدائمة لجميع عينات الكبد والكليتين مع تصميم الحمض النووي لخلايا الكبد ودورة التسنين العصبي ويوجد أعداد كبيرة من المشتقات داخل نوى الخلايا العصبية المبطنة للنطاق الكليوية للقرن. وأعداد أقل من المشتقات داخل نوى الخلايا الكندية مع تصميم توضيح النبيبات الجانبية للخلايا وحولها إلى القلب الزجاجية.  

تتأثر خلال الدراسة، بوصورات نفاد في الدم، ووابل آفات أظهر تختصس ودورة شديدة خلال الأداء الأخير من التجربة مع تكون الدم خارج النقي وتعتبر أعداد كبيرة من الخلايا النجو كما أن هناك ضمور في جزء من الأنسجة العصبية وتكاثر نسيج مكونات الدم مع وجود علامات تدل على حصول تخلخل الجسم.  

تميز وجود أعداد كبرى من الخلايا النجو كما ان هناك ضمور في جزء من الأنسجة خارج النقي وتعتبر أعداد كبيرة من الخلايا النجو كما ان هناك ضمور في جزء من الأنسجة.  

تؤكد هذه الدراسة على أهمية تقييم التسمم بنواجع مختلفة من خلال دراسة تأثيرات التسمم في الجردان النامي والتأثير الافتقاني.  

Introduction

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Early reports of toxicity in adult metal workers suggest that they suffered from lead poisoning. Compared with adult lead poisoning, pediatric lead poisoning is a somewhat newer problem (1). Populations are exposed to lead chiefly via paints, cars, plumbing fixtures, and leaded gasoline (2). The intensity of these exposures, which recently decreased by regulatory actions, remains high in some segments of the populations because of the deterioration of lead paints and vehicle exhaust into soil and house dust. Many industries such as battery manufactures, demolitions, painting, paint removal and ceramics continue to pose significant risk of lead exposure to workers and surrounding communities. Lead poisoning occurs when a person swallows or inhales lead in any form, multiple target organs can be affected such as central nervous and peripheral, kidney, liver bone, gastro intestinal tract blood vessels, reproductive and endocrine system appear in acute, subacute and chronic form (3). The toxicity result from the interaction of the metal sulfhydral groups, which interferes with multiple enzymes and enzymatic processes such as inhibition of δ-ALAD and ferrochelatase. L-cysteine is an important free SH-containing amino acid (4). Therefore the presence of a sulfhydral group in the structure of L-cysteine gives it the potential to function as a chelating agent(5). Studies on nutritional supplements found that increasing dietary calcium intake has been associated with reduced absorption of lead from the GIT (Gastrointestinal tract) in children. There is no study found which attempted to show the effect of toxic doses of lead acetate on growing rats in Iraq and the effect of nutritional supplementation on the lead intoxication. So the aim of this study is to accomplish this task and study the toxicopathological effects in different systems.

Materials and Methods

- **Experimental Animals:** Eighty four albino rats aged 6-7 weeks and weight 45-60 gms. Rats obtained from (Animal house colony of Embryo Research and Infertility Treatment Institute/Al-Nahrain University, housed in a plastic cages 50×30×10 cm and placed in the room for 10 days for adaptation. Room temperature was maintained at 21 ± 3 °C, the air of the room was changed continuously by using ventilation vacuum and with light\ dark cycle of 12:12 hrs per day. The litter of the cages was changed every week. The animals hosted in animal house at College of Veterinary Medicine, Baghdad University, and were fed on pellet.

- **Dose calculation:** Lead acetate at a dose of 75 mg\ Kg. BW. has been given as a solution which prepared by dissolving of 3 grams of lead acetate in sterile distilled water then completed to 400 ml. This dose of 75 mg\ kg. BW. of lead acetate has been used by previous studies to show the toxic effects of lead poisoning (6). The prepared drug was given orally by using stomach tube at a dose level of 1 ml/100g. BW. L-cysteine was used in a dose of 200 mg/Kg. BW. (7) and prepared by dissolving of 600 mg of pure L-cysteine in a sterile distilled water and completed to 30 ml. The prepared drug was given orally by using stomach tube at a dose level of 1 ml/100g. BW.A dose of calcium gluconate which used is 500 mg/Kg. BW. (8) which prepared by adding sterile distilled water to 1.5 g. of pure calcium gluconate to complete the volume to 60 ml. The prepared drug was given orally by using stomach tube at a dose level of 2 ml/100g. BW.

- **Experimental Design:** Eighty four albino rats divided into six groups, each group consist of 14 rats divided as follows. The first group daily given for 2 months via stomach tube with toxic dose of lead acetate (75 mg/kg B.W.). The second group daily given for 2 months via stomach tube with toxic dose of lead acetate (75 mg/kg B.W.) and (200 mg/kg B.W.) of L-cysteine. The third group daily given for 2 months via stomach tube with toxic dose of lead acetate (75 mg/kg B.W.) and (500
mg/kg B.W.) of calcium gluconate. The fourth group daily given for 2 months via stomach tube with (200 mg/kg B.W.) of L-cysteine as positive control. The fifth group daily given for 2 months via stomach tube with (500 mg/kg B.W.) of calcium gluconate as positive control. The sixth group is a control group given via stomach tube with distilled water.

- **Clinical Signs:** Clinical signs and abnormalities of behavior were observed along the period of experiment (60 days).

- **Histopathology:** At the end of each sacrifice (every 20 days), four animals from each group were sacrificed by intramuscular injection of high dose of ketamin hydrochloride. The macroscopic appearances were recorded to detect any abnormal gross changes in internal organs. Specimens were taken from internal organs and the tissues were kept in 10% formaldehyde solution, for fixation, and then processed routinely by using the histokinette. Tissue sections were embedded in paraffin, and sectioned by microtome with hematoxylin and eosin (9), then examined under a light microscope.

### Results and Discussion

**Pathological Changes**

**Macroscopical Findings**

**Control group:** There are non-significant macroscopic changes in the control group used in the experiment.

- **Lead Poisoning: First Period (20 day)**
  
  **Liver:** Hepatomegaly with pale color (Fig. 1). Kidneys: nephromegaly with pale color, and stretched capsule. Spleen: Atrophied or enlarged in size with severe congestion and dark or pale in color. Brain: Swollen, congested and oedematous with flattening of convolution and compression of sulci. Heart: Enlarged and congested with rounded apex.
  
  **Second Period (40 day)** Similar lesions to the pathological changes of the first period but only the spleen was pale in color.

  **Third Period (60 day)** Similar lesions to the previous periods but the spleen was with marked atrophy in size, pale in color, with sharper edges, femur bone fragile and easily broken. These lesions may be attributed to the direct toxic effect of lead acetate on body organs. These lesions were classic for lead toxicity as previously described (10, 11, 12).

**Lead and Cysteine:**

**First Period:** Liver: Slightly pale in color. Kidneys: No changes. Spleen: Enlarged with dark color (Fig. 2). Brain and Heart: No changes. **Second Period:** Similar to the first period but the spleen enlarged in size with granular surface. **Third Period:** Similar to the previous periods.

**Lead and Calcium:** First, Second, and Third Periods Similar pathological changes as in group of lead with cysteine.

**Microscopical Findings**

**Lead Poisoning**

**Brain:** **First Period:** Mild perineuronal and perivascular oedema (widening of Virchow–Robin space). Slight oedema between the molecular and granular layer of cerebellum with degeneration and necrosis of purkinje cells and demyelination of white matter. Degeneration and necrosis of neurons of spinal cord, brain stem and cerebrum characterized by karyolysis of nuclei of many central chromatolysis of neurons (Fig. 3). Congestion of blood vessels and infiltration of inflammatory cells in meninges. **Second Period:** Increase in the amount of perivascular and perineuronal oedematous fluid accompanied with hyperatrophy and hyperplasia. Diffuse infiltration of microglial and
astrocytes cells. **Third Period:** In addition to previous pathological lesions seen in the first and second periods, there are severe demyelination of the brain stem and white matter appeared with polymicrocavitation. The cerebellum. The cerebrum shows the presence of multifocal variable sized areas of malacia (Fig. 4). Necrosis of cerebrum neurons is more severe than previous periods with infiltration of microglial cells (neurophagia) (Fig. 5). Oedema and congestion are also more severe than previous period. There are hypertrophy and hyperplasia of endothelial cells lining the blood vessels (Fig. 6). **Spinal Cord:** **First Period:** Slight demyelination of nerve fibers in the spinal cord. **Second Period:** Similar to the first period. **Third Period:** Severe demyelination characterized by loss of myeline sheath and swelling of the axons (Fig. 7).

**Kidney:** **First Period:** The main microscopic findings are acute cellular swelling with vacuolar degeneration and necrosis of epithelial lining of proximal and distal convoluted tubules. Many cortical renal tubules undergo dilation, with atrophy of glomerular tuft, with deposition of albumin droplets within the lumen of renal tubules. All sections show the presence of large numbers of acidophilic intranuclear inclusion bodies within the epithelial cells of the proximal and distal convoluted tubules (Fig. 8).

**Second and Third Periods:** Severe necrosis of epithelial lining of renal tubules which show detachment from basement membranes in many sections. Marked dilation of medullary renal tubules containing hyaline cast in their lumina (Fig. 9).

**Liver:** **First Period and Second Period:** Extensive areas of coagulative necrosis especially in the midzone and peripheral zone with presence of apoptotic cells (Fig. 10). Other areas show swelling of hepatocytes with enlarged nuclei containing more than nucleoli acidophilic intranuclear inclusion bodies were noted, but it was not as conspicuous as kidney. Dilation and congestion of central veins and sinusoids with congestion of blood vessels of portal areas. **Third Period:** Similar lesions as in the first and second period with the presence of multiple hyperplastic nodules. Hepatocytes have no arrangements, the cells undergo fatty change. These nodules causing the pressure atrophy to the adjacent hepatic parenchyma (Fig. 11). Other sections show presence of serum protein within the hepatic sinusoids.

**Spleen:** **First and Second Period:** Moderate depletion of white pulp lymphoid tissue with presence of large numbers of megakaryocytes (Fig. 12). **Third Period:** Severe depletion of the lymphoid follicles of white pulp, many sections showing that the arterioles surrounded by only few lymphocytes (Fig. 13).

**Pancreas:** **First Period and Second:** The pancreas show marked atrophy of islets of langerhans (Fig. 14), in addition to severe congestion in other sections. **Third Period:** Similar to the previous periods with proliferation of the fibrous connective tissue (fibrosis), within the pancreatic lobules causing pressure atrophy of the pancreatic tissue (Fig. 15).

**Heart:** **First Period** There are degeneration and necrosis of myocardium with slight oedema between muscle fibers. **Second and Third Period:** Moderate fibrosis of myocardium with infiltration of mononuclear cells (Fig. 16).

**Bone:** **First and second Period:** Moderate hyperplasia of hemopoietic tissue with proliferation of megakaryocytes and the presence of thin trabeculae of calcified cartilage covered by a thin layer of bone. The bars of mineralized cartilage which result from impaired resorption of osteoclasts are wide and project further into the metaphyseal marrow cavity than normal (Fig. 16). **Third Period:** In addition to previous findings there is marked depletion of hemopoietic tissue and increase in the sinus numbers. **Brain:** Edema may be attributed to the increase in the permeability of the Blood Brain Barrier (BBB) leading to disturbances in the blood dynamics and escape of fluids to the nervous tissues and that agreed with previous studies which
showed that the first step in the neurotoxic effects of lead might be primarily related to damage to the permeability of BBB (13,14). The degenerative and necrotic changes noticed in the neurons of spinal cord, brain stem, cerebrum and purkinje cells of cerebellum represent ischemic changes resulting from injury of blood vessels. Furthermore lead accumulates in and damages mitochondria. Biosynthesis, a function of neuronal mitochondrial activity, is affected by lead, with disruptive effects on synaptic transmission in the brain. Lead also killing brain cells via excitotoxicity and apoptosis (15,16). The neuropathia which noticed in brain of animals of later stages of lead toxicity represent advanced stage of nervous tissue response to neurons necrosis. The microglial cells surround the degenerated neurons in a process called satellitosis and when the degenerated cells die the microglial cells will infiltrate the necrotic area this agreed with Jones and (17). The focal encephalomalacia was due to necrosis of the neurons and glial cells and the pressure resulting from the severe oedema and that agreed with (18), who noticed focal malacia in subacute and chronic lead toxicity in rabbits which may be caused by severe oedema and focal hemorrhage. The hyperplasia of endothelial cells lining the blood capillaries of cerebrum and brain stem represent the response to the injury and continuous damage caused by lead and due to irritation of free radicals similar lesion noticed by (19) in natural and experimental lead poisoning in cattle the most obvious and consistent lesion in the brain were in the blood vessels. Demyelination of the spinal cord and brain stem considered one of the important lesions. This is occur due to the toxic effects of lead on Schwann cells this agreed with (20) who predicted that if Schwann cell damage were to be implicated in lead neuropathy. **Kidney:** The pathological changes may be attributed to damage caused by lead since it is well known that lead was a nephrotoxic element causing extensive damage to tubular cells by necrosis and apoptosis this result similar to (21) who a progressive loss of cell viability together with a significant increase in the number of apoptotic and necrotic cells and lactate dehydrogenase release in primary cultures of rat proximal tubular (rpt) cells treated with different concentration of lead acetate (0.25, 0.5 and 1mM). The kidney sections show presence of eosinophilic intranuclear inclusion bodies. They are more numerous at the first and second periods than at the third period because of the severe necrosis of epithelial cells at the later stages of experiment. These inclusions are intranuclear protein matrices upon which metallic ions are deposited and are considered a pathognomonic lesion for lead poisoning (22). **Liver:** The most significant changes were the cellular degeneration and coagulative necrosis especially in the mid zone and peripheral zone and that agreed with (23) they reported that acute toxic hepatitis characterized by the presence of hepatic cells undergo necrosis and others undergo degenerative changes. The extent of necrosis vary according to the length of exposure, it become more severe especially at the later stages of experiment, and it appeared as a constant feature of all examined sections and that because of the accumulative ability of lead these results similar to that noticed by (24,18). Tissue sections show apoptosis of hepatocytes. Apoptosis is an active and highly regulated form of cell death responsible for the cellular default demise of the hepatocytes which occur due to the toxic effect of lead which agreed with (25) who referred to that Lead and Arsenic induce apoptosis by different mechanisms. The formation of hyperplastic nodules. May be related to the ability of the liver to replace lost cells through liver regeneration this agreed with previous studies that noticed in young adult rats at least all hepatocytes have the potential to re-enter the cell cycle (26). Almost all cases this is achieved by an increased incidence of apoptosis, and this occurs in rat livers after lead nitrate ingestion (27), or it might be due to mutagenic effect of lead acetate since contradictory results have been published to increased occurrence of chromosome
aberrations in workers occupationally exposed to lead (28). Intranuclear inclusion bodies were seen in hepatocytes of animals especially at first period and not in the later stages because of the extensive hepatocellular necrosis and they are not conspicuous as in kidney sections and that agreed with (29).

**Spleen:** Depletion of lymphoid tissue was seen 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. The present studies show that there is a decrease in erythropoiesis which is clear in bone marrow of third period which explained the presence of large numbers of megakaryocytes in the red pulp region indicating that extramedullary hemopoiesis takes place in the spleen as a secondary mechanism (30).

**Pancreas:** Atrophy of islets of langerhans especially B-cell region was due to the toxic effects of lead on its cells which lead to reduce cells number by necrosis. The atrophy become more severe at the end of the experiment accompanied with chronic fibrous pancreatitis. Cytoplasmic vacuoles, and prominent subcapsular and interacinar fibrosis were seen in four waterfowl collected in the Tri-State mining District (Oklahoma, Kansas and Missouri, USA), an area known to be contaminated with (30). The above lesions agreed with the present results of pancreas.

**Heart:** The changes in myocardium due to the toxic effect of lead acetate on myocardial cells leading to their necrosis which replaced by fibrosis and the infiltration of inflammatory cells these results agreed with the recent study which demonstrate that the cardiovascular system is just as affected by acute and chronic levels of lead as any other systems in the body (24).

**Bone:** The hyperplasia of hemopoietic tissue may be due to hemolytic anemia and it is a usual physiological response in order to increase the production of blood cells. In other words the bone marrow of toxicated group far from being depressed is in fact producing red cells at a considerable faster rate than normal and this is of a great value in helping the animal maintaining its hematological indices to meet the continuous blood loss. Stimulation and toxic effect of the drug resulting in exhaustion and suppression of hemopoietic tissue. However, there is ample evidence that the membranes of circulatory red cells are injured and that their life-span is shortened. The resulting hemolysis provokes an increase in the bone marrow but lead also affects heme synthesis by binding to sulfhydral groups in the enzyme aminolevulinic acid dehydratase (ALAD) as well as globin synthesis so that premature death of nucleated red cell precursors in the bone marrow also occurs (ATSDR). In turn, this triggers an increase in stem-cell replication and differentiation in order to compensate for the decreased effectiveness of erythropoiesis. long term exposure to even small amount of lead may increase lead in bones and lead become stored in bones, which acts like a sort of reservoir. Thin bone trabeculae, delay of calcification and osteoporosis attributed to the effect of lead on calcium absorption from intestine and competition between lead and calcium in bone formation in which lead reduce deposition of calcium.

**Lead and Cysteine**

**Brain:** First Period: Oedema is less in amount than in the lead treated group with diffuse infiltration of glial cells and astrocytes. **Second and Third Periods:** In addition to the lesions of first period tissue sections show focal aggregation of microglial cells (gliosis) forming nodular like structures (Fig. 18). **Spinal Cord First, Second, and Third periods:** Similar lesions as in the lead treated group. **Kidney:** First Period: There is perivascular aggregation of lymphocytes in perirenal adipose tissue. **Second and Third Periods:** In addition to the first period, sections show mild necrosis of epithelial lining of renal tubules, with nearly normal size of glomerular tuft compared with lead treated group. **Liver:** First Period: Similar lesion as in the lead treated group of the first period. **Second Period:** Similar lesion as in the first period in addition there is proliferation of kupffer cells with slight infiltration of lymphocytes in sinusoids (Fig. 19), with perivascular cuffing, and acute cellular swelling with minimal vacuolation of hepatocytes. **Third Period:** Similar to the second period with the presence of focal
aggregation of mononuclear cells within the parenchyma forming nodular like structure (Fig. 20).

**Spleen: First Period:** Mild depletion of white pulp. **Second and third Period:** Marked hyperplasia of lymphoid follicles (20) with proliferation of megakaryocytes. **Pancreas: First Period:** No pathological changes. **Second and Third Period:** Presence of normal sizes of islets of langerhans as compared with lead treated groups (Fig. 21).

**Heart: First Period:** Infiltration of lymphocytes between the muscle fibers of myocardium (Fig. 22). **Second and Third Periods:** Similar to the first period.

**Bone: First Period:** There is hyperplasia of the hemopoietic tissue. **Second and third Period:** There are moderate thicknesses of bone trabeculae in secondary spongiosa which show regular laminated bone with large areas of primary spongiosa which contain cartilage in center. The hemopoietic tissue was markedly hyperplastic containing large numbers of megakaryocytes (Fig. 23).

Cysteine is a semi-essential amino acid, which means that human can synthesize it but in a limit amounts (31). The presence of SH group in the structure of L-cysteine gives it the potential to function as a chelating agent (32). An adaptive and protective response to toxic metal exposure is the induction of metallothionein (MT) synthesis.

**Lead and Calcium**

**Brain: First, Second, and Third Periods:** Similar lesions as in the lead and cysteine.

**Spinal cord: First, Second, and Third Periods:** Similar to the lead treated group.

**Kidney: First Period:** Mild necrosis of the epithelial lining of renal tubules, with nearly normal size of glomerular tuft compared with lead treated groups. **Second and Third Periods:** Focal infiltration of the mononuclear cells within the interstitial tissue with dilation of cortical renal tubules (Fig. 24).

**Liver: First and Second Periods:** Tissue sections show similar lesions as in the second period of lead and and cysteine. **Third Period:** The hepatic parenchyma show extensive hydropic degeneration leading to narrowing and stenosis of the sinosoids (Fig. 25).

**Spleen: First Period:** Similar pathological changes seen in lead and cysteine. **Second and Third Periods:** No pathological changes. **Pancreas: First, Second, and Third Periods:** There is marked hyperplasia of lymphoid tissue of the organ. **Heart: First Period:** There is mild infiltration of lymphocytes between muscle fibers. **Second and Third Periods:** Similar to the first period. **Bone: First Period:** Similar to the first period of lead and cysteine. **Second and Third Periods:** Thick irregular trabeculae of spongiosa of bone, with less cartilage absorption. Presence of osteoclasts in blood vessels, with marked hyperplasia of hemopoietic tissue and presence of large numbers of megakaryocytes. Research studies conducted on animals as well as human indicated that calcium and lead interact in a negative (antagonistic) manner. The exact mechanisms by which calcium interacts with lead are not all known (33). Laboratory animal research indicates that calcium decreases lead absorption (34) promotes urinary excretions of lead (35). (36) proposed that calcium may interact with lead by binding lead in the gut, competing with lead for absorption, altering intestinal cell activity for lead, and altering affinity of target tissue for lead. The present study demonstrates that the ingestion of calcium with lead acetate limits the absorption of lead in rats. This was shown via activation of defense mechanism of the body (infiltration of mononuclear cells in soft tissue and hyperplasia of lymphoid tissue), and the degenerative changes which affect the liver which means that the concentration of the toxin become mild. Similarly biochemical parameters and histopathological studies on the liver confirmed the protective potential of calcium and magnesium on the hepatotoxicity arising from cadmium and lead using a rat model. In addition to hyperplasia of hemopoietic tissue, there is increase in thickness of bone trabeculae and formation of lamellar bone (37).
Fig. (1) Viscera of one rat treated with 75 mg/Kg. BW/day of lead acetate for 20 days showing hepatomegaly with pale color ( ) compared with control ( ).

Fig. (2) Histopathological section of brain of rat treated with 75 mg/Kg.BW/day of lead acetate for 20 days show central chromatolysis of neurons ( ) nearly complete absence of others (ghost) ( ) (H & E X 400).

Fig. (3) Histopathological section of brain of rat treated with 75 mg/Kg.BW/day of lead acetate for 60 days show focal encephalomalacia ( ) and congestion ( ) (H & E X 400).

Fig. (4) Histopathological section of brain of rat treated with (75 mg/Kg.BW/day) of lead acetate for 60 days show neurophagia ( ) (H & E X 400).

Fig. (5) Histopathological section of brain of rat treated with 75 mg/Kg.BW/day of lead acetate for 60 days show hyperplasia and hyperatrophy of endothelial cells lining the blood vessels ( ) (H & E X 400).

Fig. (6) Histopathological section of spinal cord of rat treated with 75 mg/Kg.BW/day of lead acetate for 60 days show severe demylination of nerve fiber ( ) (H & E X 400).
Fig. (7) Histopathological section of kidney of rat treated with 75 mg/Kg.BW/day of lead acetate for 20 days show intranuclear inclusion bodies in epithelial cells lining of the proximal and distal convoluted tubules (H&E X 1000).

Fig. (8) Histopathological section of liver of rat treated with 75 mg/Kg.BW/day of lead acetate for 20 days show extensive area of coagulative necrosis (H & E X 400) with presence apoptotic cells (H & E X 400).

Fig. (9) Histopathological section of liver of rat treated with 75 mg/Kg.BW/day of lead acetate for 20 days show extensive area of coagulative necrosis (H & E X 400) with presence apoptotic cells (H & E X 400).

Fig. (10) Histopathological section of spleen of rat treated with 75 mg/Kg.BW/day of lead acetate for 20 days show decrease in the numbers of lymphocytes with proliferation of megakaryocytes (H & E X 400).

Fig. (11) Histopathological section of spleen of rat treated with 75 mg/Kg. BW/day of lead acetate for 20 days show depletion of lymphoid follicle of white pulp (H & E X 400).

Fig. (12) Histopathological section of pancreas of rat treated with 75 mg/Kg. BW/day of lead acetate for 20 days show marked atrophy of islets of Langerhans (H & E X 400).
Fig. (13) Histopathological section of pancreas of rat treated with 75 mg/Kg.BW/day of lead acetate for 60 days show the disorganization of acini, cytoplasmic vacuolation ( ), and prominent interacinar fibrosis ( ) with infiltration of chronic inflammatory cells (H & E X 400).

Fig. (14) Histopathological section of heart of rat treated with 75 mg/Kg.BW/day of lead acetate for 40 days show moderate fibrosis of myocardium ( ) with infiltration of mononuclear cells ( ) (H & E X 400).

Fig. (15) Histopathological section of bone of rat treated with 75 mg/Kg.BW/day of lead acetate for 20 days show thin trabeculae ( ) of calcified cartilage covered by a thin layer of bone ( ) (H & E X 400).

Fig. (16) Histopathological section of brain of rat treated with 75 mg/Kg. BW/day of lead acetate, and 200 mg/Kg. BW/day of cysteine for 40 days show focal gliosis due to proliferation of microglial cells ( ) (H & E X 400).

Fig. (17) Histopathological section of liver of rat treated with 75 mg/Kg. BW/day of lead acetate, and 200 mg/Kg. BW/day of cysteine for 40 days show marked proliferation of kupffer cells ( ) with slight infiltration of lymphocytes in sinosoids ( ) (H & E X 400).

Fig. (18) Histopathological section of liver of rat treated with 75 mg/Kg. BW/day of lead acetate, and 200 mg/Kg. BW/day of cysteine for 60 days show focal aggregation of mononuclear cells within the parenchyma ( ) (H & E X 400).
Fig. (19) Histopathological section of spleen of rat treated with 75 mg/Kg.BW/day of lead acetate, and 200 mg/Kg.BW/day of cysteine for 40 days show marked hyperplasia of lymphoid follicle (H & E X 400).

Fig. (20) Histopathological section of pancreas of rat treated with 75 mg/Kg.BW/day of lead acetate, and 200 mg/Kg.BW/day of cysteine for 40 days show normal size of islets of langerhans due to hyperplasia of its cell as compared with lead treated groups (H & E X 400).

Fig. (21) Histopathological section of heart of rat treated with 75 mg/ Kg. BW/ day of lead acetate, and 200 mg/ Kg. BW/ day of cysteine for 20 days show moderate infiltration of lymphocytes between muscle fibers (H & E X 400).

Fig. (22) Histopathological section of bone of rat treated with 75 mg/ Kg. BW/ day of lead acetate, and 200 mg/ Kg. BW/ day of cysteine for 40 days show moderate thickness of bone trabeculae in secondary spongiosa with regular laminated bone (H & E X 400). Moderate hyperplasia of hemopoietic tissue and proliferation of megakaryocytes (H & E X 400).

Fig. (23) Histopathological section of kidney of rat treated with dose 75 mg/ Kg. BW of lead acetate, and 500 mg/ Kg. BW of calcium gluconate for 40 days show focal infiltration of mononuclear cells within the interstitial tissue (H & E X 400) with dilatation of cortical renal tubules (H & E X 400).

Fig. (24) Histopathological section of liver of rat treated with dose 75 mg/ Kg. BW of lead acetate, and 500 mg/ Kg. BW of calcium gluconate for 60 days show hydropic degeneration of hepatocytes (H & E X 400) with stenosis of sinusoids (H & E X 400).
References


