



## Histological Changes Associated with Exposure to Lead Acetate and Evaluation of the Therapeutic Role of Berbin Extract in Female Albino Rats and Their Fetuses

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### ABSTRACT

The current study aims to investigate the effect of lead acetate on pregnant female rats and their fetuses, as well as the protective role of the aqueous extract of the albino plant against lead exposure. This experiment was conducted in the animal house of the College of Veterinary Medicine at Tikrit University. Thirty female albino rats, weighing between 180-200 grams and three months old, were placed in plastic cages and paired with males of the same strain. They were then randomly divided into three groups, each consisting of five pregnant females. The first group served as the control. The second group was treated with lead acetate from days 10 to 19 of pregnancy. The third group received lead acetate during days 10 to 19 of pregnancy and also received the aqueous extract of the albino plant throughout the pregnancy. The daily oral dose of lead acetate 50 mg/kg and the berberine extract was 200 mg/kg. Histological examination of the control group's liver showed hepatocyte rows and a network of blood sinusoids containing Kupffer cells. In the kidneys, the capsular space and Bowman's capsule were nearly normal, as were the convoluted tubules. In the second group, hepatocytes experienced atrophy and dilatation of the blood sinusoids containing Kupffer cells. The kidneys of this group showed leukocyte infiltration, congestion with fibroblast proliferation, thickening of the capillary walls, and irregular tubule sizes. In the liver of the third group, the central vein exhibited blood hyperemia and leukocyte infiltration. This group showed limited atrophy, slight dilatation of the capsular space, limited epithelial cell sloughing, and near-normal convoluted tubules.

## 1. Introduction

Heavy metals are among the most significant pollutants found in various environments, including air, water, and soil, and are generated as a result of both natural and human activities. Through these environments, human and animal health are affected by heavy metals directly through direct exposure, and indirectly through their impact on the growth of plants that humans and other living organisms consume (1). Rapid technological development has been accompanied by an increase in the use of these metals, as many industrial activities and certain natural processes significantly contribute to environmental contamination with these heavy metals. Pollution has reached dangerous levels, posing a significant environmental burden, with humans themselves being victims of this environmental pressure. Long-term exposure to such pollutants leads to health risks and severe conditions that can sometimes lead to death (2). Heavy metal toxicity causes an imbalance in oxidative stress. These metals increase the production of free radicals and reduce antioxidant levels, reducing the body's ability to combat damage caused by free radicals. These metals are characterized by their ability to interact with DNA and nuclear proteins, leading to the deterioration of the biological processes of large molecules (3). Industrial activities over the past century have significantly increased human exposure to heavy metals such as lead. The accumulation of these elements in the body causes various toxic effects that affect organ tissues. These elements are sources of stress resulting from active oxygen and free radicals, which hinders the processes of growth, reproduction and differentiation, disrupts tissue repair, and may lead to programmed cell death (4) Lead is considered one of the heavy metals widespread everywhere and is a toxic element and a danger to the lives of living organisms. It causes many physiological risks to humans and animals (5) including the liver, kidneys, cardiovascular system, and reproductive system (6). There are many adverse health effects caused by lead, such as weakness of the reproductive organs (Ambiguous) and an increase in the number of deaths due to the increase in cancer cases (7). Medicinal plants have attracted great interest recently, as they have been used as a treatment for many diseases. They have become one of the preventive and health methods in many countries (8). After the portulaca plant, *Portulaca oleracea* is one of the medicinal plants and belongs to the *Portulacaceae* family. It is distributed globally in several countries such as Iraq, Palestine, Lebanon,

Arab Europe, North Africa, and others. It is a wild herbaceous plant that grows naturally. Previous studies have shown the medical importance of the barberry plant, as the ethanolic extract of the leaves and flowers of the barberry plant was used to treat urinary disorders, kidney stones, diabetes, and colitis. (9) hematochezia and seromas

## Materials and Methods

### Ethical Approval

The Habwan Ethics Committee at the College of Veterinary Medicine, Tikrit University, approved all animal experimental procedures in accordance with international guidelines for the care of laboratory animals (approval date: December 20, 2024). (Approval number: Veterinary Medicine, Tuesday, 76)

### Plant Collection

The barberry plant was collected from Salah al-Din Governorate (Sharqat District) during the month of September. The urban part of the plant was taken and washed well to remove impurities from it. Then the plant was left in a dry environment away from sunlight. The plant was crushed in an electric grinder after it dried. The powder was placed in a dark, tightly sealed box at room temperature until the extraction process was carried out

### 2.2 Animals used in the experiment

30 female white rats weighing between 180 and 200 g were used. They were raised in the animal house of the College of Veterinary Medicine at Tikrit University, where the animals were placed in plastic cages for a week before the experiment to prepare them. The animals were subjected to appropriate laboratory conditions of temperature and ventilation. The animals were fed the designated feed, which was 35% yellow corn, 20% concentrated protein, 1% dry milk, and 20% soybeans. And 35% wheat, taking into account giving it free access to water throughout the experiment period

### Identifying the dose of

The safe and effective treatment dose used in the trial was determined based on the method:(10) The oral dose is calculated based on the equivalent human dose. The body weight dose for lead acetate was determined to be 0.5 mg/kg, as was the case for the aqueous extract of berberine.

### Experimental Design

The study was designed based on the use of 30 female white rats, which were randomly distributed into groups according to the following treatments:

- (1) The first group (control): was dosed with normal drinking water.
- (2) The second group: treatment with lead acetate at a rate of 0.5 mg/kg from day (10-19) of pregnancy
- (3) The third group was treated with lead acetate at a rate of 0.5 mg/kg from day (10-19) of pregnancy, along with aqueous extract of the barberry plant at a rate of 200 mg/kg throughout the pregnancy period.

### Mating

White female rats of the Sprague Dawley strain, aged 10-12 weeks and weighing 180-200 g, were placed in a plastic cage with male rats of the same strain overnight and examined the following morning. Vaginal plugs or the presence of sperm in the vaginal fluid were considered evidence of fertilization, and females were considered to be on day zero of pregnancy (11)

### Anesthesia and Dissection

Pregnant females were placed inside a sealed box containing cotton filled with chloroform. The females were left in it until they were anesthetized. The animals were dissected, and the fetuses were extracted from their stomachs for morphological study and to observe the changes caused by lead acetate. The liver and kidneys were also removed for histological sectioning.

### Preparation of histological sections

Liver and kidney tissues were fixed in 10% neutral buffered formalin and stored temporarily. They were then permeabilized with alcohol in ascending concentrations from 50% to 100%, followed by tissue debridement with xylene. The samples were then embedded in paraffin wax, and the wax blocks were cut into 6-micrometer-thick sections. Finally, the tissue sections were stained with hematoxylin and eosin for microscopic examination (12)

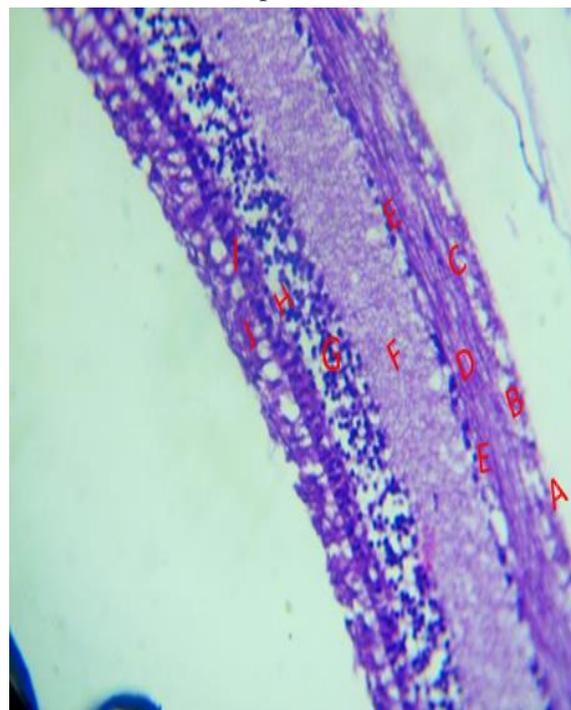
## Result and discussion

### Liver

Figure (1) shows the control group. Figure (2) illustrates the effect of treatment with lead acetate at a dose of 200 mg/kg on the liver in pregnant female white rats. Figure (3) demonstrates the protective role of *Portulaca oleracea* at a dose of 200 mg/kg in reducing the effect of lead acetate on the liver.

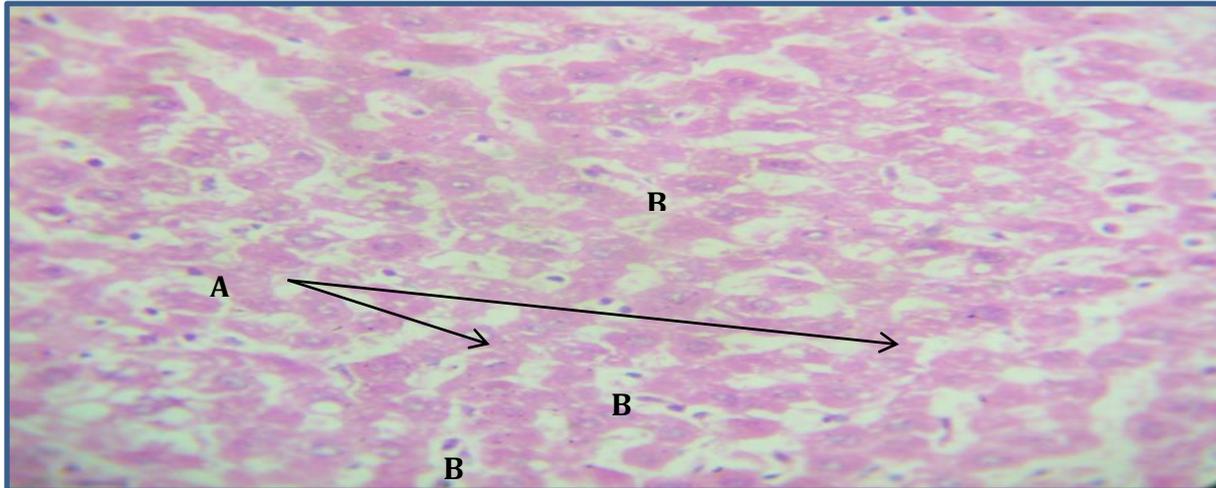
Figure (1) depicts liver tissue with rows of hepatocytes next to blood sinusoids, which appear as a network of blood vessels containing large, spherical Kupffer cells. Figure (2) shows atrophy

in some liver cells and severe nuclear degeneration, with spaces forming within the nuclei. The blood sinusoids contain Kupffer cells, have wide lumens, and appear as a network of channels. Figure (3) depicts the central vein filled with decomposed blood, with a wide lumen and walls surrounded by white blood cells. The hepatocytes are arranged in long, radial rows around the vein. Each vein appears normal and polygonal. The blood sinusoids are wide in lumen and contain some Kupffer cells.

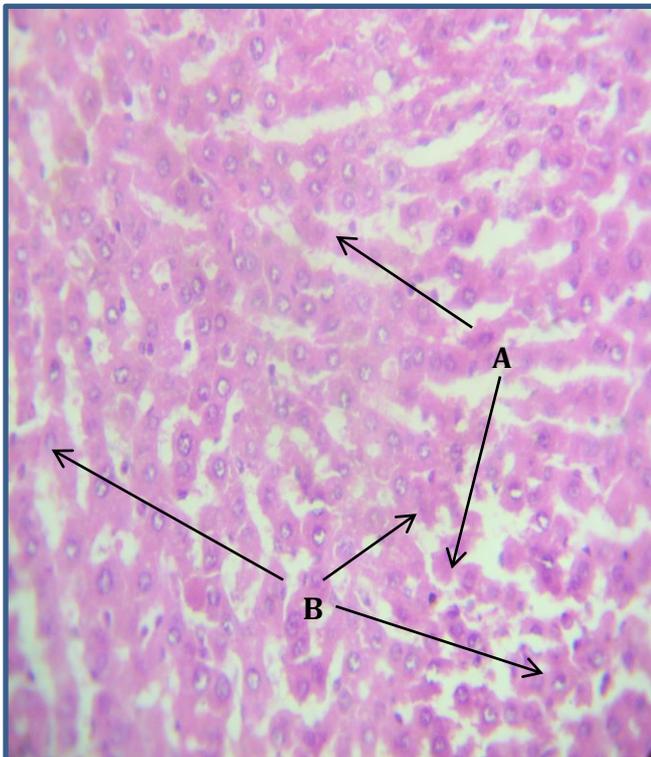


**Figure (1):** Retina of chicken, inner limiting membrane (A) nerve fiber layer (B) ganglionic cell layer (C) inner plexiform layer (D) inner nuclear layer (E) outer plexiform layer (F) outer nuclear layer (G) outer limiting layer (H) photoreceptors (I) retinal pigmented epithelium (J) H&E (X10).

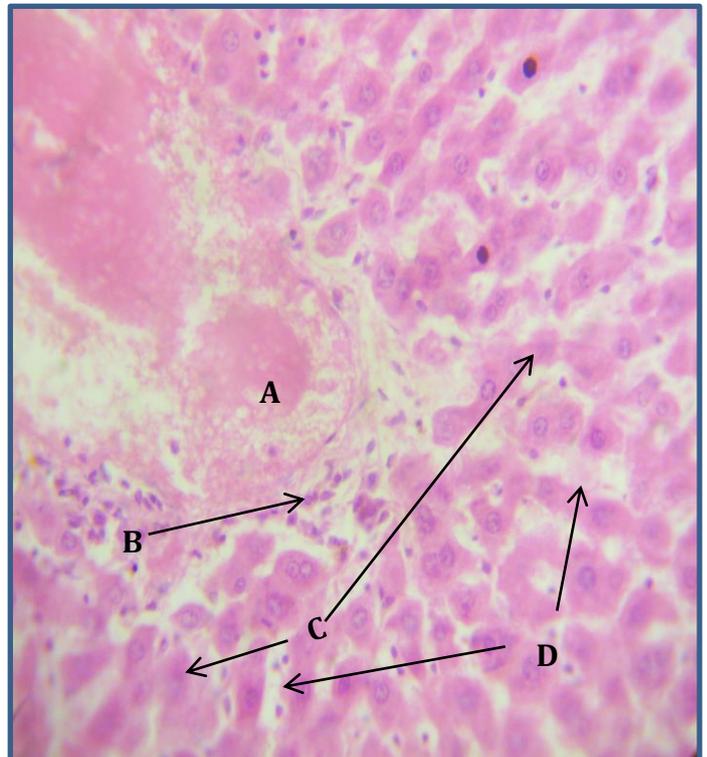
The chicken retina consists of layers that correspond to standard vertebrate retinal arrangements that link neurons between the inner nuclear layer and outer nuclear layer, and the ganglionic layer properly. The organization serves an essential role in proper signal processing, together with visual perception (16). The numerous cones within the photoreceptor layer enable chickens to detect an extensive spectrum of colors, which extends to ultraviolet wavelengths. A significant trait in chicken vision is tetrachromatic sight, which serves multiple roles in both food search activities and social relationships (17). The retinal pigmented epithelium (RPE) maintains photoreceptors through its dark color and serves to protect the receptors by absorbing light and recycling



**Figure (1)** Section of liver tissue showing rows of hepatocytes arranged in different directions (A) and a network of blood sinusoids containing Kuffer cells (B) (40X, E &H).



**Figure (2)** Section of liver tissue showing atrophy of hepatocytes (A) and dilatation of the sinusoids containing Kuffer cells (B) (40X, E & H).



**Figure (3)** Section of the central vein containing lytic hyperemia (A) Limited infiltration of white blood cells around the vein wall (B) Radial rows of hepatocytes (C) Blood sinusoids containing Kuffer cells (D) (X40, E & H).

## Kidney

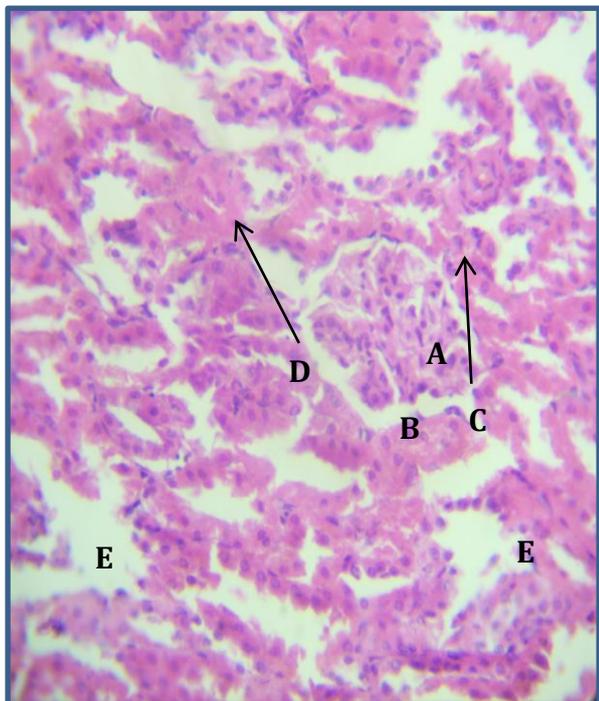
Figure (4) shows the control group. Figure (5) illustrates the effect of treatment with lead acetate at a dose of 200 mg/kg on the kidneys of pregnant white female rats. Figure (6) demonstrates the protective role of the *Portulaca oleracea* plant at a dose of 200 mg/kg in reducing the impact of lead acetate on the kidney.

Figure (4) depicts the renal cortex with incompletely lobulated renal glomeruli,

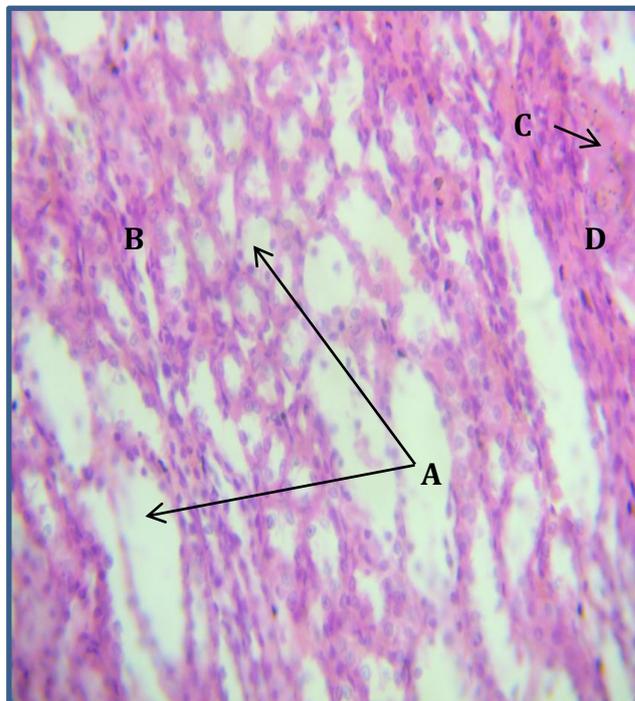
surrounded by the capsular space and Bowman's capsule. The glomeruli are encompassed by the proximal convoluted tubules, lined with pyramidal cells. These tubules have narrow lumens, while the distal convoluted tubules, lined with simple cuboidal cells, exhibit wider lumens. Figure (5) displays the renal medulla, containing compact renal tubules of various sizes, and the interstitial tissue is thickened with colloidal fibers that contain white blood cells. Figure (6) shows the

renal cortex with incompletely lobulated, atrophic glomeruli, a wide-bore capsular space outside Bowman's capsule, normal-shaped proximal convoluted tubules with narrow lumens, and normal-shaped distal convoluted tubules with

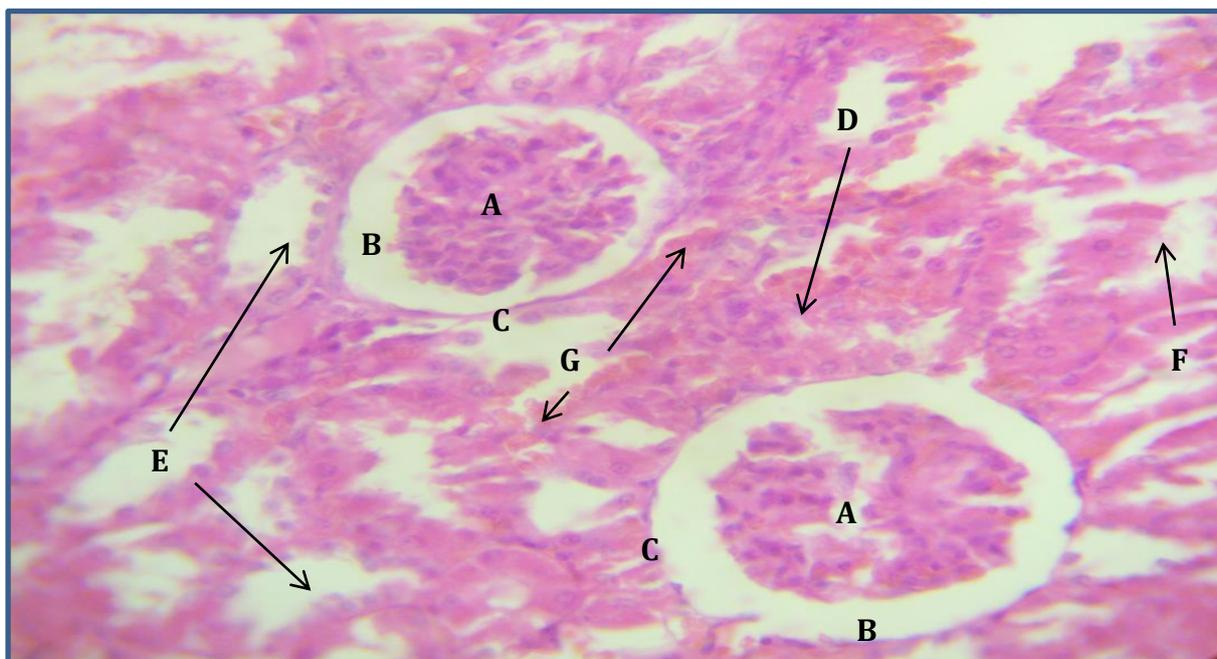
wide lumens. Glomerular filtrate was observed in the distal convoluted tubule, and some sloughed epithelial cells were present in the lumen of this tubule.



**Figure (4)** Section of the renal cortex showing semi-lobulated glomeruli (A), capsular space (B), Bowman's capsule (C), proximal convoluted tubules (D), proximal convoluted tubules (E)



**Figure (5)** Section of the renal medulla showing irregular tubule sizes (A), infiltration of white blood cells (B), congestion (C), thickening of capillary walls with fibroblast proliferation (D) (40X, E & H).



**Figure (6)** Section of the renal cortex showing renal glomeruli with limited atrophy (A) Slight dilatation of the capsular space (B) Bowman's capsule (C) Proximal convoluted tubules (D) Nearly normal distal convoluted tubules (E) Limited sloughing of epithelial cells (F) Hemorrhage (G) (40X, E & H).

The liver is one of the most complex organs in the body due to the numerous enzyme pathways it contains that are essential for its functions. Because of this, many toxins and drugs can impact these pathways and modify how they work (13). Absorbed lead is stored in soft tissues such as the liver (14). Cytotoxicity occurs in the lysosomal membrane, resulting in the production of large amounts of reactive oxygen species (ROS), like hydrogen peroxide and highly reactive hydroxyl radicals. This process is accompanied by a decrease in zinc and copper levels, along with an increase in free radical production (15.16). Histological examination in the present study showed cellular damage in the livers of pregnant female albino rats treated with lead acetate at a dose of 200 mg/kg. This damage was manifested by dilated blood sinusoids and hepatocellular atrophy. When liver cells are injured, they begin an abnormal regeneration process, leading to cell degeneration and enlargement of liver tissue. This indicates a defect in the metabolic processes within the cells, and this depends on the dose taken. As the condition deteriorates and cell nuclei degenerate in advanced stages, liver cells appear more susceptible to damage. This is due to the direct access of substances to the liver via the portal circulation from the gastrointestinal tract, which places them in constant contact with harmful substances. This continuous exposure may exacerbate the damage as a result of nutritional disorders or the accumulation of chemicals and toxins, especially with prolonged exposure (17). An aqueous extract of berbin at a dose of 200 mg/kg has shown an effective role in protecting and treating liver cells and reducing their injury caused by lead acetate. Berbin contains minerals, vitamins, omega-3 fatty acids, and a wide range of phytochemicals such as flavonoids and alkaloids (18). In a study conducted by Prabha Karan et al. (19), it was proven that administering a methanol extract of berberine at oral doses of 200 and 400 mg/kg demonstrated hepatoprotective effects against 5 D-Palaciosamine. Consistent with the results of the current study, (20) demonstrated that berberine inhibits paracetamol-induced hepatotoxicity by reducing it. The changes that occurred in the kidneys were represented by leukocyte infiltration, hematuria, thickening of capillary walls, and proliferation of fibroblasts. The results of the current study are consistent with those of (21) where the functional deterioration resulting from lead acetate in the quail kidneys

was characterized by tubular dilatation, congestion, degeneration, and cellular changes within the intertubular spaces in the kidneys of chickens treated with lead acetate. The incidence of histopathological changes increases with increasing dose. The kidneys of rats treated with high doses showed cell atrophy, infiltration of multinucleated cells, and hemorrhage of cellular debris (22.23.24). These results are consistent with the results of the current study. The role of the aqueous extract of berbin in reducing the effects of lead acetate is evident. The antioxidant effect of the aqueous extract of berbin is due to its phenolic compounds content, due to its high ability to produce diverse antioxidants, which can be based on its chemical structure through membrane-stabilizing activity by generating ROS (25).

### **Conclusion**

The current study concluded that treating female albino rats with lead acetate during pregnancy for only 10 days resulted in histological changes in the liver, including hepatic cell atrophy and dilated sinusoids. Treatment with an aqueous extract of the plant barberry throughout pregnancy reduced the damage caused by lead acetate to the liver, as results showed limited infiltration of white blood cells and the appearance of radial hepatocytes. Histological results also demonstrated damage to kidney tissue during treatment with lead acetate for the same period. Results showed irregular hepatic fibroblast chains, congestion, thickening of capillary walls, and proliferation of fibroblasts. Treatment with the aqueous extract of the plant barberry also revealed atrophy of the kidney glomeruli, dilated capsular space, and limited epithelial cell shedding.

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## التغيرات النسجية المصاحبة للتعرض لخلات الرصاص وتقييم الدور العلاجي لمستخلص نبات البربين لأنات الجرذان البيض وأجنتها

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فرع التشريح والأنسجة ، كلية الطب البيطري ، جامعة تكريت ، تكريت ، العراق

### الملخص

هدف البحث الحالي إلى دراسة تأثير خلات الرصاص على اناث الجرذان الحوامل وأجنتها والدور الوقائي للمستخلص المائي لنبات البربين في الأثار الناجمة عن التعرض للرصاص . تمت هذه التجربة في البيت الحيواني التابع لكلية الطب البيطري في جامعة تكريت ، حيث تم أخذ 30 أنثى جرد أبيض تراوحت اوزانها ما بين 180\_200 غم بعمر 3 أشهر ووضعتها داخل اقفاص بلاستيكية ومزاوجتها مع ذكور من نفس السلالة ثم توزيعها عشوائياً إلى ثلاث مجاميع شملت كل مجموعة خمسة حيوانات اناث حوامل . المجموعة الأولى اعتبرت مجموعة سيطرة . المجموعة الثانية تمت معاملتها بخلات الرصاص خلال الفترة 10\_19 يوم من الحمل . المجموعة الثالثة تمت معاملتها بخلات الرصاص خلال الفترة 10\_19 يوماً من الحمل وكذلك عوملت بالمستخلص المائي للبربين طيلة فترة الحمل . تمثلت الجرعة اليومية الفموية من خلات الرصاص 50 ملغم / كم ومستخلص نبات البربين ب 200 ملغم / كم . حيث اظهر الفحص النسجي لمجموعة السيطرة للكبد صفوف الخلايا الكبدية وشبكة الحبيبات الدموية الحاوية على خلايا كوفر أما في الكلى فتبين الحيز المحفظي ومحفظة بومان بشكل قريب من الطبيعي وكذلك النيبات الملفوفة . أما في المجموعة الثانية فقد اظهرت خلايا الكبد ضمور وتوسع للحبيبات الدموية المحتوية على خلايا كوفر. أما كلى هذه المجموعة فقد تبين أنها تعرضت لأرتشاح خلايا الدم البيض وأحتقان دموي مع انتشار للأرومات الليفية وتخن جدران الشعيرات الدموية وعدم انتظام احجام نيبات الكلى. أما كبد المجموعة الثالثة فقد احتوى الوريد المركزي على فرط دم متحلل وكذلك ارتشاح لخلايا دم بيض. أما هذه المجموعة فقد عانت ضمور محدود وتوسع طفيف للحيز المحفظي وإنسلاخ محدود لخلايا ظهارية والنيبات الملفوفة شبه طبيعية.

الكلمات المفتاحية: خلات الرصاص. المستخلص المائي لنبات البربين. أنات جرذان بيضاء وأجنتها.  
كبد. كلى.