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Toxic effect of lead acetate on the liver of male albino rats and evaluation of the protective role of *Portulaca oleracea* extract: A histological and biochemical study

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Abstract

Objective

By producing reactive oxygen species and impairing antioxidant defense systems, lead is a significant environmental contaminant that causes oxidative stress and hepatotoxicity. By assessing both biochemical parameters and histological changes, this study sought to determine the hepatoprotective and antioxidant effects of aqueous *Portulaca oleracea* extract on the liver tissue of male albino rats exposed to lead acetate.

Materials and Methods

Four groups (n = 7 per group) of twenty-eight male albino rats were randomly assigned. As the control group, Group I was given clean water and a typical diet. Lead acetate (25 mg/kg) was given intraperitoneally to Group II every 72 hours. For eight weeks, *P. oleracea* extract was administered orally to Groups III and IV at doses of 200 mg/kg and 400 mg/kg, respectively. Using common spectrophotometric methods, serum biochemical parameters including AST, ALT, MDA, GSH, SOD, and CAT were measured. Hematoxylin and eosin staining was used to histologically examine liver tissues in order to assess vascular changes, inflammatory infiltration, and hepatocellular structure.

Results

The changes in lipid peroxidation (MDA), antioxidant enzyme activities (GSH, SOD, and CAT), and liver enzyme activities (AST and ALT) brought on by lead acetate exposure in Group II were significantly lessened by the administration of *P. oleracea* extract in Groups III and IV. A dose-dependent restoration of these parameters was observed, with a more noticeable improvement at the higher dose. *P. oleracea's* hepatoprotective effect was confirmed by histological analysis, which revealed better hepatic architecture, less inflammatory cell infiltration, and less hepatocyte hypertrophy and degeneration when compared to the lead-treated group. These findings suggest that *P. oleracea* provides protection by stabilizing hepatocyte membranes and boosting endogenous antioxidant defenses.

Conclusion

The aqueous extract of *Portulaca oleracea* exhibits strong antioxidant and hepatoprotective properties against hepatic damage caused by lead acetate. These findings support additional experimental and clinical research into its therapeutic uses by highlighting its potential as a natural, safe, and affordable hepatoprotective agent for reducing liver damage caused by heavy metals.

Keywords: Antioxidants, hepatocyte, hepatoprotection, histology, *Portulaca oleracea*

Paper Type: Research Paper.

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Introduction

Minerals are essential for cellular signaling, enzymatic activity, and gene regulation. (Stefanache et al., 2023). However, because of their detrimental biological effects, the growing environmental contamination caused by toxic elements, especially lead (Pb), has become a major global concern (Chen et al., 2019). Lead is one of the most dangerous heavy metals; it is widely found in the environment and causes a variety of chemical, physiological, and behavioral problems in both people and animals (Melebary & Elnaggar, 2023). The soft, heavy, blue-gray metal lead (Pb) is found naturally in the Earth's crust, but human industrial processes such as mining, smelting, battery production, and the use of leaded paints and gasoline have made it more common. Lead enters the body through contaminated food, water, and air and has toxic effects

on almost every biological system due to its persistence and cumulative nature (Abdelhamid et al., 2020). It can negatively impact humans and other organisms by bioaccumulating and biomagnifying through the food chain after being introduced into the ecosystem (Yuniarti et al., 2021). Three main pathways allow lead to enter the human body: dermal absorption through the skin, inhalation through the respiratory system, and ingestion through the gastrointestinal tract (Boskabady et al., 2018). When lead enters the bloodstream, some of it attaches itself to red blood cells, and the rest travels through plasma to soft tissues like the brain, liver, and kidneys, as well as to bone, where it can stay for decades. Prolonged exposure and buildup can cause serious harm to the cardiovascular, neurological, gastrointestinal, renal, and hematopoietic systems (Natasha et al., 2020). Lead toxicity has been connected to cardiovascular problems, neurodegenerative diseases, nephrotoxicity, and carcinogenesis in both epidemiological and experimental studies. Additionally, it has been demonstrated that exposure to lead increases the generation of reactive oxygen species (ROS), which leads to oxidative stress by causing lipid peroxidation, protein oxidation, DNA damage, and impairment of the antioxidant defense system (Balali-Mood et al., 2021). When produced in excess, reactive oxygen species—which are byproducts of many metabolic and degradation processes—can upset cellular homeostasis and harm proteins, lipids, and nucleic acids (Mittal et al., 2014). One of the main mechanisms behind lead-induced hepatotoxicity is oxidative stress, which is brought on by the buildup of ROS. Being the primary organ in charge of metabolism and detoxification, the liver is especially susceptible to oxidative damage brought on by heavy metals. Biological systems use both enzymatic and non-enzymatic antioxidant defenses to combat oxidative stress. These comprise non-enzymatic compounds like glutathione (GSH) and vitamins C and E, as well as enzymes like glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD) (He et al., 2017). Oxidative damage results in inflammation, cellular dysfunction, and ultimately tissue necrosis when these antioxidant defenses are overpowered. Supplementing with natural antioxidants has garnered significant interest as a means of reducing oxidative damage. By giving free radicals electrons without becoming unstable themselves, exogenous antioxidants can neutralize them and stop oxidative chain reactions and cellular damage. The protective potential of antioxidants derived from plants in reducing the harmful effects of environmental pollutants has thus been the subject of numerous studies (Jideani et al., 2021; Rajab & Ali, 2020). Interest on medicinal plants as a source of bioactive compounds has rapidly increased due to ability of easy access, low toxicity and wide panel pharmacological activities associated with phytochemicals derivatives. As natural alternatives of synthetic additives, botanicals and medicinal plantshave been attracted much interest in the last few decades (Amirteymoori et al., 2021). Bioactive substances like flavonoids, alkaloids, phenolic acids, and essential oils are abundant in these natural products (Mohammadabadi et al., 2022), which support their antibacterial, anti-inflammatory, and antioxidant qualities (Mohammadabadi et al., 2025). As a result, phytobiotics are essential for enhancing performance, health, and product quality (Safaei et al., 2025). There are many benefits to using medicinal plants and phytobiotics as natural antimicrobial growth promoters instead of antibiotics (Vahabzadeh et al., 2020). Among these advantages are enhanced zootechnical efficiency metrics and the prevention of particular illnesses (Mohammadabadi et al., 2023),

improved liver function, increased digestive enzymes, hypocholesterolemic effects, and antimicrobial and antioxidant properties (Roudbar et al., 2015). Additionally, it has been demonstrated that phytobiotics alter the gut microbiota, improving absorption and bolstering immune system performance (Vahabzadeh et al., 2021). Additionally, by maximizing nutrient utilization, phytobiotics are linked to lowering the effects of stress, enhancing quality, and lessening the environmental impact (Mohammadabadi et al., 2024). The genus *Portulaca* is the largest and most widely distributed of the 25-30 genera and roughly 450-500 species that make up the family Portulacaceae (Amirul Alam et al., 2014). *Portulaca oleracea* L. (*purslane*) is a kind of annual succulent herb with slender and fleshy stems, smooth leaves, little yellow or white flowers and many black seeds. It has a cosmopolitan presence and is common in tropical and subtropical areas (Sangeetha et al., 2020). *P. oleracea* is an anciently used food and medicinal plant, with evidence of use in the Mediterranean, Central Asia, and Eastern Europe since ancient times (Ahn et al., 2018). It is traditionally used to cure several diseases including respiratory and gastrointestinal disorders, inflammation of the liver, urinary tract infection, fever, inflammatory symptoms (Rahimi et al., 2019). Pharmacological studies have revealed that P. oleracea has various pharmacological activities such as analgesic, antimicrobial, antiviral, antipyretic, diuretic, anti-inflammatory and wound healing effects (Ahn et al., 2018). These actions are mainly due to its various phytochemical components like....., flavonoids alkaloids and omega- 3 fatty acid etc. Plant possesses strong antioxidant effects too partly attributed to its flavonoids apigenin, quercetin, kaempferol, myricetin, luteolin, genisteinn and nut alloys including dopamine, noradrenaline and L-DOPA(3-hydroxy-4-methoxyphenylalanine) (Srivastava et al., 2023; Iranshahy et al., 2017). They act as free radicals scavengers, metal chelators and also they modulate endogenous antioxidant enzyme systems. Besides medicinal properties, *P. oleracea* might also find utility in food and feed sectors due to the presence of higher nutritional content (Majeed et al., 2017). Earlier reports have declared that lead acetate is hepatotoxic and several plant extracts such as *Moringa oleifera*, *curcumin*, *garlic* can exert hepatoprotective activity. Yet, these studies largely focused on biochemical analysis and the histological evaluation as confirmation of tissue level protection was limited. Moreover, few studies have been conducted on hepatoprotective effect of *p. oleracea* despite the presence of high amounts of flavonoids, alkaloids and natural antioxidants. The available literature further shows a lack of studies that assess not only the dose-response relationship, but also combine biochemical and histopathological endpoints. The objective of the present study was, therefore, to fill these lacunae and ascertain potential protection offered by aqueous extract of *P. oleracea* in lead acetate-induced hepatic injury in male albino rats. These findings helped us to gain a better understanding of the mechanisms of action for the hepatoprotective effect of *P. oleracea* by examining both changes in the biochemical markers of oxidative stress and histopathological alters within liver tissue. It also explores the dose response activity of its hepatoprotective nature, which gives useful information on its therapeutic potential as a natural, low cost and safe hepatoprotectant. In conclusion, the findings of this study provide further evidence for the potential role of natural antioxidants in ameliorating heavy metal-induced toxicity and open the door to future experimental and clinical studies on the medicinal use of *P. oleracea*.

Materials and Methods

Experimental animals: Male albino Wistar rats between 185 and 230 g. Animals were purchased and accommodated in the animal house of the Department of Biology, College of Education for Pure Sciences, University of Anbar, Iraq. The rats were housed in metabolic cages and kept at the controlled environment temperature (22 ± 2 °C), relative humidity (about 55%), and a 12:12-h light-dark cycle. All animals were acclimated to standard pellet diet and tap water ad libitum 7 days before the experiment. All experimental protocols were approved by the institutional committee on care and use of laboratory animals.

Preparation of plant extract: Plant material Fresh aerial parts of *Portulaca oleracea* (purslane) which is free from visible impurities were bought from the local market in Ramadi city, Anbar Governorate, Iraq. Taxonomic identification and authentication of the plant samples were determined by taxonomists from the Center for Desert Studies, University of Anbar. The aqueous extract, in which the plant material was homogenised at a concentration of 1:5 (w/v) with distilled water. The solution was allowed to stand at room temperature for 24 h and then filtered through Whatman No. 1 filter paper. The crude extract (filtrate) was preserved at 20 °C in air right containers till used for experimentation.

Chemicals: Lead acetate trihydrate [$Pb(CH_3COO)_2 \cdot 3H_2O$] was procured from Sigma-Aldrich Ltd. (UK). A stock solution of lead acetate was used, 1 g of lead acetate was dissolved in 20 mL deionized water and diluted to appropriate concentrations before administration. All other chemicals and reagents applied were of analytical grade and purchased from reputable commercial suppliers.

Experimental design: After acclimatization, twenty-eight male rats were randomly divided into four groups (n = 7 per group) based on body weight:

- Group I (Control): Received a standard diet and tap water ad libitum throughout the experiment.
- Group II (Lead acetate): Administered 25 mg/kg lead acetate via intraperitoneal injection every 72 hours for eight weeks.
- Group III (Lead + *P. oleracea* 200 mg/kg): Received an oral dose of *P. oleracea* aqueous extract (200 mg/kg body weight) along with the same lead acetate regimen as Group II.
- Group IV (Lead + *P. oleracea* 400 mg/kg): Received an oral dose of *P. oleracea* aqueous extract (400 mg/kg body weight) with the same lead acetate exposure as Group II.

All treatments were administered for eight consecutive weeks. Food and water were made freely available to all groups throughout the experimental period.

Biochemical analysis-Liver function tests: Serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities were estimated to evaluate hepatic function applying the colorimetric method of Reitman and Frankel (1957).

Assessment of oxidative stress and antioxidant parameters: Lipid peroxidation was evaluated by measuring malondialdehyde (MDA) levels in liver tissue applying the thiobarbituric acid reaction method described by Ohkawa et al. (1979). Levels of reduced glutathione (GSH) were estimated according to Ellman method (1959). The activity of catalase (CAT) was determined as the rate of H_2O_2 degradation according to Aebi (1984). The determination of superoxide dismutase (SOD) activity was carried out according to Nishikimi *et al.* (1972). All

estimations were done using the colorimetric methods according to standard laboratory procedures.

Blood sample collection: Following the experimental period, animals were anaesthetized and blood was directly sampled from the heart employing sterile syringes. The samples were allowed to clot at room temperature and centrifugated (3,000 rpm for 30min). The serum was then separated and preserved at −20 °C until being subjected to further biochemical examinations**.**

Histological study: The rats were sacrificed after blood collection, and their liver tissues were excised immediately, rinsed with normal saline, blotted dry on filter paper and fixed in 10% neutral-buffered formalin for histopathological examination. Formalin-fixed tissues were dehydrated through a series of graded ethanol solutions, cleared in xylene, and embedded in paraffin wax. Sections of 5-7 µm were sliced by a rotary microtome and stained with hematoxylin and eosin (H&E). The tissue slides were observed through a compound light microscope with digital camera to analyze the histological alterations such as hepatocellular degeneration, vascular congestion, and inflammatory infiltration.

Statistical analysis: Data are presented as the mean ± SE. Inter-group statistical comparisons were conducted using the one-way ANOVA method, followed by appropriate post hoc test if needed. All the statistical analyses were performed with SPSS software version 17 (IBM Corporation, Armonk, NY). A p-value < 0.05 was considered statistically significant.

Results and discussion

Lead is a known initiator of oxidative stress, and reactive oxygen species (ROS) in the body are an important marker of oxidative stress (Li et al., 2021). In studies on animals and humans, several antioxidant molecules and enzymatic markers have been used to assess lead-induced oxidative damage (Fan et al., 2020). Various parameters have been commonly used in tissues (tissue antioxidant levels, biochemical analysis) or blood (oxidative stress parameters), such as low GSH/GSSG and altered SOD activity (Demirci-Cekic et al., 2022). The present study indicated a statistically significant ($P \leq 0.05$) increase in the levels of liver enzymes AST and ALT on the second group treated with Lead acetate as compared to normal control. On the other hand, animals that received aqueous extract of *Portulaca oleracea* before and after lead acetate exposure had significantly lower levels of these enzymes when compared to the lead-only group (Figs 1 and 2). Lead is one of the most common industrial and environmental contaminants causing severe organ damage in human and animals. It has a major effect on the liver specifically, thus leading to high hepatic enzyme characteristics of lead. In our research, the concentrations of AST and ALT in normal control rats were significantly higher compared to rats receiving lead acetate. The lead and its metabolites, via karyorrhexis, appear to cause plasma membrane rupture and cell damage with resultant extracellular release of these enzymes and therefore explain how serum transaminases are raised. AST and ALT are marker enzymes of liver integrity and function (Abdelhamid et al., 2020). These enzymes tend to increase in acute hepatotoxicity or moderate hepatocellular injury, but may decrease as chronic intoxication or damage persists (Khamphaya et al., 2022). Meaningful elevation in total cholesterol was also observed with lead acetate treatment in our study (Al-Megrin et al., 2019). The high AST and ALT levels are related to the

poor integrity of liver structure. Lead inhibits K^+ and Ca^{2+} channels, which causes cell lysis, while cytoskeletal changes increase susceptibility to membrane rupture. Exposure to lead has been associated with DNA damage, including base pair mutations, deletions and oxidative challenges (i.e., interventions) as well as perturbations of both endoplasmic reticulum and intracellular Ca^{2+} homeostasis that negatively impact protein synthesis (Rădulescu & Lundgren 2019).

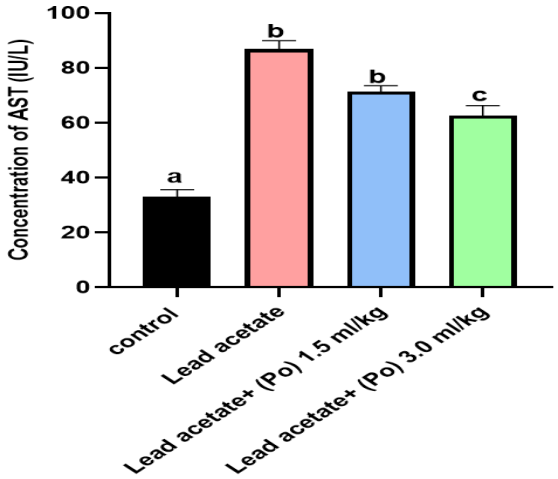

Figure 1. *P. oleracea's* impact on AST activity in albino rats given lead acetate. Significant differences between groups at P ≤ 0.05 are indicated by different letters (n = 7)

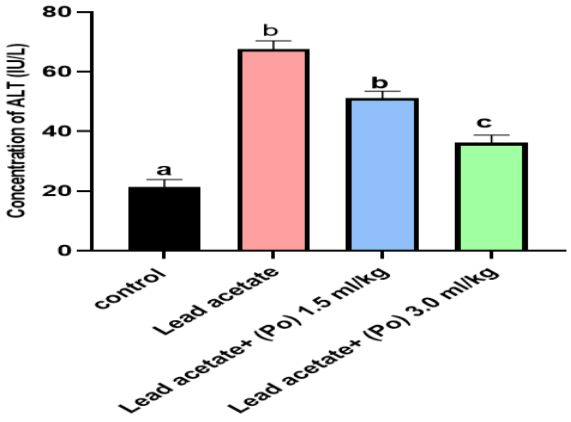

Figure 2. *P. oleracea's* impact on ALT activity in albino rats given lead acetate. Significant differences between groups at P ≤ 0.05 are indicated by different letters (n = 7)

Medicinal herbs and natural products are being receiving more attention because of their therapeutic potential and low side effects with less cost as compared to synthetic drugs (Ali, 2017). Administration of different extracts of *P. oleracea* significantly attenuated the modified biochemical parameters. CYP-450 aromatase is involved in liver regeneration and the inhibition of CYP-450 aromatase may be essential for hepato-detoxification (Srivastava et al., 2023). It is speculated that the flavonoids in P. oleracea mediated its hepatoprotective effects by inhibition of aromatase (Aini et al., 2022). Our findings suggested that treatment with *P. oleracea* extract significantly decreased the activity of AST and ALT. This decrease suggests that the bioactive

compounds, apigenin and kaempferol help in the amelioration of the tissue structural integrity and prevents lead induced hepatotoxicity (Yang et al., 2018). Various phytoconstituents of *P. oleracea*, including phenolic compounds, coumarins, monoterpene glycosides and flavonoids have been reported to demonstrate protective activity against several toxins (Kumar et al., 2022). Lead acetate caused a significant increase in MDA (a marker of lipid peroxidation) and however decreased in antioxidant, GSH, SOD and CAT levels compared to healthy controls as depicted in Figures 3-6. In contrast, animals pretreated and/or posttreated with *P. oleracea* extract showed an inverse trend where MDA levels decreased and antioxidant activity was restored.

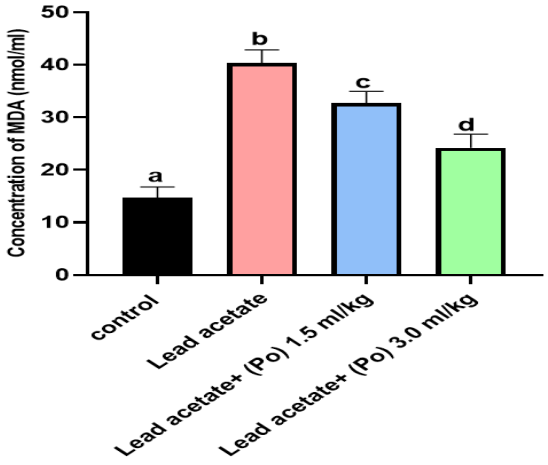

Figure 3. Effect of *P. oleracea* on MDA levels in lead acetate-treated albino rats. Different letters indicate meaningful differences among groups at $P \leq 0.05$ (n = 7)

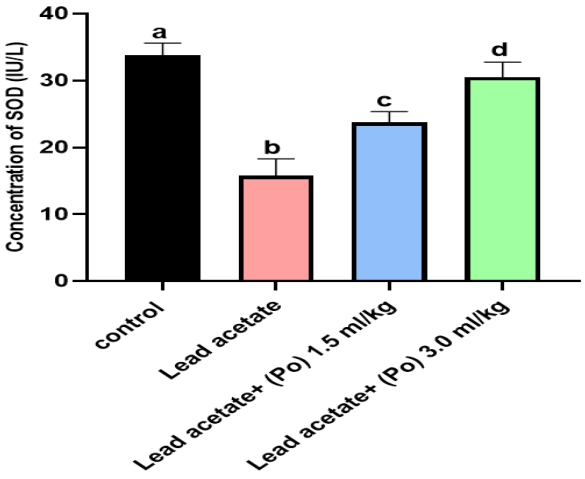

Figure 4. Effect of *P. oleracea* on SOD activity in lead acetate-treated albino rats. Different letters indicate meaningful differences among groups at $P \leq 0.05$ (n = 7)

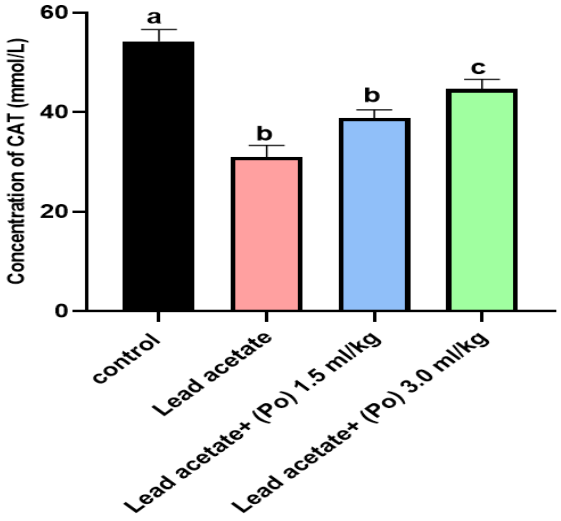

Figure 5. *P. oleracea's* impact on CAT activity in albino rats given lead acetate. Significant differences between groups at P ≤ 0.05 are indicated by different letters (n = 7)

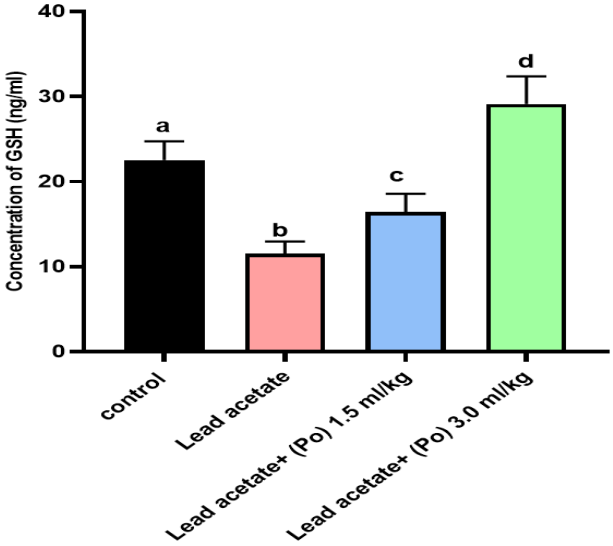

Figure 6. *P. oleracea's* impact on GSH activity in albino rats given lead acetate. Significant differences between groups at P ≤ 0.05 are indicated by different letters (n = 7)

Cellular antioxidant defense against ROS is accomplished by a robust network of antioxidants working in collaboratively and complementary mechanisms (Sachdev et al., 2021). However, when the formation of ROS exceeds these defenses, oxidative stress occurs and causes cellular damage and disease. Lead toxicity caused free radical damage through two mechanisms: direct depletion of antioxidant reserves and production of ROS (including H2O2), hydroperoxides, and dioxygen (Sies et al., 2022). Xenobiotics such as heavy metals first and foremost create oxidant injury in the cellular membrane, attacking polyunsaturated fatty acids placed in phospholipids (Ozdemir & Surmelioglu, 2023). Lead also induces the lipid peroxidation which is directly linked with the free radical mechanism and results in instability of cell

membranes (Abdelhamid et al. It has been well documented in the previous reports that lead-induced oxidative stress is highly associated with membrane lipid peroxidation. Plants containing antioxidant dietary sources have received significant attention for their capability to protect tissues from oxidative stress (Makhaik et al., 2021). Specific actions of natural products on lead acetate toxicity continue to be under study. In the present study, lead acetate significantly decreased SOD, CAT and GSH activity ($p \leq 0.05$), making the tissues susceptible to peroxidative damage. SOD and CAT are metalloproteins, actively detoxifying peroxides via enzyme action but require specific prosthetic groups and trace elements for optimal structure and activity (Mendonça et al., 2022). Lead complexes sulfhydryl proteins, it competitively inhibits the absorption of trace minerals and ultimately disrupt enzyme activity directly, which accounts for 1/2 of mechanism involved in lead toxicology (Abdulqader, 2022). GSH diminishment is another potential mechanism of lead poising. GSH, a tripeptide with reactive -SH radical has strong reductive action. Intracellular GSH depletion reduces cell's ability to cope against oxidative stress (as lead preferentially binds - SH groups) and thus deplete GSH content and disrupt antioxidant defence mechanism of the cells (Sharma et al., 2023; Singh et al., 2018). Our findings indicated that the extract of *P. oleracea* can modulate lipid peroxidation and scavenge hydroxyl radicals as well as superoxide anions (Azimi et al., 2023). The antioxidant agents of the extract seem to protect tissues against oxidative damage caused by lead acetate (Khodadadi et al., 2018). *P. oleracea* supplementation markedly restored the levels of SOD, CAT and GSH, validating the assertion that thiols (-SH groups) have a key role in defending cells from ROS. The main mechanism for the hepatoprotective action of *P. oleracea* is an increase in GSH and its dependent antioxidant enzymes (Farkhondeh et al., 2019). Histological sections of the liver in the control group showed central vein in hepatic lobules which was situated at the center of hepatic lobule and encircled by many macrophages, numerous Kupffer cells were observed within most of blood sinusoidal. Note pattern of hepatocytes arranged in a radial manner around the central vein (Fig. 7). While the liver sections of the second group (administered with lead acetate) revealed significant histopathological changes. The portal areas showed a branch of hepatic artery with thick wall and presence of blood clot, besides dilated endothelial cells. Intraductal, branches of the bile ducts were lined with columnar epithelium and were infiltrated by countless white blood cells. Hypertrophied hepatocytes were arranged around dilated small blood sinusoids with numerous Kupffer cells. Inflammatory leukocyte infiltrates were also observed around the hepatic artery, intrahepatic portal vein and bile ducts. Hepatocytes in this region presented hypertrophy with multifocal aggregations of white blood cells and congested blood sinusoids, compared with healthy controls (Figs 8-10).

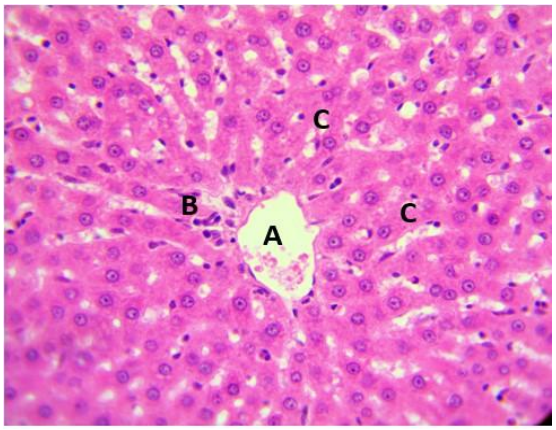

Figure 7. Cross-section of the liver of a control rat showing the central vein (A) within the hepatic lobule, Kupffer cells in the blood sinusoids (B), and hepatocytes arranged radially around the central vein (C) (H&E, ×40)

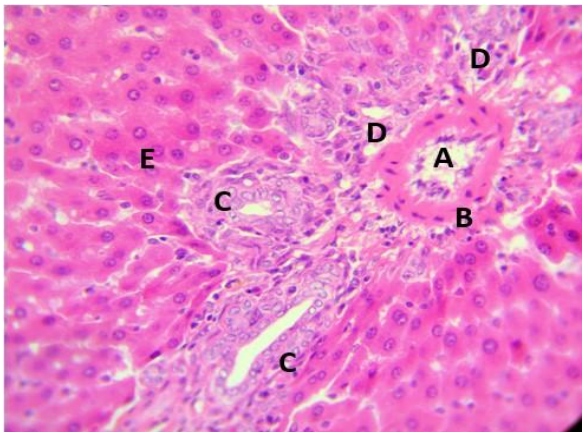

Figure 8. Cross-section of the liver of a lead acetate-treated rat showing the portal area with a branch of the hepatic artery with a thickened wall and blood clot (A), endothelial cell hyperplasia in the artery lumen (B), branches of the bile ducts with columnar epithelial cells (C), infiltration of white blood cells (D), and hepatocyte hyperplasia (E) (H&E, ×40)

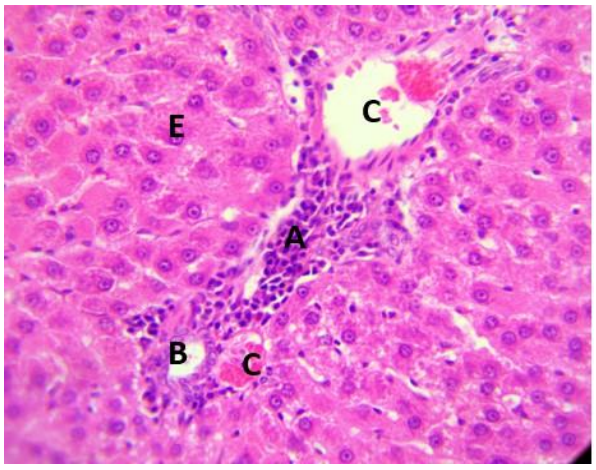

Figure 9. Cross-section of the liver of a lead acetate-treated rat showing leukocyte infiltration (A), a branch of the hepatic artery (B), portal vein (C), bile duct (D), and hepatocyte hyperplasia (E) (H&E, ×40)

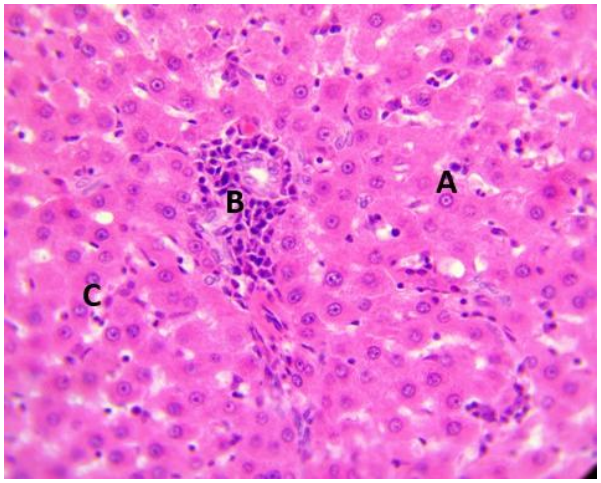

Figure 10. Cross-section of the liver of a lead acetate-treated rat showing hepatocyte hyperplasia (A), focal leukocyte infiltration (B), and blood sinusoids containing Kupffer cells (C) (H&E, ×40)

The third group, treated with lead acetate alongside *P. oleracea* extract, displayed less severe histological alterations. Mild hepatocyte hypertrophy was noted in the liver parenchyma, with focal degeneration of some cells. Kupffer cells were distributed diffusely across the blood sinusoids, proposing a partial protective effect of the extract compared to the lead-only group (Figure 11).

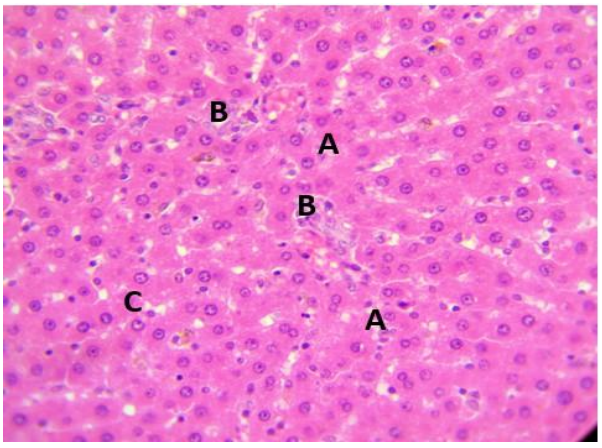

Figure 11. Cross-section of the liver of a rat from the third group showing hepatocyte hyperplasia and necrosis (A), focal degeneration of hepatocytes (B), and Kupffer cells in the blood sinusoids (C) (H&E, ×40)

Liver sections from the fourth group, receiving a higher dose of *P. oleracea*, demonstrated near-normal histology. The central vein appeared empty of blood, surrounded by minimal infiltration of white blood cells. Narrow, continuous blood sinusoids containing both small and large Kupffer cells were observed. Hepatocytes were arranged radially around the central vein, with hypertrophic cells containing large spherical nuclei. Some blood-filled central veins contained homogeneous decomposed blood with a few white blood cells (Figures 12-13). These

observations indicate meaningful tissue recovery and hepatoprotective effects at higher doses of *P. oleracea*.

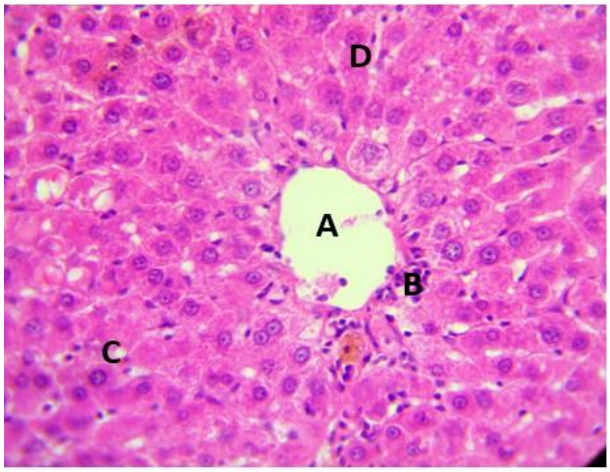

Figure 12. Cross-section of the liver of a rat from the fourth group showing an empty central vein (A), mild leukocyte infiltration around the vein (B), Kupffer cells in blood sinusoids (C), and macrocytic hepatocyte hyperplasia (D) (H&E, ×40)

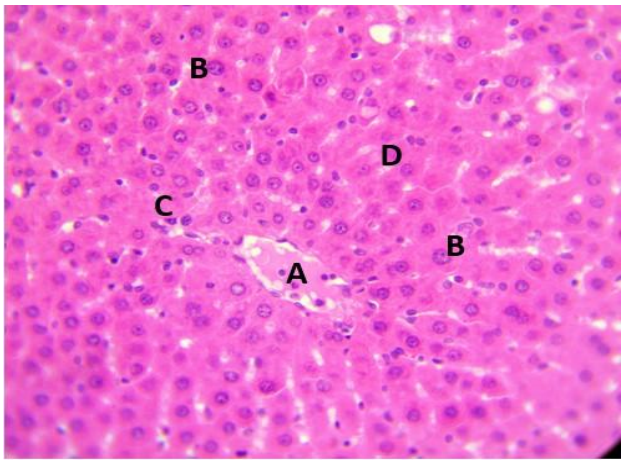

Figure 13. Cross-section of the liver of a rat from the fourth group showing the central vein containing hemolyzed blood (A), hepatocyte hypertrophy (B), Kupffer cells in blood sinusoids (C), and radial arrangement of hepatocytes (D) (H&E, ×40)

The oxidative damage observed in liver tissue following lead acetate exposure is primarily attributed to reactive oxygen species (ROS), which create membrane damage, improved plasma membrane permeability, and vascular alterations leading to bleeding (Kucukler et al., 2021; Aouacheri & Saka, 2021). These findings were in accordance with previous studies that viewed hepatocyte vacuolization and inflammatory cell infiltration being typical traits of lead-mediated hepatic damage (Abdelhamid et al., 2020). The hepatoprotective effects of *P. oleracea* may be in line with its known pharmacological properties including antibacterial, analgesic, antihypertensive, antioxidant and anti-inflammatory activities which could be due to the presence of bioactive phytochemicals (Ghorani et al., 2023). The active antioxidant and free radical

scavenging effect may have decreased the MDA concentration in the liver of lead treated rats. *P. oleracea* treatment at 400 mg/kg body weight significantly suppressed liver MDA content in lead-administered mice (Veber et al., 2020). These findings support previous studies emphasizing the anti-oxydative potential of the extract against heavy metals-induced oxidative stress (Farag et al., 2021). *P. oleracea* improves antioxidant defense system, and therefore prevents lipid peroxidation and regulates hydrogen peroxide to ameliorate liver injury (Recknagel et al., 2020). The extract treatment reduced the oxidative stress in Pb-intoxicated rats probably by suppressing SOD, CAT and GPx depletion in the serum and the liver. The free radical scavenging activity of the extract reduces cellular damage through ROS neutralization, and this may reflect its protective action.

Study limitations

1. Study was based only on short-term biochemical and histological parameters, the long term benefits of *P. oleracea* is still unknown.
2. There was only a single animal model (rats) which restricted direct extrapolation of findings to humans.
3. This study was conducted with only aqueous extract of *P. oleraceae* and other extracts or isolated bioactive compounds might overrule similar effects.
4. Molecular mechanics, including signaling pathways and the changes on gene expression were not studied.

Conclusion: In summary, lead acetate exposure resulted in significant hepatotoxicity and enhanced oxidative stress and lipid peroxidation expressed by increase in liver enzymes (AST, ALT), total cholesterol, MDA levels with decrease antioxidant enzyme activities (SOD, CAT, GSH). Oral *Portulaca oleracea* extract treatment, given either prophylactically or therapeutically, significantly reversed these biochemical imbalances; restored antioxidant mechanisms and maintained the histological architecture of liver. Hepatoprotective activity of *P. oleracea* may be attributed to cause by its bioactive compounds, which includes flavonoids, phenolic compound and monoterpene glycosides scavenge for free radicals, augment the enzymatic antioxidant capacity and uphold cellular membrane integrity. These findings contribute to the strong evidence of *P. oleracea* as a safe, cost-effective and natural candidate in ameliorating lead-induced liver damages and provide scientific basis for further studies on plant-based therapeutic approaches against heavy metal toxicity.

Author contributions

H.R.A.K.A. designed the study and drafted the manuscript. H.H.M. carried out the biochemical analyses. A.A.H. conducted the histological examinations. L.H.A. supervised the study and critically revised the manuscript. All authors have read and approved the final version of the manuscript.

Data availability statement

The datasets generated and/or analyzed through the current study are available from the corresponding authors upon reasonable request.

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Ethical considerations

The use of rats in this study was approved by the University of Anbar, Iraq (Approval Number: 243, dated 24 March 2024) and conducted in accordance with ethical guidelines for the care and use of laboratory animals.

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Conflict of interest

The authors declare no conflicts of interest related to this study.

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اثر سمی استات سرب بر کبد موش‌های صحرایی نر آلبینو و ارزیابی نقش حفاظتی عصاره گیاه خرفه (*Portulaca oleracea*) : مطالعه‌ای با رویکرد بافت‌شناسی و بیوشیمیایی

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چکیده

هدف: سرب یکی از آلاینده‌های عمده محیط‌زیست است که از طریق تولید گونه‌های فعال اکسیژن و تضعیف سامانه‌های دفاعی آنتی‌اکسیدانی، موجب سمیت کبدی و استرس اکسیداتیو می‌شود. هدف از این پژوهش، بررسی اثرات آنتی‌اکسیدانی و محافظت‌کننده کبدی عصاره آبی گیاه خرفه (*Portulaca oleracea*) بر بافت کبد موش‌های صحرایی نر آلبینو در معرض استات سرب بود؛ این ارزیابی با بررسی شاخص‌های بیوشیمیایی و تغییرات بافت‌شناسی انجام گرفت.

مواد و روش‌ها: بیست‌وهشت موش نر آلبینو به‌صورت تصادفی به چهار گروه (هر گروه ۷ موش) تقسیم شدند. گروه اول به‌عنوان شاهد، رژیم غذایی استاندارد و آب سالم دریافت کرد. گروه دوم هر ۷۲ ساعت، استات سرب با دوز ۲۵ میلی‌گرم بر کیلوگرم به‌صورت درون‌صفاقی دریافت نمود. گروه‌های سوم و چهارم به‌ترتیب دوزهای ۲۰۰ و ۴۰۰ میلی‌گرم بر کیلوگرم عصاره خرفه را به‌صورت

خوراکی و به‌مدت هشت هفته دریافت کردند. شاخص‌های بیوشیمیایی سرم شامل AST ، ALT، MDA، GSH، SOD و CAT با روش‌های استاندارد اسپکتروفوتومتری اندازه‌گیری شدند. نمونه‌های بافت کبد نیز با رنگ‌آمیزی هماتوکسیلین و ائوزین برای ارزیابی ساختار سلول‌های کبدی، نفوذ سلول‌های التهابی و تغییرات عروقی مورد بررسی قرار گرفتند.

نتایج: تجویز عصاره خرفه در گروه‌های سوم و چهارم باعث بهبود معنی‌دار تغییرات ایجادشده در فعالیت آنزیم‌های کبدی (AST و ALT) ، پراکسیداسیون چربی‌ها (MDA) و فعالیت آنزیم‌های آنتی‌اکسیدانی (GSH ، SOD و CAT) در اثر مواجهه با استات سرب در گروه دوم گردید. این شاخص‌ها به‌صورت وابسته به دوز به حالت طبیعی بازگشتند، به‌طوری‌که دوز بالاتر اثر بهبودی بیشتری نشان داد. بررسی‌های بافت‌شناسی نیز اثر محافظتی خرفه را تأیید کرد و بهبود ساختار بافت کبد، کاهش نفوذ سلول‌های التهابی و کاهش هیپرتروفی و دژنراسیون سلول‌های کبدی را در مقایسه با گروه تیمارشده با سرب نشان داد. این یافته‌ها نشان می‌دهد که خرفه از طریق تقویت دفاع آنتی‌اکسیدانی درون‌زا و تثبیت غشای سلول‌های کبدی، اثر محافظتی خود را اعمال می‌کند.

نتیجه‌گیری: عصاره آبی گیاه خرفه دارای فعالیت‌های قوی محافظت‌کننده کبد و آنتی‌اکسیدانی در برابر آسیب کبدی القاشده توسط استات سرب است. این نتایج، پتانسیل این گیاه را به‌عنوان یک عامل طبیعی، ایمن، کم‌هزینه و مؤثر در پیشگیری از آسیب‌های کبدی ناشی از فلزات سنگین برجسته می‌سازد و از انجام تحقیقات تجربی و بالینی بیشتر درباره کاربرد درمانی آن حمایت می‌کند.

کلمات کلیدی: آنتی‌اکسیدان‌ها، سلول کبدی، محافظت کبد، بافت‌شناسی، *Portulaca oleracea*

نوع مقاله: پژوهشی

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