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RESEARCH ARTICLE OPEN ACCESS

Therapeutic Potential of Ephedra alata Alcoholic Extract Against Lead Acetate-Induced Nephrotoxicity in Male Albino Rats: Biochemical, Histological, and Antioxidant Insights

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Abstract
Objective: This study investigated the nephroprotective potential of Ephedra alata alcoholic extract against lead acetate-induced toxicity in rats.
Methods: Forty male albino rats were assigned to five groups: control, lead acetate only, E. alata only, therapeutic (lead + E. alata), and preventive (E. alata + lead). Kidney function, oxidative stress, cytokine levels, and tissue morphology were evaluated.
Results: Lead acetate significantly increased serum urea, creatinine, MDA, IL-1β, and TNF-α levels ($p<0.05$), while reducing GPx. Co-administration of E. alata reversed these alterations. Histologically, E. alata mitigated renal damage and preserved tissue structure.
Conclusion: Ephedra alata extract exhibits strong antioxidant and anti-inflammatory properties, effectively protecting against lead-induced nephrotoxicity in rats.

Key words: Nephrotoxicity, Ephedra alata, Lead acetate, Antioxidants, Inflammation, Cytokines

Plain English Summary

This study investigated how E. alata extract impacts the function and tissue structure in male albino rats. We used 40 male rats weighing between 185 and 215 grams and randomly split them into five groups with eight rats each. Our results suggest that E. alata extract can help protect against the damaging effects of lead acetate in these animals.

Background

One of the most detrimental metals to human systems is lead (Pb); it is extensively found in the environment and leads to numerous physiological, biochemical, and behavioural issues (1). The Earth's crust naturally holds lead, a malleable, dense, blue-grey metal that has been spread across the environment due to various human activities (2). Lead was previously used in gasoline and household paint. Lead is still present in batteries,

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solder, ammunition, pipes, unglazed ceramics, traditional medicine, and construction materials (3). The use of surma, a cosmetic for the eyes that consists of 100% lead sulphide, has recently been shown to result in lead poisoning (4). Human exposure to lead continues to pose a major public health concern. Lead can travel through the food chain and cause harmful impacts on humans and other living creatures. It is a hazardous metal found in the environment and has adverse effects on multiple organs within the human body (3). Lead can penetrate the body via three main routes: the digestive tract, the respiratory tract, and the skin. A significant amount of evidence shows that lead functions as a toxic substance, impacting different organs such as the kidneys, liver, nervous system, immune system, and blood-producing system (5). Lead toxicity is associated with a range of physiological, morphological, and biochemical alterations, including liver dysfunction, haematological disorders, renal system impairment, irregularities in glucose metabolism, and issues in the nervous system (6).

Plants act as great sources of numerous bioactive compounds that are advantageous for enhancing and supporting human health (7). Herbal medicine is considered helpful for addressing different human ailments, such as oxidative stress, which includes free radicals and reactive nitrogen or oxygen species, which are responsible for inflicting harm on cells and diseases. Antioxidants represent a group of substances used to control or avert oxidants due to their ability to safeguard against disease and tissue harm (8).

Ephedra alata (referred to as Alanda in Arabic) is a perennial species belonging to the Ephedraceae family and Gnetales order, noted for its light green colouration, dense branching offshoots, dioecious
characteristics, and small stature, typically reaching heights of around 50–100 cm (9). It produces a potent pine aroma and possesses an astringent taste. This ephedra variety is indigenous to Iran, Palestine, Saudi Arabia, Algeria, Morocco, Egypt, Tunisia, Libya, Lebanon, Jordan, and Iraq. It flourishes extensively in arid settings on gravelly, rocky, sandy, and clay-rich soils, frequently located near moving dunes (10). E. alata is among the herbal plants employed in traditional medicine, particularly its stem infusion, to treat various health issues such as bronchial asthma, disorders of the circulatory system, complications of the digestive system, cancer, and infections caused by fungi and bacteria (11).

Current therapeutic modalities for lead-induced nephrotoxicity predominantly encompass chelation therapy and the cessation of exposure to lead. Nevertheless, these interventions exhibit considerable constraints. Chelating agents such as calcium disodium EDTA and dimercaptosuccinic acid (DMSA) demonstrate efficacy in instances of acute poisoning; however, their effectiveness in ameliorating chronic renal impairment is markedly limited. Furthermore, the early identification of lead nephropathy remains a formidable challenge attributable to the insidious progression of the disease and the absence of sensitive biomarkers. For example, urinary N-acetyl-β-D-glucosaminidase (NAG) levels are elevated in the early stages of lead nephropathy, yet its reliability as a biomarker for chronic exposure is subject to scepticism (12). Consequently, there exists an urgent imperative for the development of alternative therapeutic strategies that can effectively mitigate both oxidative stress and chronic inflammation associated with lead-induced renal pathology.

In the Middle East, particularly within the geographic confines of Iraq, the persistent exposure to environmental lead constitutes a substantial public health dilemma. Prolonged periods of conflict have engendered extensive lead pollution, particularly in proximity to industrial facilities and densely populated urban locales, consequently resulting in heightened blood lead concentrations among the populace (13). Empirical studies have documented an average blood lead concentration of 13.9 µg/dL among elementary-age children residing in Baghdad, which exceeds established international safety limits. Furthermore, analyses of soil samples collected from Baghdad have disclosed lead concentrations reaching as high as 9,350 mg/kg in areas adjacent to battery manufacturing plants, thereby signifying acute environmental degradation (14). These empirical findings accentuate the pressing necessity for the implementation of efficacious interventions aimed at alleviating lead exposure and its concomitant health hazards within the region.

In this study, an alcoholic extract was chosen because ethanol is an efficient solvent for extracting a wide range of both polar and nonpolar compounds, ensuring the recovery of important phytoconstituents like flavonoids and alkaloids with established reno-protective potential. Ephedra alata is characterised by its rich composition of bioactive phytochemicals, including ephedrine alkaloids, flavonoids, tannins, and phenolic compounds, many of which are known for their strong antioxidant and anti-inflammatory qualities.

Numerous studies carried out both in vivo and in

vitro on various extracts of this plant have shown a range of biological effects linked to the phytoconstituents, which include antibacterial, anti-inflammatory, antioxidant, anti-obesity, antidiabetic, antiviral, and anticancer activities (15). The health advantages of E. alata plant extracts are linked to their wealth of bioactive constituents, including phenolic acids, tannins, flavonoids, cardiac glycosides, alkaloids, reducing sugars, and saponins. Ephedrine, pseudoephedrine, and norpseudoephedrine are the main chemical substances identified and obtained from Ephedra extract as a member of the alkaloid category (16). Consequently, this research is focused on the assessment of the antioxidant activity of E. alata on the toxic effects caused by the administration of lead acetate to experimental animals.

Materials and Methods

Animals

Forty (40) adult male albino rats weighing between 185 and 215 grams were selected for this research. The rats were sourced from the Animal Research Centre at Tikrit University in Iraq and were kept in the animal house of the College of Education for Pure Sciences at Anbar University. Before conducting the experiments, the animals were given a period of acclimatisation in the lab for 10 days. They had unrestricted access to food and water and were kept under standard laboratory conditions, which comprised a 12-hour light-dark cycle and a temperature range of 25 to 28 °C. Throughout the acclimatisation period, the rats were housed in polycarbonate cages and were provided with a suitable pellet diet and tap water available ad libitum. All experimental methods were conducted by international guidelines for the ethical treatment of laboratory animals and in line with sanitary regulations for the maintenance of experimental biological facilities.

Prior toxicological research has shown that the chosen dosage of lead acetate (120 mg/kg) given for six weeks is effective in producing sub-chronic nephrotoxicity without resulting in a high death rate, enabling the evaluation of preventative measures. Renal oxidative stress and histopathological alterations, as well as ecologically relevant lead exposure, have all been replicated using this dosage and exposure duration (17). Oral gavage was used to give lead acetate, which is the recommended method for simulating natural ingestion and guaranteeing uniform dosage (18). To ensure reliable measurement of renal function markers and minimise variability in serum biochemical data, the rats were fasted for 12 hours before blood collection

Plant materials

The research was conducted utilizing the aerial components (twigs and leaves) of *Ephedra alata*, which were obtained from the Western Desert in Anbar Governorate, Iraq. The species was recognized and verified taxonomically by Professor Dr. Muhammad Othman Musa from the Desert Studies Center at the University of Anbar, Iraq.

Preparation of the extract

The plant samples were subjected to drying for 15 days at room temperature, protected from both light and moisture. The dried aerial parts obtained were milled to create a powder. The ethanolic extract was prepared by macerating 50 g of the plant powder in 150 ml of 70% ethanol, using magnetic stirring for 72 hours at room temperature, protected from light to prevent oxidation. After filtering the macerate through Whatman filter paper, the resulting filtrate was stored in a dry place, and the remaining material was macerated a second time following the same previously described methods. Subsequently, the two filtrates were mixed. Finally, the extract was evaporated at 50 °C with a Rotary evaporator model (Laborota 400) and stored in the refrigerator until it was required (19).

70% ethanol, a hydroalcoholic solvent that efficiently extracts a wide variety of phytoconstituents, including both polar and moderately nonpolar compounds like flavonoids, phenolic acids, and alkaloids, was used to prepare the ethanolic extract of Ephedra alata. The maceration process involved magnetic stirring at room temperature (~25 °C) for 72 hours, protected from light to prevent oxidative degradation of sensitive phytochemicals. While the precise agitation speed was not specified, standard magnetic stirring usually ranges between 300 and 600 rpm, guaranteeing optimal solvent penetration and compound release.

The extraction yield was roughly 12% w/w after twofold maceration and solvent evaporation at 50 °C using a rotary evaporator. Major bioactive groups, such as flavonoids, phenolic compounds, alkaloids, saponins, and tannins, were found by preliminary phytochemical screening. According to quantitative measurements, the extract had a total flavonoid content of 42.1 ± 1.7 mg QE/g extract and a total phenolic content of 78.5 ± 2.3 mg GAE/g extract, suggesting substantial antioxidant capacity that is consistent with nephroprotective effects.

Experimental design

The animals were randomly divided into five groups, each containing eight rats and the following: Group I (control) received 5 ml/kg of

distilled water. Group II (lead acetate group): received 120 mg/kg orally dissolved in distilled water. Group III: received 30 mg/kg of *E. alata* orally (1 ml per rat). Group IV: received lead acetate first for six weeks, then received *E. alata* extract at the same concentration above for six weeks. Group V: received *E. alata* extract first, and after two hours, received lead acetate at the same concentration as above.

Blood sample collection

Upon the conclusion of the experiment, the animals were subjected to starvation and anaesthetised through inhalation of diethyl ether. Subsequently, blood was extracted directly from the heart and placed in a centrifuge operating at 3000 rpm to isolate the serum. The serum was then preserved at a temperature of -20°C until the biochemical analyses were conducted.

Biochemical and oxidation balance analyses

Sera were used for the determination of serum creatinine and blood urea nitrogen levels, using commercially available diagnostic kits (Biodiagnostic, Egypt). Malondialdehyde (MDA) and glutathione peroxidase (GPX) were measured in serum by colourimetric method and assay kit, respectively (20, 21).

Table 1: Kit catalogue numbers and validation details

Oxidative stress markers	**Validation details**
Malondialdehyde (MDA) Assay Kit	
Catalogue Number	abx052570
Manufacturer	Abbexa
Detection Method	Colorimetric
Assay Range	0.1–10 nmol/ml
Sensitivity	0.1 nmol/ml
Validation	Validated for serum and plasma samples; inter-assay CV <10%
Glutathione Peroxidase (GPx) Assay Kit	
Catalogue Number	abx052570
Manufacturer	Abbexa
Detection Method	Colorimetric
Assay Range	0.5–50 mU/ml
Sensitivity	0.5 mU/ml
Validation	Validated for serum and plasma samples; inter-assay CV <10%
Pro-inflammatory Cytokines	
Human Interleukin 1 Beta (IL-1β) ELISA Kit	
Catalogue Number**:**	abx050327
Manufacturer**:**	Abbexa
Detection Method**:**	Sandwich ELISA, Colourimetric
Assay Range**:**	15.6–1000 pg/ml
Sensitivity**:**	<10 pg/ml
Validation**:**	Validated for serum and plasma samples; inter-assay CV <10%
Human Tumour Necrosis Factor Alpha (TNF-α) ELISA Kit	
Catalogue Number	abx050328
Manufacturer	Abbexa
Detection Method	Sandwich ELISA, Colourimetric
Assay Range	15.6–1000 pg/ml
Sensitivity	<10 pg/ml
Validation	Validated for serum and plasma samples; inter-assay CV <10%

Immunological analysis

Measurement of IL-1β and TNF-α in rat blood plasma was carried out using the enzyme-linked immunosorbent assay (ELISA) method with a protocol according to the rat ELISA kit (ABclonal Biotechnology, Cat No: RK00020 for IL-1β and Cat No: RK00050 for TNF-α). Microplates were coated with antibodies specific for IL-1β or TNF-α.

Histopathological study

The kidneys obtained from each group were preserved in a 10% formalin solution. Subsequently, the fixed kidneys were embedded in paraffin, and serial sections were prepared for histopathological examination, which included staining with hematoxylin and eosin. The stained specimens were then examined under a light microscope (20).

A semiquantitative histopathological scoring system based on the degree of glomerular degeneration, tubular necrosis, interstitial inflammation, and vascular alterations was used to evaluate kidney damage.

The following is how scores were allocated: According to Bancroft and Gamble, 0 indicates normal histology, 1 indicates mild damage (such as slight glomerular shrinkage or tubular dilatation), 2 indicates moderate damage (such as glomerular atrophy, moderate tubular necrosis), and 3 indicates severe damage (such as complete glomerular collapse, extensive necrosis, and interstitial infiltration) (20).

Statistical analysis

All data were expressed as means ± standard deviation. We employed One-way ANOVA for analysis, and LSD was performed to compare the groups (SPSS version 17.0).

Results

The results shown in Tables 1 and 2 highlight a significant increase in the level of both urea and creatinine in the second positive group that received lead acetate orally at a concentration of 60 mg/kg compared to the negative healthy control group. Whereas, the levels of urea and creatinine were decreased slightly in the third group that received the extract of the *E. alata*, while the results showed an improvement in the above indicators in the fourth and fifth groups, as the results showed a significant decrease in the level of urea and creatinine when the extract of the *E. alata* was administered either before or after administering lead acetate.

Table 2: Effect of *Ephedra alata* Extract on Serum Creatinine Concentration in Male Albino Rats Exposed to Lead Acetate

Group	Serum Creatinine (Mean ± SD)
Control	1.2 ± 0.1
Lead Acetate	3.8 ± 0.2
E. alata Extract	1.0 ± 0.1
Lead + *E. alata*	2.4 ± 0.1
Vitamin C	1.6 ± 0.1

Table 2 shows the effect of the extract of E. alata on creatinine concentration in rats treated with lead acetate. Number of rats per group = 8. Different lowercase letters indicate a significant difference ($p<0.05$) between the groups. Similar letters indicate no significant difference

Table 3: Effect of *Ephedra alata* Extract on Serum Urea Concentration in Male Albino Rats Exposed to Lead Acetate

Group	Serum Urea (Mean ± SD)
Control	22 ± 1.5
Lead Acetate	45 ± 2.0
E. alata Extract	20 ± 1.2
Lead + *E. alata*	33 ± 1.7
Vitamin C	27 ± 1.5

Table 3 shows the effect of the extract of E. alata on the Urea concentration in rats treated with lead acetate. Number of rats per group = 8. Different lowercase letters indicate a significant difference ($p<0.05$) between the groups. Similar letters indicate no significant difference.

Assessing oxidation balance, the results of our current study showed a significant increase in the level of malondialdehyde (MDA) with a significant decrease in the level of glutathione peroxidase (GPX) in the serum of the rats of the group treated with lead acetate. The results showed the same indicators in their normal state in the third group that received the extract of the *E. alata* alone, and were close to the healthy control group. A significant decrease in the level of MDA was observed with an increase in the level of antioxidants GPX in the fourth preventive group and the fifth therapeutic group after or before administering the extract of the *E. alata* with lead acetate, noting that the results improved in the fifth therapeutic group, as shown in Table 4 and Figure

1.

Table 4: Effect of *Ephedra alata* Extract on Serum Malondialdehyde (MDA) Levels in Male Albino Rats Exposed to Lead Acetate

Group	Serum MDA (Mean ± SD) (nmol/mL)
Control	2.1 ± 0.2
Lead Acetate	5.7 ± 0.3
E. alata Extract	1.9 ± 0.1
Lead + *E. alata*	5.2 ± 0.2
Vitamin C	4.1 ± 0.2

Table 4 shows the effect of the extract of E. alata on MDA Level in rats treated with lead acetate. Number of rats per group = 8. Different lowercase letters indicate a significant difference (p<0.05) between the groups. Similar letters indicate no significant difference

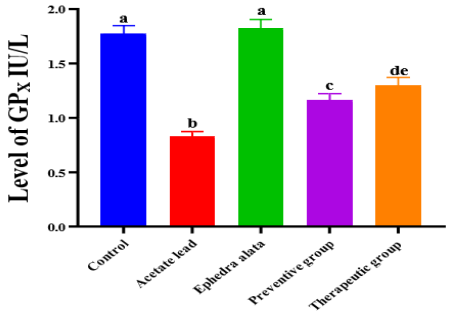

Figure 1: Effect of Ephedra alata Extract on Serum Glutathione Peroxidase (GPx) Activity in Male Albino Rats Exposed to Lead Acetate

Figure 2 shows the effect of the extract of E. alata on GPX Level in rats treated with lead acetate. Number of rats per group = 8. Different lowercase letters indicate a significant difference (p<0.05) between the groups. Similar letters indicate no significant difference

While the immunological tests showed an increase in the levels of interleukin-1 beta and tumour necrosis factor alpha in the second group that was administered oral lead acetate, compared to the first group and the healthy control. The results of the immunological tests for the third group that received oral extract of the *E. alata* were similar and without significant differences when compared to the control group. The results showed an improvement in the above indicators, as a significant decrease was observed in the levels of both IL-1β and TNF-α in the blood serum of the fourth and fifth groups that were given the extract of the *E. alata* either before or after lead acetate compared to the second group, as shown in Figures 5 and 6.

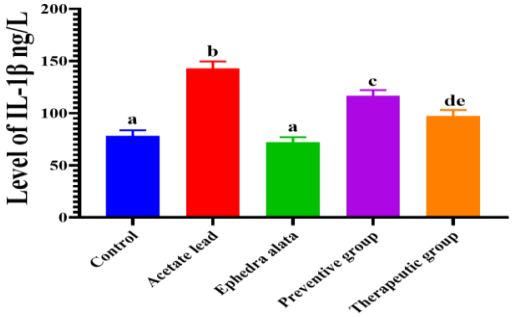

Figure 2: Effect of Ephedra alata Extract on Serum Interleukin-1β (IL-1β) Levels in Male Albino Rats Exposed to Lead Acetate

Figure 2 shows the effect of the extract of E. alata on IL-1β Level in rats treated with lead acetate. Number of rats per group = 8. Different lowercase letters indicate a significant difference (p<0.05) between the groups. Similar letters indicate no significant difference

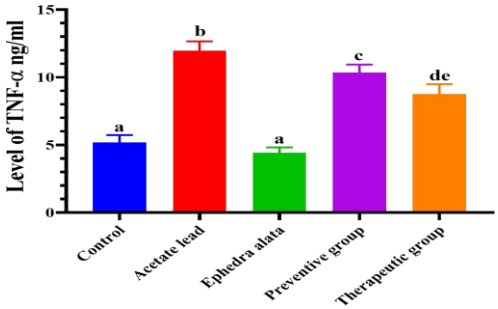

Figure 3: Effect of Ephedra alata Extract on Serum Tumor Necrosis Factor-α (TNF-α) Levels in Male Albino Rats Exposed to Lead Acetate

Figure 3 shows the effect of the extract of E. alata on TNF-α levels in rats treated with lead acetate. Number of rats per group = 8. Different lowercase letters indicate a significant difference ($p<0.05$) between the groups. Similar letters indicate no significant difference

Microscopic examination of kidney tissue showed pathological changes in the rat group that was administered lead acetate alone orally. The changes showed degeneration of the glomerulus (GD) with severe blood congestion (CON), inflammatory infiltration (LI) of white blood cells (as shown in Image 1). The histological examination results also indicated complete lysis of some glomeruli (GL), hemorrhage(H), cellular degeneration(D) with deposition of necrotic materials(NM) in the lumen of the convoluted tubules (CT) Images 2 and 3, also showed the presence of tubular epithelial sloughing (SL) lining, necrosis of cells compared with the control group, which showed the normal histological pattern of the glomerulus (G) with its normal shape and size within Bowman's capsule (BC), and the convoluted tubules (CT) (Image 4). The group that was administered with the extract of *Ephedra alata* showed the normal shape of the kidney tissue, similar to the control group (Image 5).

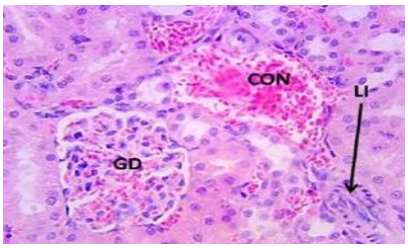

Image 1: Cross section of a lead acetate group rat kidney showing glomerular degeneration (GD), severe congestion (CON), and inflammatory infiltration (LI), (hematoxylin and eosin stain, 400x)

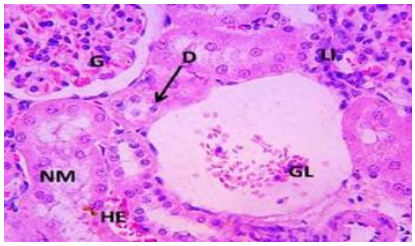

Image 2: A cross section of a kidney of a rat in the lead acetate group showing complete glomerular Lysis (GL), haemorrhage (HE), inflammatory infiltration (LI), cell degeneration (D), necrotic material (NM) (hematoxylin and eosin stain, 400x)

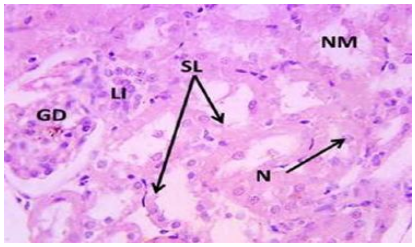

Image 3: A cross section of a lead acetate group rat kidney showing glomerular degeneration (GD), sloughing of the convoluted tubule lining (SL), inflammatory infiltration (LI), necrosis of N cells, necrotic material (NM) (hematoxylin and eosin stain, 400x)

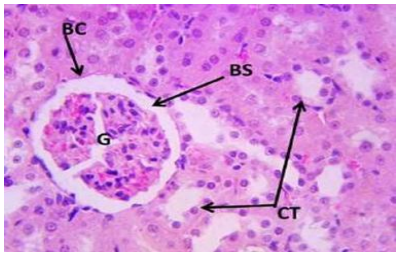

Image 4: A cross section of the kidney of a rat from the control group showing the glomerulus G, Bowman's space BS, Bowman's capsule BC, and the urinary convoluted tubules CT (hematoxylin and eosin stain. 400X)

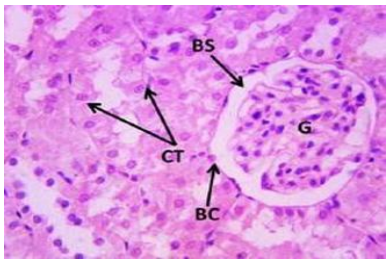

Image (5) Across section of a kidney of a rat from the Ephedra alata group showing the glomerulus G, Bowman's space BS, Bowman's capsule BC, and the urinary convoluted tubules CT (hematoxylin and eosin stain, 400x)

The histological sections of the preventive group that was first administered the extract of *Ephedra alata* + lead acetate showed the appearance of a histological pattern like that of the healthy control group, except for the presence of histological changes represented by the presence of sloughing of the lining of Bowman's capsule (SBC) with expansion of Bowman's space (BS). Also, there was dilatation of some convoluted urinary tubules (DCT), and presence of remnants of blood haemorrhage and simple inflammatory infiltration (as shown in images 6 and 7). The treatment group (lead acetate first + extract of *Ephedra alata*) produced better results, as it showed the return of the normal pattern of the glomeruli (G) and convoluted urinary tubules (CT) in a manner close to the control, with the presence of remnants of histological changes, including simple necrosis (N) of some cells, as well as the presence of a simple inflammatory infiltration (LI) (Image 8).

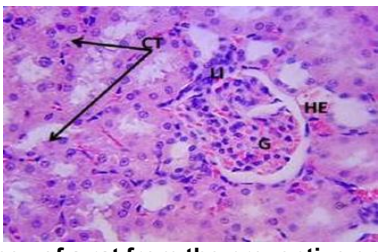

Image 6: A cross section of the kidney of a rat from the preventive group showing the glomerulus G in its almost normal shape, the convoluted tubules CT, haemorrhage HE, simple inflammatory infiltration LI

(hematoxylin and eosin stain, 400x)

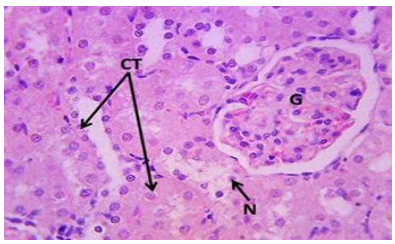

Image 7: A cross-section of the kidney of a rat in the lead acetate + Ephedra alata (Therapeutic group), showing the glomerulus G in its almost normal shape, the convoluted tubules CT, and slight cell necrosis N (hematoxylin and eosin stain, 400x)

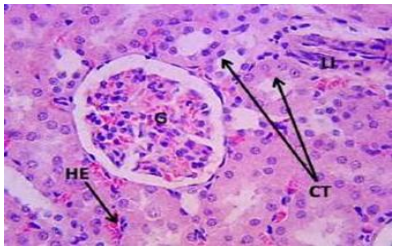

Image 8: A cross section of the kidney of a rat in the Therapeutic group, showing the glomerulus G in its almost normal shape, convoluted tubules CT, slight haemorrhage HE, slight inflammatory infiltration LI (hematoxylin and eosin stain, 400x)

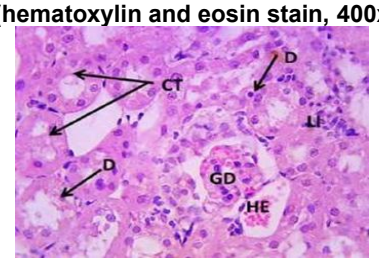

Image 9: A cross section of the kidney of a rat in the lead acetate group showing glomerular degeneration (GD), degeneration of D cells, inflammatory infiltration (LI), and haemorrhage (HE) (hematoxylin and eosin stain, 400x)

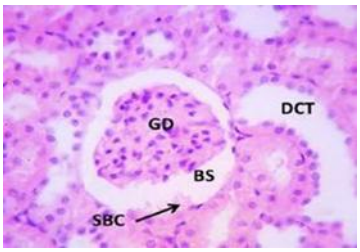

Image 10: A cross section of the kidney of a rat from the preventive group (Ephedra alata + Lead Acetate) showing degeneration of the glomerulus (GD), dilation of the convoluted tubules (DCT), shedding of the lining of Bowman's capsule (SBC), and dilation of Bowman's space (BS) (hematoxylin and eosin stain. 400X)

Discussion

Toxic metals, especially lead, present a significant danger to the well-being of living beings. In diverse ecosystems, lead coexists with a variety of other heavy metals, often detected alongside numerous elements at trace levels of pollution; therefore, lead's presence in nature contributes nothing advantageous to biological systems. It can result in chronic health problems, with a notable prevalence of ailments such as liver, nervous system, and renal disorders.

The current research revealed heightened levels of creatinine and Blood Urea Nitrogen (BUN) in the serum of rats administered lead acetate when contrasted with negative controls. A comparable finding was documented by Abdel-Daim *et al.*

(2020) (22), who noted that treatment with lead acetate led to a marked increase in serum creatinine and BUN activities. Elevated urea levels during oxidative stress stem from the depletion of a direct energy source, prompting the animal to utilise proteins as a substitute energy source, subsequently leading to the production of substantial amounts of urea (23). Free radicals contribute to the oxidation of proteins and amino acids, consequently, causing an upsurge in urea and creatinine concentrations in the blood serum (24). The application of lead acetate markedly raised creatinine and BUN levels, which may signify impaired renal function in cases of nephrotoxicity (25). Monitoring serum creatinine and BUN is advised for evaluating kidney damage in preclinical studies, as they provide a more precise and sensitive measure of renal injury (26).

Biological antioxidants are currently areas of research interest due to their safety and positive effects on a variety of diseases associated with oxidative stress (27). Many medicinal plants contain many antioxidants that can weaken and neutralise free radicals. *E. alata* is one of the most used plants for the treatment of diabetes, cardiovascular diseases, and various cancers (28). Previous co-administration of *E. alata* extracts normalised plasma concentrations of urea and creatinine. The protective effect of *E. alata* seed extract may be a result of improving the structure of the renal membrane, thereby improving the normal functioning of the tissue (29). The protective effect of *E. alata* may be related to its content of phytochemicals such as flavonoids, alkaloids, tannins, etc. (25). Recent reports have studied the protective effects of natural compounds against kidney damage (30).

MDA serves as the ultimate byproduct of lipid peroxidation and the direct reduction of antioxidant reserves because lead toxicity triggers oxidative stress (31). Our study findings indicate a notable elevation in MDA levels within the serum of rats exposed to lead acetate compared to the negative control group. This observation implies that lead acetate may heighten oxidative stress in rats. Furthermore, the current investigation revealed that lead acetate treatment suppressed the activity of GPx in rat serum. These findings align with earlier studies (32). This indicates that lead acetate toxicity can interfere with the function of the antioxidant enzyme (GPx), potentially leading to oxidative stress. Elevated levels of lead provoked oxidative harm through augmented free radical production while simultaneously compromising the cellular antioxidant defence system by diminishing (GPx) levels, inhibiting sulfhydryl-dependent enzymes or antioxidant enzyme activities, and/or escalating lipid peroxidation (27).

The administration of *E. alata* enhanced lipid peroxidation status, clearly demonstrating its potent chemopreventive and antioxidant abilities against renal oxidative stress instigated by Lead (Pb) in the progeny of adult rats. As previously stated, *E. alata* displayed an antioxidant function through the presence of biomolecules recognised for their capacity to scavenge ROS (27). Moreover, prior co-administration with E. *alata* reduced renal oxidative stress by elevating the activities of SOD and CAT, along with increasing the GPX levels in blood serum (35). The influence of *E. alata* as a robust antioxidant and free radical scavenger could lower MDA levels in the kidneys of lead-exposed rats (34). The intake of *E. alata* led to a reduction in MDA levels when the rats were administered lead acetate. This indicates that *E. alata* diminished the toxic impact of lead acetate through its antioxidant properties. The protective antioxidant mechanism scavenges free radicals and alleviates oxidative stress, which contributes to kidney injury and consequently reduces lipid peroxidation (MDA) (35).

Our findings suggested that *E. alata* may suppress oxidative stress by lowering MDA levels in rats treated with lead acetate. Moreover, *E. alata* was highly successful in attenuating lead hepatotoxicity, nephrotoxicity due to its high total phenol and flavonoid contents. The suggested three mechanisms for this attenuation include, first, lowering the oxidative stress, second, increasing the oxidant enzymes level, and third, acting as a chelating agent for lead ions (33).

Following the administration of acetate lead, the levels of pro-inflammatory cytokines in blood serum, specifically TNF-α and IL-1β, showed a significant increase (36) documented that exposure to lead enhances the activation of NF-κB and further inhibits the phosphorylated form of adenosine monophosphate-activated protein kinase (AMPK), which in turn raises the levels of reactive oxygen species and inflammatory cytokines in both serum and tissues. These results corroborate the findings of Harshitha *et al.* (37), who observed a marked increase in tumour necrosis factor-alpha and caspase-3 levels in the livers of rats subjected to lead acetate poisoning at a dosage of 100 mg/kg daily for up to four weeks. Similarly, Kucukler *et al.* (38) reported that rats suffering from lead acetate toxicity exhibited elevated levels of IL-1β and TNF-α in serum. Additionally, treatment with E. alata, at both high and low doses, significantly decreased the activities of IL-1β and TNF-α in serum, aligning with

findings from the existing literature. The precise mechanism through which E. alata reduces the expression of IL-1β and TNF-α remains unclear and warrants additional investigation. Nonetheless, it has been suggested that E. alata inhibits the production of IL-1β and TNF-α by blocking the NF-kB pathway, with the plant exhibiting anti-inflammatory effects due to its active chemical constituents, including flavonoids, sphingolipids, and derivatives of ephedrine (37).

Using a microscope with 400x magnification, the acetate Lead induction group showed changes in tissue structure. The noticeable rise in plasma creatinine and blood urea nitrogen levels was likely linked to damaged glomeruli and renal tubules, which was supported by the findings from the histopathological analysis of our work. Furthermore, the injury to the renal tubules caused by high lead exposure is thought to be related to the amount of lead that builds up in the kidneys. Various studies have shown that oxidative damage from lead might be the primary cause of kidney and liver toxicity linked to lead (38, 39).

The process by which lead causes renal impairment is linked to its interaction with mitochondrial components. This interaction leads to heightened oxidative stress and the generation of reactive oxygen species, along with other free radicals. Consequently, this results in an increase in renal markers such as urea and creatinine levels. Exposure to lead acetate is correlated with the rise in superoxide anion production and the peroxidation of lipids (40). Additionally, lead toxicity might contribute to a reduction in the tissue-specific functionalities of enzymatic antioxidants like superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH). The levels of expression of these antioxidants may reflect the degree of cytotoxic damage to renal tissues (41). This indicates that lead acetate toxicity may hinder the function of the antioxidant enzyme (GPx), potentially leading to oxidative stress.

The administration of *E. alata* reduced the buildup of free radicals, which could enhance GPx activity in the kidneys of rats treated with lead. *E. alata* serves as a neutraliser for oxygen-derived free radicals, thereby shielding cells from harm (42). The influence of *E. alata* extract in combating lead acetate-induced liver injury might be linked to the plant's antioxidant characteristics (43). Considering the antioxidant and anti-inflammatory effects of *E. alata* extract documented in numerous studies and validated in our research, it can be posited that *E. alata* extract may protect against nephron injury and dysfunction by lowering the levels of inflammatory cytokines, suppressing free radicals and ROS, and boosting the antioxidant potential of both tissue and serum (44). Thus, the results of the current research demonstrate that *E. alata* can safeguard against the histological changes induced by Pb-related hepatotoxicity in animals. Additionally, they support the outcomes of previous studies showing that *E. alata* can effectively avert Pb-triggered nephrotoxicity, along with findings by other investigators who noted that administering 60 mg/kg/rat inhibited the rise in lipid peroxidation levels in Pb-exposed rats (43).

Conclusion

Because of its strong anti-inflammatory and antioxidant qualities, the study shows that E. alata extract provides significant protection against lead acetate-induced nephrotoxicity in rats. Ephedrine and flavonoids, two phytochemicals found in E. alata, are essential for reducing the harmful effects of lead exposure. Reactive oxygen species (ROS) can be scavenged by flavonoids, which is well known for lowering oxidative stress and lipid peroxidation, two major causes of kidney injury. Ephedrine, on the other hand, is believed to alter the NFκB pathway, which is connected to the inflammatory reaction. Ephedrine may lessen the synthesis of pro-inflammatory cytokines like TNF-α and IL-1β, which are increased in lead-induced nephrotoxicity, by blocking the NF-κB pathway.

Although the current investigation on E. alata shows promise in reducing nephrotoxicity caused by lead acetate, dose-response data are conspicuously lacking. Determining the ideal dosage of E. alata that optimises therapeutic benefits while limiting potential side effects requires a thorough dose-response relationship. Furthermore, because chronic administration of E. alata may disclose cumulative toxicities that may not be evident in short-term investigations, long-term toxicity assessments are required to determine the safety of prolonged usage of the drug in clinical or preclinical settings.

Furthermore, it is important to take into account any confounding variables such as rat strain diversity. The reported results may be influenced by the physiological reactions of various rat strains to lead exposure and E. alata therapy. Variations in the efficacy of the medication may be caused by genetic diversity in immunological responses, metabolism, and antioxidant enzyme activity. Future research must therefore take these parameters into consideration to guarantee that the results are generally relevant and unaffected by strain-specific variations.

Consequently, this *E. alata* dosage effectively mitigates Pb-induced oxidative stress and damage

in affected animals. We conclude from our findings that *E. alata* extract plays a significant therapeutic role in alleviating functional and tissue impairment in the kidneys due to exposure of test animals to lead acetate.

List of abbreviations

BUN: Blood Urea Nitrogen
ELISA: Enzyme-linked immunosorbent assay
GPX: Glutathione peroxidase
IL-1β: Interleukin-1 beta
MD: Malondialdehyde
Pb: Lead
TNF-α: Tumour Necrosis Factor-Alpha

Declarations

Ethical approval and consent to participate

All experimental procedures involving animals were carried out in strict accordance with the recommendations of the ARRIVE guidelines and the Guide for the Care and Use of Laboratory Animals published by the U.S. National Institutes of Health. The study protocol was reviewed and approved by the Institutional Animal Ethics Committee of the University of Anbar, College of Education for Pure Sciences (Approval No: UoA/CEPS/ANB-2023-07). Every effort was made to minimise the number of animals used and to alleviate pain and distress throughout the experiment. This study did not involve human participants.

Consent for publication

All the authors gave consent for the publication of the work under the Creative Commons Attribution-Non-Commercial 4.0 license.

Availability of data and materials

The data and materials associated with this research will be made available by the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Author contributions

HAA and ALH made substantial contributions as follows:
Conceptualisation and Study Design: HAA; Methodology and Investigation: HAA and ALH; Data Acquisition and Curation: HAA; Histological and Statistical Analysis: ALH; Interpretation of Results: HAA and ALH; Writing—Original Draft Preparation: HAA; Writing—Review and Editing: ALH; Supervision and Project Administration: ALH. Both authors have read and approved the final manuscript and agree to be accountable for all aspects of the work.

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