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Emerging and Re-emerging Animal Health Challenges in Low and Middle-Income Countries

Nephroprotective Effect of Alcoholic Extraction of *Tribulus terrestris* Against Lead Acetate Toxicity on Kidney in Male Rats

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Abstract | Lead is a heavy metal that occurs naturally in the earth's crust; however, it is infrequently encountered as a metal. Lead compounds are formed when mixed with two or more elements, and these compounds are used as a pigment in paints, dyes, ceramic glazes, and caulk. To limit the hazardous effects of lead on humans and animals, the quantity of lead used in these items has recently been lowered. The kidney and liver are very sensitive organs to chronic Pb toxicity. Lead produces free radicals and causes oxidative stress, both of which are linked to renal damage. *Tribulus terrestris* (TT) has anti-inflammatory and antioxidant effects. The hydroalcoholic extract of TT has been investigated against lead acetate-induced nephrotoxicity. In experimental animals, forty adult male albino rats were utilized. Four groups of animals were used in the study. Ten male rats each group are used: Group 1 (control group): For 28 days, the animals were given distilled water orally. Group 2 (lead acetate group): For seven days, animals in this group received intraperitoneal injections of lead acetate at a dose of 30 mg/kg B.W. every day, depended on LD50. Group 3 (*Tribulus Terrestris* group) animals received extraction of T.T orally at a dose of 250mg/kg / B.W. for 28 days. Group 4 (lead acetate+ *Tribulus Terrestris*) received lead acetate (30mg/kg B.W.) for 7 days + *Tribulus Terrestris* (250mg/ kg B.W) daily for 28day. In the current investigation, *T. terrestris* plant extract has been shown to provide protection against lead acetate-induced toxicity. It is abundantly obvious that administering lead acetate causes a substantial change in the structure and activities of the kidneys. The alcoholic extract of *T. terrestris* fruit containing lead acetate has demonstrated a significant protective impact on renal function and tissue structure. *T. terrestris* extract can raise the amounts of the biochemical indicators including urea and creatinine, and it is efficient in considerably reducing some of the physiological effects of liver and renal lead acetate toxicity, which is further supported by the significant histological alterations of *T. terrestris* extract, in contrast to lead acetate, which displayed some detrimental effects.

Keywords | Lead acetate, *Tribulus terrestris*, Plant extract, Nephrotoxicity, Antioxidant

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Kidneys are vital organs in the body that eliminate waste products of metabolism through urine. Our bodies are routinely exposed to a wide range of chemicals that damage the kidneys. Medications, natural products, industrial chemicals, environmental pollutants, insecticides, and other substances harm numerous organs in the body on a variety of levels (Gupta and Gupta, 2020). Heavy metal-induced nephrotoxicity contributes to the high incidence and prevalence of acute kidney injury (Al-Mukhtar *et al.*, 2016). The Earth's crust contains small amounts of lead, a common element. It is frequently utilized in industry, particularly for construction materials, paint, batteries, and pipework. Lead is considered a strong occupational toxin, with well-documented toxicological effects (Rahman and Singh, 2019). Lead's lengthy environmental persistence is mostly due to its nonbiodegradable nature. Lead can be exposed to animals through a variety of procedures and materials, including paints made of lead, pipes made of lead or solder made of lead in water supply systems, batteries recycled, grids, and bearings (Mahdi and Ghadhban, 2022a). Lead causes health problems such as toxicity of the liver, kidneys, hematopoietic system, and nervous system (Shukla *et al.*, 2018). Lead exposure has a number of harmful consequences on the liver, kidneys, and haematopoietic system, mostly via increased oxidative stress (Flora *et al.*, 2012). The way that diseases manifest is significantly influenced by these changes. A treatment method for lead poisoning involves altering cellular thiols to guard against reactive oxygen species (ROS) (Unsal *et al.*, 2020). The liver is the primary organ affected by Pb-induced oxidative stress, and these changes were followed by a significant drop in Glutathione (GSH) levels in the blood (Matović *et al.*, 2015). The oxidative stress induced by lead on other enzymes (Mahdi and Ghadhban, 2022b). In the liver, Pb-induced oxidative stress produces alterations in membrane integrity and composition of fatty acids, which are related to elevated levels of plasma Malondialdehyde (MDA) in the liver. Lead Effects on the hematopoietic system after (Mahdi and Ghadhban, 2022b; Abubakar *et al.*, 2020).

Tribulus terrestris generally known as Gokshur or Gokharu or Punvture vine, is a member of the family Zygophyllaceae and is very common in Indian and Chinese traditional system of medicine (Azam *et al.*, 2019). TT has been reported to have anti-inflammatory, antioxidant, diuretic, antihypertensive and anti-urolithiatic efficacy (Mohamed *et al.*, 2023). It is considered to be an important component in Ayurvedic and Unani systems for the treatment of renal diseases (Singh *et al.*, 2023). TT is effective against ROS-induced damage in the renal endothelium and inhibits vascular endothelial dysfunction (Zhang *et al.*,

2023). *Tribulus terrestris* is one of the most significant plants that is widely utilized throughout the world. It is a plant that grows in many places, including Europe, Iraq, South Africa, Australia, and India. This plant has a lot of components and chemicals that are biologically active. These substances can be found in fruits, flowers, and leaves (Ştefănescu *et al.*, 2020; Zhu *et al.*, 2017). Numerous studies have been conducted on *Tribulus terrestris* chemistry (Ştefănescu *et al.*, 2020). Of the many different kinds of constituents, steroidal saponins and flavonoids are the two most important metabolites with different bioactivities (Zhu *et al.*, 2017). The most unique compounds in TT are believed to be the saponins furostanol and spirostanol. It is believed that TT's distinct biological characteristics are derived from these two steroidal saponins (Chhatre *et al.*, 2014). Most of the flavonoids in TT are derivatives of isorhamnetin, kaempferol, and quercetin (Semerdjieva and Zheljazkov, 2019).

Extracts from *Tribulus terrestris* have produced a wide range of components with different chemical structures and pharmacological properties. The most significant components, flavonoids and steroidal saponins, have a variety of biological functions. The plant sections analyzed and the extraction technique both affect the extract's chemical composition (Zhu *et al.*, 2017). It has been demonstrated through research that *Tribulus terrestris* possesses antibacterial, antioxidant, anticancer, and hepatoprotective properties (Abdulqawi and Quadri, 2021; El-Chaghaby *et al.*, 2024; Abbas *et al.*, 2022; Ali *et al.*, 2018).

For both males and females, the herb has been used to treat impotence, infertility, urinary problems, itchy skin, and blood purification. In addition to being used as a remedy for antioxidative stress, TT fruits are utilized as a source of antiaging, cardiovascular, hepatic, and renal issues (Semerdjieva and Zheljazkov, 2019).



Figure 1: *Tribulus terrestris* Linn natural habitat (Chaudhary *et al.*, 2023).



Figure 2: *Tribulus terrestris* Linn dried fruits (Chaudhary et al., 2023).

AIM OF THE STUDY

The current investigation emphasized the importance protective effects *Tribulus terrestris* on the rat kidney and liver against lead acetate toxicity.

MATERIALS AND METHODS

Preparation of hot ethanolic extract of *Tribulus terrestris* (TT) fruits ethanolic extract of dried fruits of *Tribulus terrestris* was prepared using soxhlet apparatus by hot percolation method. The dried fruits were grinded in an electrical grinder to a coarse powder and sieved. About 45-50 g of coarsely ground powder of *Tribulus terrestris* fruits was placed in a porous cellulose thimble in soxhlet apparatus (capacity 500 ml). Thimble was placed in the extraction chamber above the flask containing 500 ml of 70% ethanol. The flask was heated, and the solvent was allowed to evaporate. The temperature (40 °C) was adjusted according to the boiling temperature of solvent in the flask. Extraction process was allowed to continue for 36 hours involving 15-18 cycles till the solvent in siphon tube of extractor became completely colorless.

LEAD ACETATE PREPARATION

Lead acetate powder manufactured by Sigma-Aldrich in Germany was used to prepare a solution of lead acetate according to the dose determined in this experiment. It dissolved with distilled water after weighing on a delicate scale in the recommended concentration to be used as a single daily dose at the dose rate of 1/20 LD 50 of (30mg/kg) (Mahdi and Ghadhban, 2022).

ANIMAL OF THE STUDY

The present study was carried out in the animal house of the

physiology department at the University of Kerbala College of Veterinary Medicine. In the current investigation, forty adult male albino rats (*Rattus rattus*) were utilized. The animals ranged in age from 8 to 10 weeks, with an average weight of 200±20g. Prior to the experiment, they were housed for two weeks to help them adjust. Each of the ten animals was kept in a separate 15 x 35 x 50 cm plastic cage. They were given an unlimited supply of the standard diet pellet provided by the Institute for Public Accuracy, Free access to drinking water was provided to them, and they were maintained at a precise temperature of 22–25 °C. and light, the regime of 14 hours of light and 10 hours of darkness.

THE BODY WEIGHT MEASUREMENTS

At the beginning of the experiment, the initial body weight of adult rats was recorded and then obtained until the end of the investigation; body weight gain was also recorded.

EXPERIMENTAL DESIGN

Four groups of animals were used in the study. Ten male rats each group are used in the following ways for experiment design:

- Group 1 (control group): For 28 days, the animals were given distilled water orally.
- Group 2 (lead acetate group): For seven days, animals in this group received intraperitoneal injections of lead acetate at a dose of 30 mg/kg B.W. every day. Depended on LD50.
- Group-3 (*Tribulus Terrestris* group) animals received extraction of T.T orally at a dose of 250mg/kg B.W. for 28 days
- Group-4(lead acetate + *Tribulus Terrestris*) received lead acetate (30mg/kg B.W.) for 7 days + *Tribulus terrestris* (250mg/ kg B.W) daily for 28 days.

SAMPLE COLLECTION

After the treatment period, the rats were dissected to collect samples. The animal was anaesthetized before the blood sample was taken via cardiac puncture. A 5 ml disposable syringe was used to gather samples. To extract the serum, 5 milliliters of the drawn blood were centrifuged at 3000 rpm for 15 minutes in a non-heparinized plane tube. After that, the serum was put into Eppendorf tubes and kept cold (-4°C) until it was time to measure the ALT in the liver and the urea and creatinine in the kidneys to assess oxidative stress (MDA and CAT). The kidney was also removed for microscopic analysis.

RESULTS AND DISCUSSION

THE BODY WEIGHT GAIN

The effect of lead acetate, T.T or their combination with lead on initial and final body weight in adult male rats. There was a significant decrease ($P < 0.05$) in final body weight (Table 1)

Table 1: The effect of lead acetate, T.T on the body weight gain in adult male rats.

Group name	Number of rats	Initial body weight (gm)	Final body weight (gm)	Mean body weight gain
Control group	10 rats	214.2	228.5	14.3
Lead acetate group	10 rats	219.6	223.7	4.1
T.T group	10 rats	211.7	232.9	21.2
T.T + Lead acetate group	10 rats	209.6	219.1	8.5

Table 2: Effect of lead acetate alone and in combination with *Tribulus terrestris* extract on the liver enzyme in male rats.

Groups	Parameters (Mean $\pm$ SD) No=10		
	AST (U/L)	ALT (U/L)	ALP (U/L)
Control	152.8 $\pm$ 5.69 c	105 $\pm$ 3.73 c	101.4 $\pm$ 3.9 c
Lead acetate (U/L)	207.5 $\pm$ 9.53 a	166.6 $\pm$ 6.49 a	143.5 $\pm$ 4.95 a
<i>T. terrestris</i> extract (U/L)	159.3 $\pm$ 6.3 c	115.3 $\pm$ 5.34 c	106.6 $\pm$ 6.29 c
Lead + <i>T. terrestris</i> extract (U/L)	167.4 $\pm$ 7.29 b	135.7 $\pm$ 5.18 b	117.8 $\pm$ 8.74 b
LSD	7.4	10.2	5.3

*The different small letters a show significant difference at (P<0.05).

was observed in treated groups of lead acetate and lead acetate + T.T. with mean values were (223.7gm), (2219.1gm), respectively as compared with Control, and T.T group (228.5gm), and (232.9gm) respectively. A significant decrease (P<0.05) in body weight gain was observed in the lead acetate group (4.1gm) as compared to control (14.3gm), and T.T (21.2gm), and T.T + Lead acetate (8.5gm) groups as shown in Table 1.

LIVER ENZYMES

When comparing the result of the lead acetate group to the other experimental groups, it was shown that the liver's enzyme activity was significantly elevated (P<0.05) in conjunction with the AST, ALT, and ALP enzyme activities. In the *Tribulus terrestris* extract group, there was a substantial (P<0.05) decrease in AST, ALT, and ALP. revealed a significant P<0.05 decrease in the lead + *Tribulus terrestris* extract group when contrasted with the lead acetate group, suggesting that the *Tribulus terrestris* extract possesses greater mitigating effects on the toxicity of lead in animals, as shown in Table 2.

Table 3: Effect of lead acetate alone and in combination with *Tribulus terrestris* extract on some kidney serum function in male rats (Mean $\pm$ SE).

Groups	Parameters (Mean $\pm$ SD) No=10	
	Urea (mg/dl)	Creatinine (mg/dl)
Control	26.6 $\pm$ 7.69 c	0.37 $\pm$ 0.014 b
Lead acetate (U/L)	39.6 $\pm$ 2.52 a	0.41 $\pm$ 0.025 a
<i>T. terrestris</i> extract (U/L)	24.3 $\pm$ 1.47 c	0.36 $\pm$ 0.024 d
Lead + <i>T. terrestris</i> extract (U/L)	31.00 $\pm$ 2.56 b	0.39 $\pm$ 0.071 c
LSD	4.43	0.21

*The different small letters a show significant differences at (P<0.05).

KIDNEY FUNCTION

Table 3 illustrates the characteristic features of kidney function (Creatinine and Urea). The results showed the serum creatinine and urea levels increased significantly (P<0.05) when lead acetate was administered compared to the other groups. Rats' levels of creatinine and urea were significantly (P<0.05) reduced when using *Tribulus terrestris* extract with lead acetate; however, this effect was less pronounced in the *Tribulus terrestris* extract group than in the control group.

Table 4: Effect of lead acetate and in combination with *Tribulus terrestris* extract on the enzymatic antioxidants in male rats.

Groups	Parameters (Mean $\pm$ SD)	
	MDA (nmol/ml)	GHPx (Pg/ml)
Control	2.31 $\pm$ 0.36c	93.23 $\pm$ 6.39 c
Lead acetate (U/L)	4.9 $\pm$ 0.47a	65.04 $\pm$ 5.29d
<i>Tribulus terrestris</i> extract (U/L)	2.73 $\pm$ 0.40c	105.33 $\pm$ 5.78a
Lead acetate+ <i>Tribulus terrestris</i> (U/L)	3.96 $\pm$ 0.56b	79.24 $\pm$ 4.96b
LSD	1.27	7.39

*The different small letters a show significant difference at (P<0.05).

ENZYMATIC ANTIOXIDANTS

Table 4 presented the results, which showed that the lead acetate group had a significantly (P<0.05) higher serum MDA level (4.9 $\pm$ 0.47 nmol/ml) than the other treatment groups and the control group (2.31 $\pm$ 0.36 nmol/ml). In contrast, the MDA level in the group treated with *Tribulus terrestris* extract did not change significantly (P<0.05) from that of the control group. In contrast, a significant (P<0.05) decrease in serum MDA level in Lead acetate + *Tribulus*

terrestris extract treated (3.96 ± 0.56) groups compared with the lead acetate group (4.9 ± 0.47) also resulted in revealed a significant ($P < 0.05$) decrease in serum glutathione level recorded in the Lead acetate group ($65.04 \pm 5.29d$) compared with the control group (93.23 ± 6.39) and other treated groups, While a significant ($P < 0.05$) increase in serum glutathione level was recorded in (Lead acetate + *Tribulus terrestris* extract) treated ($79.24 \pm 4.96b$) Pg /ml groups, (*Tribulus terrestris* extract) treated (105.33 ± 5.78) when compared with the lead acetate group ($65.04 \pm 5.29d$) Pg/ml.

HISTOPATHOLOGICAL CHANGES

KIDNEY

The histological section of the kidney is shown in **Figure 3A** (10X H and E) sections of kidney from male rats (control group) showed the normal renal glomerulus and showed the normal renal tubules; **Figure 3B** sections of kidney (*Tribulus terrestris* extract group), showed focal interstitial nephritis appears as aggregations of inflammatory cells, some tubules appears loosen its lumen and desquamated epithelial lining due to necrosis; **Figure 3C** sections of kidney treated with (lead acetate group) showed hyaline casts appears with hemorrhagic changes enclosed to focal aggregation of monocytes (Yellow arrow), (40X H and E). **D**: Sections of kidney treated with (lead acetate + *T. terrestris* extract group) fibrosis appears very clear near to inflammatory cells foci which reveals presence of chronic inflammation (Blue arrow), (40X H and E).

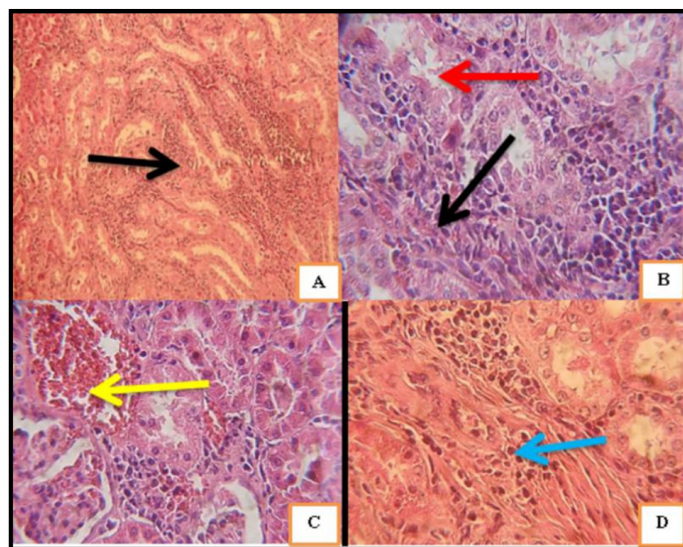


Figure 3: A: (10X H and E) sections of kidney from male rats (control group) showed the normal renal glomerulus (black arrow) and showed the normal renal tubules. B: sections of kidney (*T. terrestris* extract group), showed focal interstitial nephritis appears as aggregations of inflammatory cells (Black arrow) some tubules appears loosen its lumen and desquamated epithelial lining due to necrosis (Red arrow). C: Sections of kidney treated with (lead acetate group) showed hyaline casts appears with hemorrhagic changes enclosed to focal aggregation of monocytes (Yellow arrow), (40X H and E). D: Sections of kidney treated with (lead acetate + *T. terrestris* extract group) fibrosis appears very clear near to inflammatory cells foci which reveals presence of chronic inflammation (Blue arrow), (40X H and E).

casts appears with hemorrhagic changes enclosed to focal aggregation of monocytes, (40X H and E); **Figure 3D** sections of kidney treated with (lead acetate + *Tribulus terrestris* extract group) fibrosis appears very clear near to inflammatory cells foci which reveals presence of chronic inflammation, (40X H and E).

The present study results showed in **Table 1** that administration of lead acetate showed a significant decrease in body weight gain in rats compared to control and *T. terrestris* groups. We notice a significant decrease in rat weight in this study, resulting from reduced food intake and an increased catabolic state. Decrease of food intake and increased catabolic state due to the high duration of exposure to lead acetate and lack of balance in the metabolism resulting from changes in the zinc-dependent enzyme (Mahdi and Ghadhbhan, 2022a). Simultaneously, the elevation of serum and tissue ROS levels is associated with a decreased SOD concentration and a decline in NO. Various mitochondrial antioxidant enzymes may lead to the loss of animal appetite due to stress. Insulin-like growth factor (IGF-1) and insulin-like growth factor binding protein 3 (IGFBP-3) may have a role in the mechanism of action of TT drugs. Growth hormone IGF-1 has been shown to have the ability to increase skeletal muscle mass and slow the ageing process (Kilany *et al.*, 2020).

The suppression of cyclooxygenase-2 (COX-2) by TT may also lead to an increase in the generation of nitric oxide (NO). NO has the ability to modulate enhanced mitochondrial respiration, greater muscular energy metabolism, and blood flow activation (Fernández-Lázaro *et al.*, 2021).

Increased levels of circulating testosterone and IGF-1, as well as increased levels of AR and IGF-1R protein expression in the gastrocnemius, which leads to increased muscle weight and increased MHC in the gastrocnemius, may be the cause of the effect of TT extracts on the performance of high intensity exercise rats. enhances hunger and relieves stomach discomfort (Katiyar *et al.*, 2024).

According to the study's findings, the impact of TT extracts on rats performing high-intensity exercise may be related to the increased levels of circulating testosterone and IGF-1 as well as the increased expression of AR and IGF-1R proteins in the gastrocnemius, which increases the muscle mass and myosin content of the region (Wu *et al.*, 2017).

The liver has a high concentration of ALT, ALP, and AST enzymes, as shown in **Table 2**. These enzymes are often seen in trace concentrations in the bloodstream due to liver development and repair. Blood liver enzymes (AST, ALT, and ALP) increased significantly when administered lead

acetate in comparison to a control group; the liver plays a role in the detoxification of dangerous substances like lead acetate, which it removes after metabolism and breakdown. Cell membrane rupture brought on by this mechanism may raise serum liver enzyme levels. Additionally, rats given lead treatment experienced histological abnormalities, liver damage, and elevated blood levels of ALT, AST, and ALP. Lead-related liver damage This increase can be attributed to the accumulation of lead in the liver tissue, which damages the liver and releases enzymes into the bloodstream from the necrosis of the liver tissue (Mahdi *et al.*, 2022c).

Plasma biochemical indicators showed that the combination of *Tribulus terrestris* and lead considerably reduced liver damage and the repercussions of acute toxic liver injury. According to plasma biochemical markers, when combined with lead, greatly lowered liver damage and the effects of acute toxic liver damage. According to plasma biochemical markers on hepatic tissue in rats with chronic liver injury induced by lead acetate, the protective effect of when given in addition to lead significantly reduced liver damage and decreased the consequences of acute toxic liver damage. This was attributed to the inhibition of lipid peroxidation.

Moreover, it has been demonstrated that *Tribulus terrestris* increases the activity of the antioxidant enzymes CAT and superoxide dismutase, protecting liver tissues against ischemia/reperfusion injury.

The activities of plasma ALT, ALP, and AST increased significantly in our study of the progression of liver damage caused by repeated administration of PbAc. PbAc induced a rise in plasma ALT, ALP, and AST activity, and *Tribulus terrestris* appeared to improve liver damage caused by lead acetate considerably.

As an antioxidant, *Tribulus terrestris* extract reduced tissue oxidative damage and functional declines. Lead acetate's prooxidant qualities are widely known. HgCl₂ exposure is commonly associated with an imbalance in the antioxidant protection mechanisms that results in oxidative stress in the cells (Sugunavarman *et al.*, 2010). A significant increase in the generation of reactive oxygen species is accompanied by a significant decrease in the antioxidant enzymes catalase and glutathione peroxidase. There are three possible explanations for this: either the enzymes expression is lost, reactive oxygen species directly affects the enzymes, or lead acetate directly inhibits the enzymes, impairing their antioxidant capacity and increasing the production of reactive oxygen species. Fruit of *Tribulus terrestris* aqueous extract's antioxidant capacity in spleen cells Protection against oxidative stress-driven apoptosis was demonstrated by both AAPH and scavenged reactive

oxygen species (ROS) produced by γ -radiation. In spleen cells, it also demonstrated mitogenic activity (Amanullah *et al.*, 2021).

Our findings demonstrate that treatment with *Tribulus terrestris* considerably reduces MDA formation, suggesting a decrease in lipid peroxidation and cellular injury that shields the tissues from oxidative damage caused by lead acetate. As a result, functional parameters were also enhanced.

Tribulus terrestris reduced malondialdehyde by lowering the quantity of free radicals that cause lipid peroxidation. This indicates that *Tribulus terrestris* may be able to scavenge free radicals and reduce damage caused by free radicals induced by GNT, which is further supported by the histology results.

The waste product of protein metabolism in the blood, blood urea nitrogen, is measured in relation to blood protein levels. Because the kidneys remove urea from the bloodstream, urea is created by the liver and transported by the blood to the kidneys for excretion. The quantitative BUN test quantifies the blood's urea nitrogen content. The ability to determine how renal tissue functions is also helpful (Sugunavarman *et al.*, 2010). A BUN test is commonly performed to evaluate renal function. Moreover, one of the waste products of protein metabolism that the kidney excretes in urine is creatinine. Kidney function can be assessed by measuring BUN and creatinine. The markers of renal function are BUN and creatinine. When free radicals attack lipids, a chemical process known as lipid peroxidation takes place that has the power to damage the structure and functionality of cellular membranes (Sugunavarman *et al.*, 2010). Interstitial nephropathy, which can be either reversible or irreversible, is one type of renal injury. Long-term occupational lead exposure increases the risk of reversible interstitial nephropathy Petering (Mahdi and Ghadhbhan, 2022c). A growing number of humans and animals are suffering from environmental lead poisoning, and prolonged exposure to high amounts of lead has a negative impact on renal function (Collin *et al.*, 2022). In the absence of acute intoxication, lead-induced kidney damage may still develop, making it difficult to recognize concealed lead nephropathy as such (Amin *et al.*, 2023). Chronic lead poisoning impairs renal function (Gidlow, 2015). The kidneys eliminate urea and creatinine as waste products of amino acid metabolism. The development of renal toxicity seems to be linked to oxidative stress. The renal systems of humans and animals are severely damaged by environmental lead exposure (Assi *et al.*, 2016). Significant decreases in blood urea and creatinine were observed in the *Tribulus terrestris* treated groups following the administration of both *Tribulus terrestris* and lead, indicating that the herb protects renal

function from lead toxicity. *Tribulus terrestris* extract raises plasma parameters like blood urea nitrogen and creatinine. It also increases renal blood flow by inhibiting the activity of the angiotensin-converting enzyme, which in turn causes the vascular endothelium to release nitric oxide. Additionally, it causes the vascular smooth muscles to become hyperpolarised, which causes vasodilatation (Gunarathne *et al.*, 2023).

Rats exposed to a heavy metal had testicular architecture in their cross-sections that revealed histological changes. Due to seminiferous tubule atrophy and irregularity, there are no spermatogonia in the lumen of the tubules, a heavy metal can also cause vacuolization with a small number of spermatogonia by causing degenerative testes and the loss of germinal epithelial cells, which can disrupt the basement membrane and harm the male reproductive system (Hamza *et al.*, 2024).

The results of this study on lead's effects were in line with those of earlier studies (Balachandar *et al.*, 2020; Al-Megrin *et al.*, 2020).

The testes histopathological changes were significantly improved when *T. terrestris* extract was administered in conjunction with lead acetate, bringing them close to normal and shielding them from oxidative damage. This may be because *T. terrestris* extract has the ability to scavenge free radicals, as evidenced by the reorganisation of seminiferous tubule cells and the restoration of their activity. Reducing ROS levels would prevent lipoperoxidation and its detrimental effects on cells (Rasolifoshazeh *et al.*, 2020).

CONCLUSIONS AND RECOMMENDATION

In experimental animals, *T. terrestris* plant extract has been shown to provide protection against lead acetate-induced toxicity. It is abundantly obvious that administering lead acetate causes a substantial change in the structure and activities of the kidneys. The alcoholic extract of *T. terrestris* fruit containing lead acetate has demonstrated a significant protective impact on renal function and tissue structure. *T. terrestris* extract can raise the amounts of the biochemical indicators including urea and creatinine, and it is efficient in considerably reducing some of the physiological and histological effects of renal lead acetate toxicity.

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NOVELTY STATEMENT

The results showed that alcoholic extract of *T. terrestris* fruit has demonstrated a significant protective impact on renal function and tissue structure against lead acetate toxicity. We aimed to investigate how alcoholic extract of *T. terrestris* fruit reversed the negative effects of lead acetate-induced nephrotoxicity. All authors read, reviewed, and revised the manuscript and approved the final version.

AUTHORS CONTRIBUTION

MSH developed the offered concept and prepared the experiments. HTM and SRA-S formulated the theory and did the physiology, carried out the experiment, and authored the report with input and support from all authors, as well as the data analysis did by HTM.

CONFLICT OF INTEREST

The authors have declared no conflicts of interest.

REFERENCES

- Abbas MW, Hussain M, Akhtar S, Ismail T, Qamar M, Shafiq Z, Esatbeyoglu T (2022). Bioactive compounds, antioxidant, anti-inflammatory, anti-cancer, and toxicity assessment of *Tribulus terrestris* in vitro and in vivo studies. *Antioxidants*, 11(6): 1160. <https://doi.org/10.3390/antiox11061160>
- Abdulqawi LNA, Quadri SA (2021). Evaluation of antibacterial and antioxidant activities of *Tribulus terrestris* L. fruits. *Res. J. Pharma. Technol.*, 14(1): 331-336. <https://doi.org/10.5958/0974-360X.2021.00061.5>
- Abubakar K, Mailafiya MM, Chiroma SM, Danmaigoro A, Zyouid TY, Rahim AE, Zakaria ABMZ (2020). Ameliorative effect of curcumin on lead-induced hematological and hepatorenal toxicity in a rat model. *J. Biochem. Mol. Toxicol.*, 34(6): e22483. <https://doi.org/10.1002/jbt.22483>
- Ali SI, Gaafar AA, Abdallah AA, El-Daly SM, El-Bana M, Hussein J (2018). Mitigation of alpha-cypermethrin-induced hepatotoxicity in rats by *Tribulus terrestris* rich in antioxidant compounds. *Jordan J. Biol. Sci.*, 11(5).
- Al-Megrin WA, Soliman D, Kassab RB, Metwally DM, Moneim AEA and El-Khadragy MF (2020). Coenzyme Q10 activates the antioxidant machinery and inhibits the inflammatory and apoptotic cascades against lead acetate-induced renal injury in rats. *Front. Physiol.*, 11: 64. <https://doi.org/10.3389/fphys.2020.00064>
- Al-Mukhtar N, Mohammed A, Mahdi H (2016). The effects of potassium dichromate on testes, accessory sex glands, liver and kidney in adult male rats treated with ascorbic acid. *Austral. J. Basic Appl. Sci.*, 10(4): 99-108.
- Amanullah KZ, Zeenat F, Ahmad W, Firoz S, Naeela A (2021). A comprehensive review on a Unani medicinal plant: *Tribulus terrestris* Linn. *Int. J. Herb. Med.*, 9(1): 23-28.
- Amin R, Alam F, Dey BK, Darwin R, Ahmed SA, Thalluri C, Mandhadi JR (2023). Sources, blood concentrations, and approaches for reducing exposure to lead: A critical appraisal on lead poisoning. [https://doi.org/10.18006/2023.11\(3\).506.519](https://doi.org/10.18006/2023.11(3).506.519)

- Assi MA, Hezmee MNM, Sabri MYM, Rajion MA (2016). The detrimental effects of lead on human and animal health. *Vet. World*, 9(6): 660. <https://doi.org/10.14202/vetworld.2016.660-671>
- Azam F, Munier S, Batoo M, Ahmad B, Abbas G (2019). A review on advancements in ethnomedicine and phytochemistry of *Tribulus terrestris* a plant with multiple health benefits. *Int. J. Biosci.*, 14(1): 21-37. <https://doi.org/10.12692/ijb/14.1.21-37>
- Balachandar R, Bagepally, BS, Kalahasthi R, Haridoss M (2020). Blood lead levels and male reproductive hormones: A systematic review and meta-analysis. *Toxicology*, 443: 152574. <https://doi.org/10.1016/j.tox.2020.152574>
- Chhatre S, Nesari T, Somani G, Kanchan D, Sathaye S (2014). Phytopharmacological overview of *Tribulus terrestris*. *Pharmacogn. Rev.*, 8(15): 45. <https://doi.org/10.4103/0973-7847.125530>
- Chaudhary, A., Kumari, R., & Thakur, P. (2023). A review on mitigation of various ailments via a bioactive component of *Tribulus terrestris* L.-A medicinally important herb. *Ethnobotany Research and Applications*, 25, 1-17.
- Collin MS, Venkatraman SK, Vijayakumar N, Kanimozhi V, Arbaaz SM, Stacey RS, Swamiappan S (2022). Bioaccumulation of lead (Pb) and its effects on human: A review. *J. Hazard. Mater. Adv.*, 7: 100094. <https://doi.org/10.1016/j.hazadv.2022.100094>
- El-Chaghaby G, Koçer O, Korkmaz N, Imran U, Rashad S, Sevindik M (2024). Evaluation of the antimicrobial, antioxidant, and other biological activities of *Tribulus terrestris* plants collected from different countries. *Egypt. J. Vet. Sci.*, pp. 1-8. <https://doi.org/10.21608/ejvs.2024.284787.2031>
- Fernández-Lázaro D, Mielgo-Ayuso J, del Valle Soto M, Adams DP, González-Bernal JJ, Seco-Calvo J (2021). The effects of 6 weeks of *Tribulus terrestris* L. supplementation on body composition, hormonal response, perceived exertion, and crossFit® performance: A randomized, single-blind, placebo-controlled study. *Nutrients*, 13(11): 3969. <https://doi.org/10.3390/nu13113969>
- Flora G, Gupta D, Tiwari A (2012). Toxicity of lead: A review with recent updates. *Interdis. Toxicol.*, 5(2): 47-58. <https://doi.org/10.2478/v10102-012-0009-2>
- Gidlow DA (2015). Lead toxicity. *Occup. Med.*, 65(5): 348-356. <https://doi.org/10.1093/occmed/kqv018>
- Gunarathe R, Nadeeshani H, Lu A, Li J, Zhang B, Ying T, Lu, J (2023). Potential nutraceutical uses of *Tribulus terrestris* L. in human health. *Food Rev. Int.*, 39(8): 5326-5355. <https://doi.org/10.1080/87559129.2022.2067172>
- Gupta PK, Gupta PK (2020). Target organ toxicity. Problem solving questions in toxicology: A study guide for the board and other examinations, pp. 83-117. https://doi.org/10.1007/978-3-030-50409-0_7
- Hamza MA, Hassan MS, Mousa RF (2024). The role of ginseng extract green nanoparticles as an antioxidant on physiological parameters and fertility in male rats exposed to potassium dichromate. *Nusant. Biosci.*, 16: 219-227.
- Katiyar D, Saxena R, Kumar A, Bansal P, Prakash S, Ghosh D, Nagarajan K (2024). A comprehensive review of the protective effects of herbals against toxicity of Bisphenol A. *Toxin Rev.*, pp. 1-29. <https://doi.org/10.1080/15569543.2024.2329907>
- Kilany OE, El-Beltagy MA, El-Sherbeeney NA (2020). *Tribulus terrestris* ameliorates carbon tetrachloride-induced hepatotoxicity in male rats through suppression of oxidative stress and inflammation. *Environ. Sci. Pollut. Res.*, 27: 24967-24981. <https://doi.org/10.1007/s11356-020-08826-w>
- Mahdi HT, Ghadhban RF (2022a). L-carnitine nanoparticles effects on physiology and histology changes induced by lead acetate in male rats. *Chinese J. Med. Genet.*, 32(3).
- Mahdi HT, Ghadhban RF (2022b). Physiological effects of nano L-carnitine on the gonadal pathway in adult male rats exposed to lead acetate. *Rev. Electron. Vet.*, pp. 188-209.
- Mahdi HT, Ghadhban RF (2022c). Protective effect of L-carnitine nanoparticles Vs carnitine on lead acetate-induced toxicity in male rats. *J. Adv. Biotechnol. Exp. Ther.*, 5(3): 590-604. <https://doi.org/10.5455/jabet.2022.d138>
- Matović V, Buha A, Đukić-Čosić D, Bulat Z (2015). Insight into the oxidative stress induced by lead and/or cadmium in blood, liver and kidneys. *Food Chem. Toxicol.*, 78: 130-140. <https://doi.org/10.1016/j.fct.2015.02.011>
- Mohamed SS, Zeima NM, El-Melmoslemany AM (2023). The effect of *Tribulus terrestris* fruits and *Urtica dioica* leaves extracts on renal calculus induced by sodium oxalate in experimental rats. *Bull. Natl. Nutr. Inst. Arab Republ. Egypt*, 61(1): 100-137. <https://doi.org/10.21608/bnni.2023.291548>
- Rahman Z, Singh VP (2019). The relative impact of toxic heavy metals (THMs) arsenic (As), cadmium (Cd), chromium (Cr) (VI), mercury (Hg), and lead (Pb)) on the total environment: An overview. *Environ. Monit. Assess.*, 191: 1-21. <https://doi.org/10.1007/s10661-019-7528-7>
- Rasolifoshazeh A, Abedi B, Matinhomae H, Farzanegi P (2020). The effect of aerobic training and *Tribulus terrestris* extract on some oxidative stress indices in the heart tissue of male wistar rats poisoned with hydrogen peroxide. *Gene Cell Tissue*, 7(4). <https://doi.org/10.5812/gct.103794>
- Semerdjieva IB, Zheljazkov VD (2019). Chemical constituents, biological properties, and uses of *Tribulus terrestris*: A review. *Natl. Prod. Commun.*, 14(8): 1934578X19868394. <https://doi.org/10.1177/1934578X19868394>
- Shukla V, Shukla P, Tiwari A (2018). Lead poisoning. *Indian J. Med. Special.*, 9(3): 146-149. <https://doi.org/10.1016/j.injms.2018.04.003>
- Singh J, Kaur J, Kaur M, Rana A, Rasane P, Kaur S (2023). *Tribulus terrestris*: Pharmacological and nutraceutical potential. *Harvesting Food from Weeds*, pp. 79-112. <https://doi.org/10.1002/9781119793007.ch3>
- Ștefănescu R, Tero-Vescan A, Negroiu A, Aurică E, Vari CE (2020). A comprehensive review of the phytochemical, pharmacological, and toxicological properties of *Tribulus terrestris* L. *Biomolecules*, 10(5): 752. <https://doi.org/10.3390/biom10050752>
- Sugunavarman T, Jagadeesan G, Samipillai SS (2010). *Tribulus terrestris* extract protects against mercury-induced. *J. Ecobiotechnol.*, 2(1).
- Unsal V, Dalkıran T, Çiçek M, Kölükçü E (2020). The role of natural antioxidants against reactive oxygen species produced by cadmium toxicity: A review. *Adv. Pharma. Bull.*, 10(2): 184. <https://doi.org/10.34172/apb.2020.023>
- Wu Y, Yang H, Wang X (2017). The function of androgen/androgen receptor and insulin growth factor-1/insulin growth factor-1 receptor on the effects of *Tribulus terrestris* extracts in rats undergoing high intensity exercise. *Mol. Med. Rep.*, 16(3): 2931-2938. <https://doi.org/10.3892/mmr.2017.6891>
- Zhang X, Zheng Y, Wang Z, Gan J, Yu B, Lu B, Jiang X (2023). Melatonin as a therapeutic agent for alleviating endothelial dysfunction in cardiovascular diseases: Emphasis on

- oxidative stress. Biomed. Pharmacother., 167: 115475. <https://doi.org/10.1016/j.biopha.2023.115475>
- Zhu W, Du Y, Meng H, Dong Y, Li L (2017). A review of traditional pharmacological uses, phytochemistry, and pharmacological activities of *Tribulus terrestris*. Chem. Central J., 11: 1-16. <https://doi.org/10.1186/s13065-017-0289-x>