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# Mitigation of Lead-Induced Renal Dysfunction and Tissue Damage by Microwave-Assisted Extracts of Silybum marianum: Impact on Systemic Growth, Blood Profiles, and Renal Architecture

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## Abstract

Lead is a widespread environmental toxicant that induces nephrotoxicity primarily through oxidative stress and disruption of cellular homeostasis. Natural antioxidants such as Silybum marianum have gained attention for their protective potential against heavy metal toxicity. Thirty adult male Wistar rats were divided into five groups (n = 6): normal control, lead acetate-treated (100 mg/kg), lead + Silybum marianum (100 mg/kg), lead + Silybum marianum (200 mg/kg), and lead + glutathione + vitamin C. Treatments were administered orally for 30 days. Biochemical parameters including serum urea, creatinine, lipid profile, and blood lead levels were measured. The kidney tissues were also examined histopathologically.Lead exposure significantly elevated blood lead levels, serum urea, creatinine, total cholesterol, LDL, VLDL, and triglycerides, while reducing HDL levels. Treatment with Silybum marianum extract demonstrated a dose-dependent protective effect, with the high dose showing near normalization of biochemical and histological parameters.Silybum marianum seed extract exerts significant nephroprotective effects against lead-induced toxicity, likely via antioxidant mechanisms, and may serve as a promising natural therapeutic agent for managing lead-induced renal damage

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## Introduction

The kidneys are vital organs responsible for maintaining internal homeostasis through their roles in filtration, detoxification, electrolyte balance and hormone regulation. However, the continuous exposure of the kidneys to circulating toxins renders them highly susceptible to damage (Soriano et al., 2023). Recent findings further highlight the kidney as a primary target of lead toxicity, often resulting in progressive structural and functional damage (Bharti and Gopalakrishnan, 2025). The mechanisms behind lead-induced kidney injury are complex, but oxidative stress stands out as a central factor. Lead exposure promotes the overproduction of reactive oxygen species (ROS), which overwhelms the body's antioxidant defenses and disturbing cellular redox balance (Ercal et al., 2001). To address these challenges there has been increasing interest in exploring natural compounds that can offer protection with minimal side-effects. Plant based medicine rich in antioxidants are becoming popular as safer and more environmentally friendly alternatives. Such traditional plant is milk thistle, which has been widely studied for its protective effect. Its active component, silymarin is composed of several flavonolignans such as silibinin, silydianin, and silychristin recognised for their strong free radical scavenging properties (Abenavoli et al., 2010). Many indications that silymarin may have a renoprotective effect against nephrotoxic drugs were found in the review of Dashti-Khavidaki et al. (2013).

With this in mind, the current study was designed to to assess the protective impact of Silybum marianum against lead-induced nephrotoxicity in Wistar rats. By examining biochemical parameters, blood lead levels, lipid profile and histopathological changes, this study aims to provide clearer insight into its potential as a natural therapeutic agent against lead-induced kidney damage.

## Material and Methods

### Chemicals and Preparation of Solutions

Lead acetate trihydrate [$Pb(OAc)_2 \cdot 3H_2O$] (El Nasr Pharmaceutical Chemicals Company, Cairo, Egypt), L-glutathione, and L-ascorbic acid (Sigma-Aldrich, Germany) were used in this study. Silybum marianum seeds were procured from a local herbal supplier (Bliss of Earth Brand, Harmony Lifesciences, India).

### Experimental Animals and Study Design

Thirty healthy adult male wistar rats (180–220 g) were obtained from the institutional animal facility and acclimatized for one week under standard laboratory conditions with free access to standard pellet diet and water. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC), NIMS University, Jaipur, Rajasthan, India (Approval No. NIMSUR/IAEC-02/2024/11), and all experimental procedures were conducted in accordance with CPCSEA guidelines. The animals were randomly divided into five groups (n = 6): Group I (Normal Control), Group II (Lead acetate, 100 mg/kg), Group III (Lead acetate + Silybum marianum extract, 100 mg/kg), Group IV (Lead acetate + Silybum marianum extract, 200 mg/kg), and Group V (Lead acetate + glutathione, 100 mg/kg + vitamin C, 1 mg/100 g body weight). All treatments were administered orally by gavage once daily for 30 consecutive days.

- **Group I**: Normal Control
- **Group II**: Lead acetate-treated Disease Control (100 mg/kg)
- **Group III**: Lead + Silybum marianum extract (100 mg/kg)
- **Group IV**: Lead + Silybum marianum extract (200 mg/kg)
- **Group V**: Lead + Glutathione (100 mg/kg) + Vitamin C

Treatments were administered orally once daily for 30 days.

All rats were euthanized using diethyl ether at the end of the experiment. EDTA and Red top tube (serum tube) were used for the collection of blood and biochemical analysis. kidney and liver tissues were harvested and stored in formalin for histopathological examination and frozen at -80°C for biochemical analysis.

### Biochemical Analysis

Blood was coagulated at room temperature and subsequently centrifuged at 3000 rpm for 10 minutes. Serum was separated and kept at -20°C for further biochemical analysis. Liver and kidney tissue was removed and washed with the help of ice- cold normal saline, blotted dry and stored at -20°C.

## Kidney Function Tests (KFTs)

Serum levels of creatinine, and urea were determined using standard enzymatic and colorimetric methods provided in commercial diagnostic kits. The procedures were Urea: Diacetyl monoxime or Berthelot enzymatic method, for Creatinine: alkaline picrate method and for Uric acid :Uricase-peroxidase method (Enzymatic - colorimetric method)[15] .All biochemical estimations were performed using a semi-automated biochemistry analyzer (e.g., Erba Chem 5x or equivalent model).

## Estimation of Blood Lead Concentration

Blood lead level were measured using Atomic Absorption Spectroscopy (AAS) GBC 932+ (Central Instrumentation Lab of Guru Jambheshwar University, Hisar) following sample digestion. Approximately 1 ml of whole blood was collected in heparinized tubes and the samples underwent wet acid digestion, for which a digestion mixture was prepared using Perchloric and nitric acid in a 4:1 ratio. The blood samples were heated at 80-100°C on a hot plate until a clear solution was obtained. The digested samples were cooled, diluted with deionized water to a final volume of 10 milliliters, and filtered through Whatman No. 42 filter paper.

A flame Atomic Absorption Spectrophotometer(Model: GBC 932+Specification is Elemental Determination in ppm range) were used to measure the amount of lead concentration in digested blood sample, at 283.3 nm wavelength (in CIL of Guru Jambheshwar University of Science & Technology). Calibration was performed using standard lead solutions of known concentrations, and quality control was ensured by analyzing blanks and standards alongside samples. All estimations were conducted in triplicate to ensure accuracy and reproducibility. The results or data were expressed in µg/dL of blood.

## Histopathological Examination

Following standard processing, kidney tissues were embedded in paraffin wax, fixed in 10% neutral buffered formalin, sectioned at 4–5 µm, stained with hematoxylin and eosin, and examined under a light microscope.

## Statistical Analysis

The data were expressed by the mean ± standard deviation (SD) for each group of Wistar rats. ANOVA (a one-way analysis of variance) was used to analyze data from the control and experimental groups. Several comparisons were made using Tukey's test. Significance levels of $p < 0.05$ were considered. SPSS 22.0 was employed for statistical analysis.

## Results

## Body Weight

Body weight serves as the principal physiological metric for assessing the impact of lead exposure and overall health in toxicological studies. During the trial, it was watched for any signs of toxicity, metabolic problems, or cachexia. To evaluate the harmful effects of lead acetate trihydrate and the impact of antioxidant therapies, body weight was measured weekly. The stabilisation seen in the groups that were treated suggests a protective effect. A noticeable reduction in body weight, in the lead-exposed group suggests systemic poisoning.

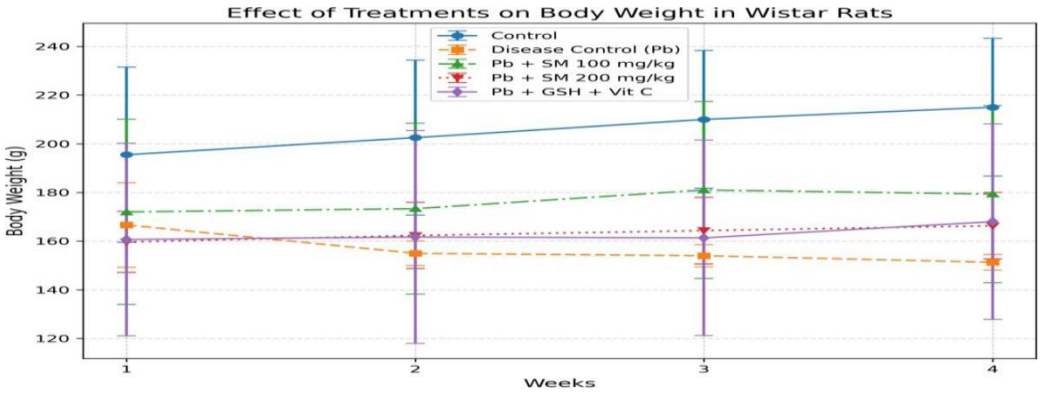



**Figure. 1.** Effect of Silybum marianum, vitamin C, and glutathione on body weight changes over four weeks in lead acetate–treated Wistar rats. Values are expressed as mean ± standard deviation (SD) for six rats in each group. One-way ANOVA and Tukey's post hoc test were used for statistical analysis (SPSS version 22.0). A p-value of less than 0.05 was considered statistically significant. Significant differences exist across groups that do not share the same superscript letter

A progressive decrease in weight was observed in disease control; in comparison to the normal control, it showed significant impairment of physiological growth caused by lead acetate ($P < 0.01$).Normal control group rats indicate consistent weight gain ($P < 0.05$), while the rats received combination of vitamin C and glutathione resulted in a moderate but significant recovery ($P < 0.05$). Supplementing with Silybum marianum provided dose-dependent protection: both low and high doses increased body weight in comparison to disease controls ($p < 0.01$), while the high-dose (200 mg/kg) group maintained weights similar to normal controls ($p > 0.05$).According to One - way ANOVA with Turkey's post hoc analysis, Silybum marianum (200mg/kg) seed extract at higher doses provided nearly complete protection against lead acetate induced toxicity in wistar rats.

## Blood Analysis

Blood analysis is a vital source of information regarding the physiological effects of toxicants and their systemic circulation. For this study, blood samples were obtained at the end of the exposure period to assess hematological alterations and systemic toxicity.

Table.1 Blood lead concentrations (µg/dL) determined by Atomic Absorption Spectrophotometry (AAS) in normal control, lead acetate, and treatment groups.

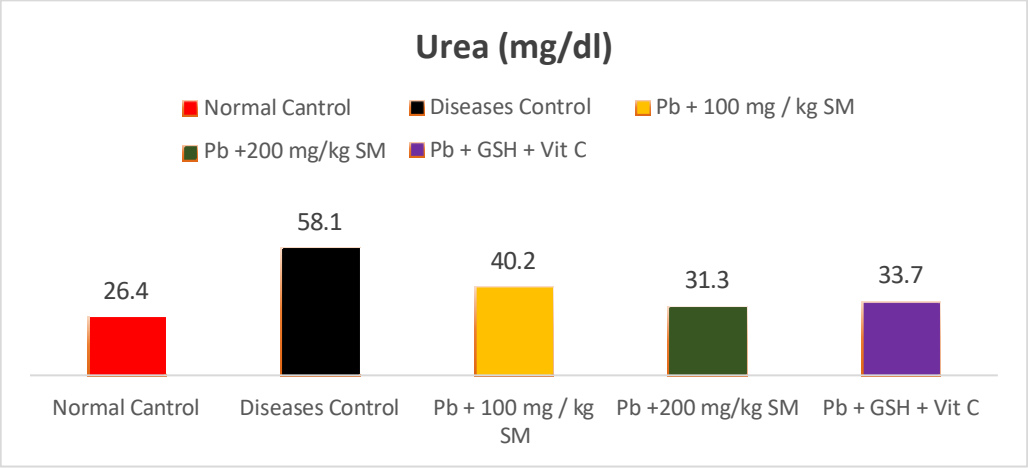
| **Groups** | **Blood Lead Level (µg/dL)** |
|---|---|
| Normal Control | 8.02 ± 0.73 |
| Diseases Control (Pb Acetate) | 67.40 ± 2.82 |
| Pb + 100 mg /Kg SM extract (Low Dose) | 38.60 ± 2.31 |
| Pb 200 mg/kg SM extract (High Dose) | 24.87 ± 1.63 |
| Pb + Glutathione + Vitamin C | 21.45 ± 1.28 |

The mean ± SD is used to represent the values ($n = 6$). One-way ANOVA followed by Tukey's post hoc test were employed for the statistical analysis. The difference between the lead acetate treated group and the normal control group was $p < 0.05$.

Exposure to lead acetate markedly increased blood lead levels in the disease control (Pb - actate treated group) relative to normal controls ($p < 0.05$), indicating that toxicity was successfully induced. While treatment groups showed significant decreases, normal controls maintained typical physiological values. Silybum marianum at 100 mg/kg exhibited a notable decrease ($p < 0.05$), indicating partial alleviation of toxicity, and the higher dose (200 mg/kg) further reduced levels ($p < 0.01$ vs. disease control). The glutathione + vitamin C combination exhibits the greatest reduction, highlighting the combined antioxidant potential to reduce lead-induced toxicity. One-way ANOVA with Tukey's post hoc analysis confirmed significant differences between the groups, with Silybum marianum showing dose -dependent potency.

## Kidney Function Profile

Serum urea and creatinine, level were used to estimate renal clearance and filtration efficiency, which is biochemically referred to as the kidney biomarkers. By measuring tubular integrity, nitrogenous waste excretion, and glomerular filtration rate (GFR), these metrics function as sensitive markers of nephrotoxicity. In toxicological investigations, high urea and creatinine levels are frequently linked to decreased renal function as a result of structural damage, oxidative stress, or inflammation brought on by toxicants. The effectiveness of therapeutic procedures intended to restore normal kidney function and the degree of renal damage are both shown by monitoring these indicators. Urea and creatinine level in serum were evaluated to analyzed renal function following lead acetate exposure and treatment interventions. Lead acetate administration resulted in a noteworthy increase in both creatinine and urea levels against the normal control group ($p < 0.05$), indicating impaired renal function (Figure 2).



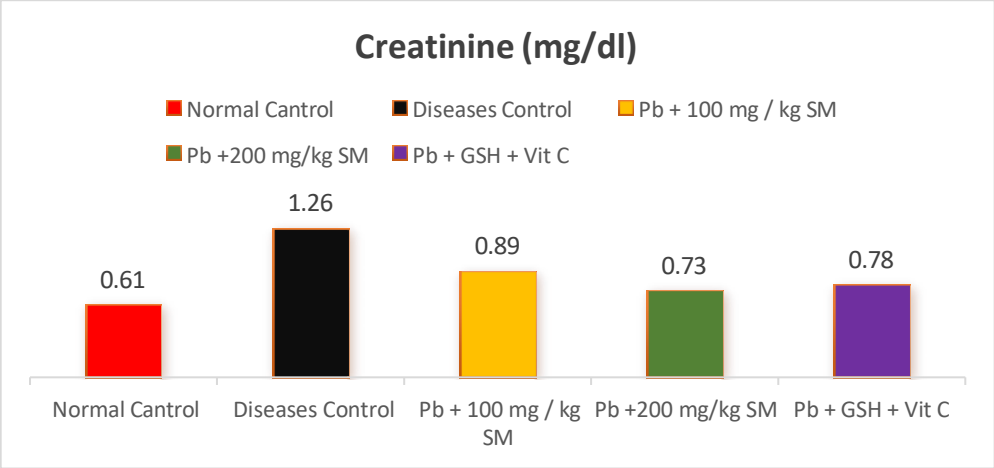




**Figure.2.** Effect of Silybum maranum extract, vitamin C, and glutathione on serum urea and creatinine levels in lead acetate–treated Wistar rats.Values are showed as mean ± standard deviation (SD) for six rats in each p. One-way ANOVA and Tukey's post hoc test were used for statistical analysis (SPSS version 22.0). A p-value of less than 0.05 ($P<0.05$) was considered statistically important.Significant differences exist across groups that do not share the same superscript letter.

Treatment with Silybum marianum extract produced a dose-dependent reduction in these parameters. The low-dose group (100 mg/kg) showed a significant decrease in serum creatinine and urea levels compared with the Pb-treated group ($p < 0.05$), although values remained higher than those of the control group. In contrast, rats treated with the higher dose (200 mg/kg) exhibited a greater reduction in both parameters, approaching values observed in the normal control group.

Similarly, co-administration of glutathione and vitamin C significantly reduced serum creatinine and urea levels relative to the Pb-treated group ($p < 0.05$). The reduction in creatinine levels in this group was comparable to that observed in the high-dose S. marianum treatment group. Overall, these results demonstrate that both S. marianum extract and antioxidant supplementation improved renal function in lead-exposed rats.

## Histopathology of Kidney Tissue

Upon histopathological examination, the normal control group's kidney sections exhibited normal glomerular architecture, including intact Bowman's capsules, clear urinary spaces, and well-organized tubular epithelium (Fig. 3-a). The group that was treated with Pb acetate showed severe tubular degeneration, interstitial inflammation, glomerular atrophy, and a loss of brush border integrity in the proximal tubules (Fig. 3-b).

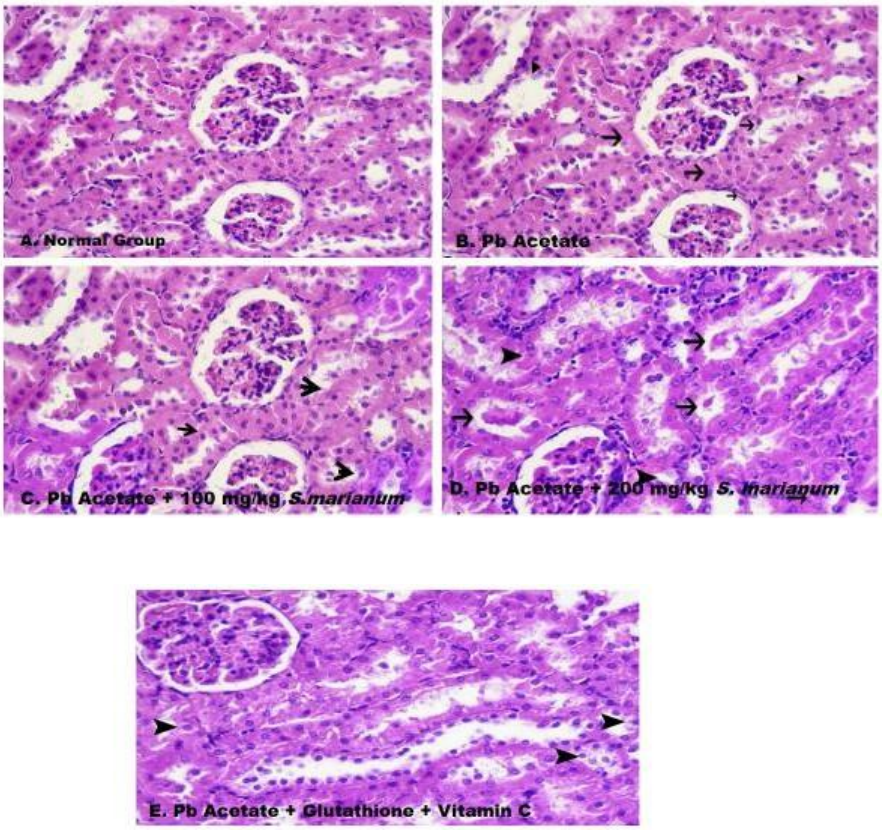



**Figure .4. Histopathological analysis of kidney sections from experimental groups (H&E stain, ×400, scale bar- 100 µm).**
**(A) Normal control: Renal tissue shows intact glomerular architecture, well-defined Bowman's capsules, clear urinary spaces, and organized tubular epithelium.**
**(B) Pb acetate-treated group: Displays severe renal damage including glomerular atrophy, loss of brush border integrity in proximal tubules, interstitial inflammation, and tubular degeneration. Kupffer cell hyperplasia is also evident, indicating systemic toxic response.**
**(C) Pb acetate + Silybum marianum (100 mg/kg): Shows partial restoration of glomerular structure and reduced tubular necrosis, suggesting moderate nephroprotective effects.**
**(D) Pb acetate + Silybum marianum (200 mg/kg): Reveals near-normal glomeruli and minimal interstitial alterations, indicating dose-dependent histological improvement.**
**(E) Pb acetate + Vitamin C + glutathione: Demonstrates almost complete renal recovery, with morphology closely resembling the Normal control group**

Tubular necrosis and partial glomerular structural restoration were significantly enhanced in rats supplemented with the low dose (100 mg/kg) of Silybum marianum extract (Fig. 4-c). In the high dose (200 mg/kg) Silybum marianum extract group, renal histology was noticeably improved, characterized by glomeruli that were nearly normal and minimal interstitial alteration (Fig. 4-d). The administration of combination of vitamin C and glutathione led to an almost complete recovery, as shown by the renal morphology.

These results are similar to the previous studies, which indicates that Silybum marianum and antioxidant combinations of vitamin C and gluathione counteract the f lead poisoning through membrane- stabilizing, tissue - regenerative mechanism and antioxidant.

## Discussion

Lead is a well-recognized environmental pollutant that accumulates in soft tissues, particularly the kidneys, where it induces structural and functional damage through oxidative stress and inflammation. In the present study, lead acetate exposure resulted in significant alterations in body weight, blood lead concentration, renal biomarkers, and kidney histology, confirming successful induction of nephrotoxicity. Similar findings have been reported in previous studies demonstrating that chronic lead exposure impairs renal function and promotes oxidative tissue injury (Patrick, 2006; Flora et al., 2012).A significant reduction in body weight gain was observed in the lead-exposed group. This decrease may be attributed to reduced feed utilization, impaired nutrient absorption, metabolic disturbances, and increased oxidative stress associated with lead toxicity. Previous investigations have reported comparable reductions in body weight following lead exposure in experimental animals (Wani et al., 2015). Administration of Silybum marianum extract improved body weight in a dose-dependent manner, suggesting restoration of metabolic activity and overall physiological status.

Blood lead concentration was markedly elevated in the disease control group, indicating substantial systemic lead accumulation. Treatment with Silybum marianum significantly reduced blood lead levels compared with the untreated lead-exposed group. This reduction may be associated with the metal-binding capacity of flavonoids

and enhanced elimination of lead from biological tissues. Similar detoxifying effects of plant-derived antioxidants against heavy metal toxicity have been documented previously (Flora et al., 2013).Serum urea and creatinine concentrations were significantly increased following lead exposure, indicating impaired glomerular filtration and renal dysfunction. Lead-induced nephrotoxicity is known to result from oxidative damage to renal tubular cells, mitochondrial dysfunction, and disruption of membrane integrity (Ercal et al., 2001). Treatment with Silybum marianum significantly restored these biomarkers toward normal values, particularly at the higher dose. The nephroprotective effect may be attributed to the antioxidant and membrane-stabilizing properties of silymarin, which protect renal cells from reactive oxygen species-mediated injury (Pradhan and Girish, 2006).

Histopathological examination further supported the biochemical findings. Kidneys from lead-treated rats exhibited glomerular shrinkage, tubular degeneration, inflammatory cell infiltration, and disruption of normal renal architecture. These pathological changes are consistent with previous reports describing lead-induced renal injury (Flora et al., 2008). In contrast, rats treated with Silybum marianum showed marked improvement in renal structure, including restoration of glomerular integrity and reduction of tubular damage. The protective effect was more pronounced in the high-dose group, indicating a dose-dependent response.

The glutathione and vitamin C-treated group also demonstrated significant recovery of renal function and tissue architecture. Both antioxidants are known to scavenge reactive oxygen species and enhance cellular antioxidant defenses, thereby reducing oxidative stress-induced tissue injury (Hsu and Guo, 2002). However, the comparable efficacy of high-dose Silybum marianum suggests that the plant extract may serve as an effective natural alternative for mitigating lead-induced nephrotoxicity.

Overall, the findings of the present study demonstrate that microwave-assisted Silybum marianum extract effectively reduces blood lead burden, improves renal function, enhances body weight gain, and preserves kidney histo- architecture in lead-exposed wistar rats. These protective effects are likely mediated through antioxidant, anti-inflammatory, and metal-detoxifying mechanisms.

## Conclusion

The present study demonstrates that lead acetate exposure causes significant nephrotoxicity, characterized by elevated blood lead concentration, impaired renal function, reduced body weight gain, and marked histopathological alterations in kidney tissue. Oral administration of microwave-assisted Silybum marianum extract significantly ameliorated these toxic effects in a dose-dependent manner. The high-dose extract (200 mg/kg) showed substantial renoprotective activity, comparable to that of glutathione and vitamin C. These findings suggest that Silybum marianum may serve as a promising natural therapeutic agent for the prevention and management of lead-induced renal damage. Further studies are warranted to investigate the underlying molecular mechanisms and evaluate its potential clinical applications.

## Declaration

### Ethical approval

The experimental protocol involving Wistar rats was reviewed and approved by the **Institutional Animal Ethics Committee (IAEC)** of **Nims University** (Approval Number: **NIMSUR/IAEC-02/2024/11**) prior to the commencement of experimental work. All procedures were conducted in strict accordance with the guidelines provided by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

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## Conflict of interest

The authors have no conflicts of interest regarding this investigation**.**

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