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Ethanol Extract of Tamarind Fruit Increased Glomerular Area in Lead-Induced Wistar Rats

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ABSTRACT

Lead (Pb) exposure is associated with nephrotoxicity, particularly structural alterations of the glomerulus mediated by oxidative stress. Tamarind fruit (Tamarindus indica L.) contains antioxidant compounds and vitamin C that may help attenuate lead-induced renal alterations. This study aimed to investigate the effect of ethanol extract of tamarind fruit on the glomerular area of Wistar rat kidneys exposed to lead.

This experimental study conducted a post-test only control group design using 20 male Wistar rats. All groups were induced with lead acetate at a dose of 10 mg/kg body weight/day for 14 days. Treatment groups received ethanol extract of tamarind fruit (TFE) at doses of 12.5, 25, and 50 mg/kg body weight/day. Glomerular area was assessed histologically, and data were analyzed using one-way ANOVA and PostHoc.

The results showed a significant difference in glomerular area among groups ($p < 0.05$). Administration of TFE at a dose of 12.5 mg/kg body weight/day demonstrated a more favorable glomerular area compared to the lead-only group, whereas higher doses did not show additional improvement. These findings suggest that low-dose TFE may influence glomerular structural changes induced by lead exposure.

Keywords : glomerulus; kidney; lead; Tamarindus indica L

INTRODUCTION

Lead (Pb) remains a major environmental toxicant with significant public health implications due to its persistence, bioaccumulation, and multi-organ toxicity. Human exposure occurs through contaminated air, water, food, and occupational activities, particularly in industrial settings. Despite regulatory efforts, lead exposure continues to contribute substantially to global morbidity and mortality, especially in low- and middle-income countries (Mani et al., 2020; Rees et al., 2020; WHO,

2024). Once absorbed, lead circulates in the bloodstream and accumulates in soft tissues, including the kidneys, where it exerts toxic effects (Yu et al., 2020).

Among target organs, the kidney is particularly susceptible to lead toxicity because of its central role in filtration and excretion. Chronic lead exposure results in renal accumulation, leading to progressive nephrotoxicity. At the cellular level, lead induces oxidative stress through excessive production of reactive oxygen species (ROS), which disrupt mitochondrial function, enhance lipid peroxidation, and impair endogenous antioxidant defense systems (Aulanni'am et al., 2019; Gao et al., 2020; Shafiekhani et al., 2019). These mechanisms contribute to structural alterations in renal tissue, including damage to the glomerulus, which plays a critical role in maintaining filtration function. Changes in glomerular structure, such as alterations in glomerular area, may serve as early indicators of renal injury.

Given the pivotal role of oxidative stress in lead-induced nephrotoxicity, antioxidant-based interventions have gained increasing attention. Natural products, particularly those rich in bioactive compounds, offer promising therapeutic potential due to their ability to scavenge free radicals and enhance cellular defense mechanisms (Obafemi et al., 2019; Riaz et al., 2020).

Tamarind (Tamarindus indica L.) fruit is widely recognized for its high content of bioactive compounds, including flavonoids, polyphenols, saponins, alkaloids, and vitamin C. These compounds exhibit strong antioxidant and metal-chelating properties, which may contribute to protection against heavy metal-induced oxidative damage (Dewi et al., 2018; Farooq et al., 2022). Previous studies have demonstrated the general antioxidant potential of tamarind; however, evidence specifically addressing its effect on renal structural changes, particularly at the glomerular level under lead exposure, remains limited.

Therefore, this study aimed to evaluate the effect of ethanol extract of Tamarindus indica fruit on glomerular area in lead-induced Wistar rats, as an indicator of its potential nephroprotective activity.

METHODS

This study used a true experimental design using a post-test only control group approach. The experiment was conducted to evaluate changes in glomerular area in male Wistar rats (Rattus norvegicus) following lead (Pb) induction and treatment with ethanol extract of tamarind fruit (Tamarindus indica L.). The study was carried out at the Pharmacology Laboratory, Faculty of Medicine, Universitas Muhammadiyah Malang, over a 14-day experimental period in December.

A total of 20 healthy male Wistar rats aged 2–3 months and weighing 100–200 g were used in this study. Sample size determination was based on the degree of freedom method, which indicated a minimum of 16 animals; additional animals were included to anticipate potential experimental loss, resulting in five rats

per group (Arifin & Zahiruddin, 2017). All animals were acclimatized prior to experimentation and maintained under standard laboratory conditions.

Experimental Grouping and Treatment Protocol

The animals were randomly divided into four experimental groups: a normal control group and three treatment groups (P1, P2, and P3). All groups were induced with lead acetate at a dose of 10 mg/kg body weight/day, administered orally using a gastric gavage for 14 consecutive days.

Following lead induction, treatment groups received TFE at different doses. The P1 group received 12.5 mg/kg body weight/day, the P2 group received 25 mg/kg body weight/day, and the P3 group received 50 mg/kg body weight/day. The extract was administered orally once daily for 14 days, concurrently with lead exposure, according to group allocation.

Dose selection was based on previous studies reporting that TFE at a dose of 25 mg/kg body weight exhibited significant antioxidant activity and enhanced endogenous antioxidant enzymes, including superoxide dismutase (SOD) and glutathione peroxidase (GPx) (Azman et al., 2012). In addition, vitamin C contained in tamarind fruit has been reported to exert metal-chelating effects and to reduce lead-induced oxidative damage by decreasing lead absorption and inhibiting lipid peroxidation (Shaban El-Neweshy & Said El-Sayed, 2011). Therefore, dose variation was applied using a half-dose (12.5 mg/kg), standard dose (25 mg/kg), and double-dose (50 mg/kg) strategy.

Histological Examination and Glomerular Area Measurement

At the end of the experimental period, the rats were euthanized, and kidney tissues were harvested for histological analysis. Haematoxylin-Eosin was used to stain renal tissues. The glomerular area was measured using Image-J software to investigate structural changes of the glomerulus under light microscopy (400x magnification).

Statistical Analysis

Data normality was assessed using the Shapiro–Wilk test, while homogeneity of variances was evaluated using Levene's test. Data were considered normally distributed and homogeneous when p-values exceeded 0.05. Differences among experimental groups were analyzed using one-way analysis of variance (ANOVA). When statistically significant differences were detected, post hoc comparisons were performed using the Bonferroni test. Linear regression analysis was additionally performed to explore the association between tamarind fruit extract dosage and changes in glomerular area.

Ethical approval

All experimental procedures involving animals were conducted in accordance with the guidelines for the care and use of laboratory animals. The study protocol was reviewed and approved by the Ethics Committee of the Faculty of Medicine, Universitas Muhammadiyah Malang (Approval No. E.5.a/080/KEPK-UMM/IV/2024).

RESULTS AND DISCUSSION

Histological analysis revealed variations in glomerular area among experimental groups. Statistical analysis showed a significant difference in glomerular area between groups ($p < 0.05$). Lead-exposed rats showed a reduction in glomerular area compared to treatment groups, indicating structural alteration of the renal filtration unit.

Administration of ethanol extract of Tamarindus indica fruit (TFE) modulated these changes. The group receiving 12.5 mg/kg body weight/day exhibited the most pronounced improvement in glomerular area (404.77 µm) compared to the lead-only group (358.62 µm). Higher doses (25 mg/kg and 50 mg/kg) showed less pronounced effects, with mean glomerular areas of 375.60 µm and 368.54 µm, respectively, indicating a non-linear dose-response pattern. These findings suggest that TFE influences glomerular structural integrity in lead-exposed Wistar rats, with the lowest dose demonstrating the most favorable outcome.

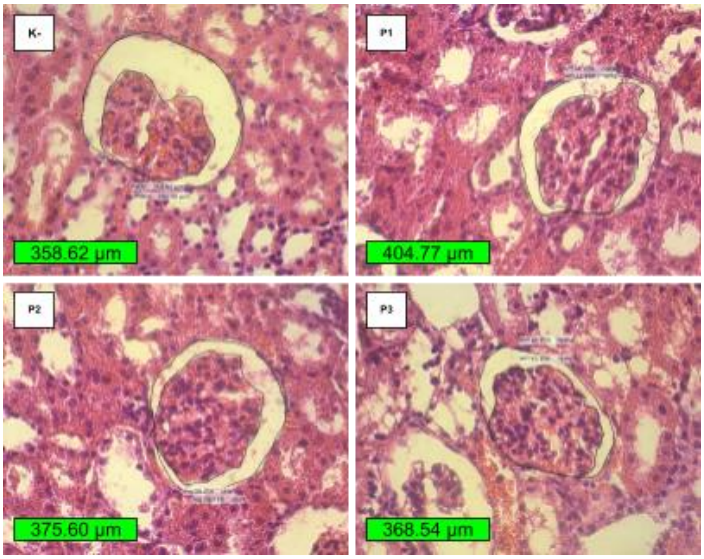

Figure 1. Histopathological analysis of glomerular morphology in lead-exposed rats treated with tamarind extract

Representative photomicrographs showing renal glomeruli stained with hematoxylin and eosin (H&E) at 400× magnification. (K-) Lead control group showing glomerular area of 358.62 µm; (P1) TFE 12.5 mg/kg body weight demonstrating increased glomerular area (404.77 µm); (P2) TFE 25 mg/kg body weight (375.60 µm); (P3) TFE 50 mg/kg body weight (368.54 µm). Green scale bars indicate measured glomerular diameter in micrometers (µm). Images demonstrate dose-dependent modulation of glomerular size in response to tamarind extract treatment.

The present study demonstrated that lead exposure induces significant structural alterations in the renal glomerulus, as evidenced by a reduction in glomerular area. This finding supports previous studies indicating that lead-induced nephrotoxicity is primarily mediated by oxidative stress mechanisms. Excessive production of reactive oxygen species (ROS) disrupts endothelial integrity, promotes lipid peroxidation, and impairs

cellular homeostasis, ultimately leading to structural damage in renal tissue (Abdel-Zaher et al., 2019; Aulanni'am et al., 2019; Collin et al., 2022).

Mechanistically, lead-induced renal damage is increasingly understood as a consequence of complex molecular pathways involving oxidative stress and inflammation. Lead exposure has been shown to inhibit the PI3K/AKT signaling pathway while activating NF-κB-mediated inflammatory responses, ultimately leading to cellular injury and disruption of renal architecture (Zhang et al., 2023). lead nephropathy is characterized by progressive structural damage that may initially present as subtle glomerular and tubular alterations before advancing to more severe functional impairment (Toniutti & Vidal, 2022).

The reduction in glomerular area observed in the lead-only group may reflect early glomerular shrinkage or collapse due to oxidative injury and impaired microcirculation. Lead accumulation in renal tissue, combined with its slow excretion, contributes to sustained cellular damage and progressive disruption of glomerular architecture (Gao et al., 2020; Levin et al., 2021). These structural changes are critical, as the glomerulus plays a central role in maintaining filtration efficiency, and even subtle alterations may indicate early renal dysfunction.

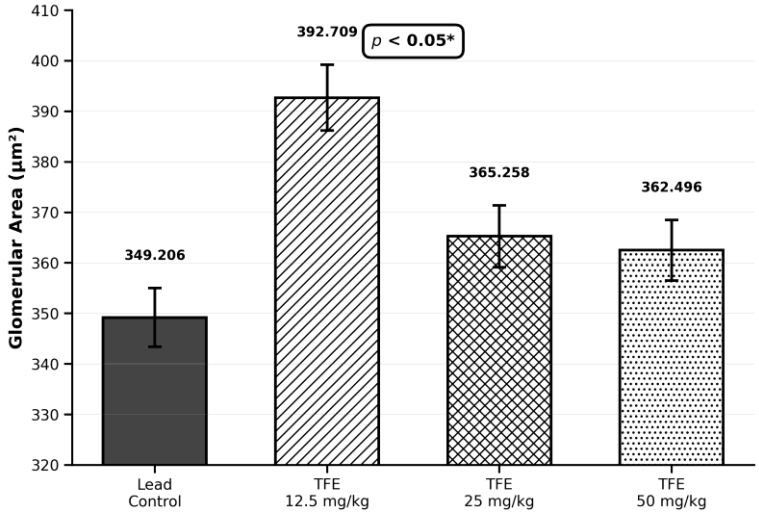

Figure 2. Effect of Tamarind Fruit Extract (TFE) on glomerular area in lead-exposed rats. Data are presented as mean ± SEM $p<0.05$ (one-way ANOVA). *, statistically significant.

The nephrotoxic effect of lead observed in this study is further supported by epidemiological and experimental evidence demonstrating a strong association between lead exposure and impaired kidney function. Chronic exposure to lead, even at low levels, has been linked to decreased glomerular filtration rate and increased risk of chronic kidney disease, particularly when combined with co-exposure to other toxic metals such as cadmium and mercury (Jain, 2019). Recent population-based data also indicate that elevated serum lead levels are significantly associated with reduced kidney function, especially in vulnerable populations such as individuals with hypertension (KOOKANOK et al., 2024).

Treatment with ethanol extract of Tamarindus indica fruit resulted in measurable improvements in glomerular area, particularly at the 12.5 mg/kg dose. This effect observed in this study may be strongly

associated with the rich phytochemical composition of *Tamarindus indica*, particularly its flavonoid and polyphenolic constituents. These bioactive compounds are widely recognized for their potent antioxidant activity, which plays a crucial role in mitigating oxidative stress–induced cellular damage. Phytochemical investigations have confirmed that tamarind extracts contain substantial levels of phenolic compounds and flavonoids, which contribute to their strong free radical scavenging capacity (Ahmed et al., 2024; Mohamed et al., 2025). In addition, tamarind fruit is a source of vitamin C, which, together with flavonoids and polyphenols, enhances antioxidant potential by stabilizing cellular membranes and reducing oxidative injury (Dewi et al., 2018; Farooq et al., 2022). Collectively, these compounds may attenuate oxidative stress by lowering reactive oxygen species (ROS) levels and supporting endogenous antioxidant defense systems.

Flavonoids and polyphenols exert their antioxidant effects through multiple pathways, including direct scavenging of reactive oxygen species (ROS), chelation of metal ions, and modulation of endogenous antioxidant defense systems. These compounds are capable of donating hydrogen atoms or electrons to neutralize free radicals, thereby preventing lipid peroxidation and preserving cellular membrane integrity (Muhammad Dahiru et al., 2023; Songkro et al., 2022). This mechanism is particularly relevant in lead-induced toxicity, where excessive ROS generation plays a central role in initiating renal cellular injury.

In addition to their antioxidant properties, flavonoids and polyphenols have been reported to support cellular repair and survival by modulating signaling pathways involved in apoptosis and inflammation. By reducing oxidative stress, these compounds help maintain mitochondrial function and prevent activation of cell death pathways, thereby promoting cell viability and tissue integrity (Bressiani et al., 2021; Komakech et al., 2019). This cytoprotective effect may explain the preservation of glomerular structure observed in the treatment groups in the present study.

Furthermore, tamarind extracts have demonstrated the ability to inhibit oxidative damage in various experimental models, including reduction of lipid accumulation and improvement of cellular metabolic balance (Harjadi et al., 2022). Antioxidant assays such as DPPH and FRAP have consistently shown strong radical scavenging activity of tamarind extracts, further supporting their role as effective natural antioxidants (Kartika Risfianty & Sanuriza, 2021; Muhammad Dahiru et al., 2023).

Importantly, the combined antioxidant and cytoprotective effects of flavonoids, polyphenols, and vitamin C suggest a multifaceted protective mechanism. In addition to scavenging free radicals, vitamin C may also exert a metal-chelating effect, reducing lead bioavailability and limiting its accumulation in renal tissue. This mechanism has been reported to decrease lipid peroxidation and attenuate cellular damage associated with heavy metal exposure (Shaban El-Neweshy & Said El-Sayed, 2011).

Interestingly, higher doses of tamarind extract (25 mg/kg and 50 mg/kg) did not produce greater improvements and appeared less effective than the lowest dose, suggesting a non-linear or biphasic dose–response relationship. At higher concentrations, antioxidant compounds may exhibit reduced efficacy or even pro-oxidant behavior, potentially disrupting redox balance and diminishing their protective effects (Ahmed et al., 2024; Mohamed et al., 2025).. Additionally, factors such as limited bioavailability, saturation of transport

mechanisms, and altered metabolic processing at higher doses may reduce intracellular effectiveness (Harjadi et al., 2022; Riaz et al., 2020). The possibility of mild cytotoxic effects at higher concentrations, as reported in previous studies, may further contribute to the reduced efficacy observed (Bressiani et al., 2021). The relatively modest contribution of TFE to variations in glomerular area suggests that renal structural changes are influenced by multiple factors, including endogenous antioxidant capacity and individual biological variability. This highlights the complexity of lead-induced nephrotoxicity and the multifactorial nature of its modulation (Collin et al., 2022) also the importance of dose optimization and suggest that the beneficial effects of *Tamarindus indica* extract occur within a specific therapeutic window.

Overall, these findings support the biological plausibility that the improvement in glomerular area observed in this study is mediated by the antioxidant and cytoprotective properties of *Tamarindus indica.* Although oxidative stress biomarkers and tissue lead concentrations were not directly assessed, the observed structural preservation of the glomerulus suggests a protective effect through these mechanisms. However, several limitations should be considered. This study was limited to histological evaluation of glomerular area without assessing renal functional parameters or key biochemical indicators of oxidative stress, such as malondialdehyde (MDA), glutathione (GSH), and antioxidant enzyme activity. In addition, the absence of tissue lead measurements restricts direct correlation between structural alterations and lead accumulation. Therefore, these findings should be interpreted as preliminary structural evidence of nephroprotection.

CONCLUSION

Ethanol extract of Tamarindus indica fruit influenced glomerular structural changes in lead-induced Wistar rats, with a more favorable effect observed at lower doses. Further studies are needed to confirm these findings using functional parameters.

Lead exposure induced early structural alterations of the renal glomerulus, as evidenced by a reduction in glomerular area. Administration of ethanol extract of Tamarindus indica fruit, particularly at a low dose, was associated with relative preservation of glomerular structure in lead-exposed rats. These findings suggest a potential modulatory role of plant-derived antioxidants in lead-induced renal injury, although the observed effects were limited and dose-dependent. Future studies are recommended to incorporate comprehensive biochemical and molecular analyses, including oxidative stress markers, inflammatory mediators, and relevant signaling pathways, alongside evaluation of renal function and dose–response optimization, to further clarify the mechanisms and confirm the therapeutic potential of *Tamarindus indica* extract in lead-induced nephrotoxicity.

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