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Renoprotective Effect of Broccoli Extract in Pre-pubertal Wistar Rats Exposed to Lead Acetate Toxicity

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ABSTRACT

Background: This study was aimed to assess the potential renoprotective properties of broccoli extract in pre-pubertal Wistar rats subjected to lead acetate-induced renal toxicity. Lead acetate poses a significant health threat due to its toxic effects on various organ systems, particularly the kidneys, which play a crucial role in waste elimination and maintaining physiological balance.

Methods: The study design involves the administration of lead acetate to induce subchronic intoxication in experimental rats, alongside treatment with broccoli extract and vitamin E to assess their protective effects.

Result: Biochemical analyses reveal significant increases in urea, uric acid, creatinine and BUN levels in the lead acetate-treated group, indicative of impaired renal function. However, treatment with broccoli extract demonstrates a significant reduction in these biomarkers, comparable to the effects observed with vitamin E treatment, suggesting potential renoprotective benefits. Histopathological examination corroborates these findings, with the lead acetate group exhibiting severe renal damage, including degeneration, necrosis and inflammatory cell infiltration, while broccoli extract treatment partially mitigates these effects.

The study highlights the importance of natural compounds like broccoli in combating heavy metal-induced renal toxicity and underscores the need for further investigation into their protective mechanisms. These findings hold implications for the development of preventive strategies and therapeutic interventions against lead-induced nephrotoxicity, offering valuable insights into the potential of broccoli extract as a natural renoprotective agent.

Key words: Broccoli extract, Kidney function test, Lead toxicity, Prepubertal rats, Renoprotective.

INTRODUCTION

Heavy metal exposure poses a significant threat to human health. Lead acetate is a toxic substance commonly found in industrial and consumer products. Exposure to lead acetate can have detrimental effects on various organ systems, particularly in young individuals. Among the vital organs vulnerable to lead toxicity, the kidneys play a crucial role in eliminating waste products and maintaining over all physiological balance (Fu *et al*., 2020; Arunachalam *et al*., 2021). Recognizing the need for effective interventions, this study investigates the potential renoprotective effects of broccoli extract in pre-pubertal Wistar rats exposed to lead acetate toxicity.

Lead (Pb) is a pervasive environmental contaminant known for its extensive toxic effects, adversely affecting various organs and causing a range of biochemical, physiological and behavioral dysfunctions. The kidney is particularly vulnerable to Pb-induced damage due to its pivotal role in eliminating Pb from the body. Proximal tubule cells play a crucial role in removing lead, but this process leads to damage to mitochondria, suppression of mitochondrial function and alterations in cell absorptive activities, resulting in renal tubular damage and failure. Prolonged exposure to Pb can also lead to the development of chronic Pb nephropathy (Dkhil *et al*., 2016; Asiwe *et al*., 2023).

Similar to other heavy metals, Pb has been identified as a generator of reactive oxygen species (ROS), contributing

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to oxidative injury in the kidney. Exposure to Pb through various routes has been linked to increased lipid peroxidation (LPO) in the kidney and depletion of glutathione (GSH). Additionally, Pb has been found to inhibit sulfhydryl-dependent enzymes, disrupt metals crucial for antioxidant enzyme activities and heighten cell vulnerability to oxidative attacks by compromising the integrity and fatty acid composition of renal cell membranes (Asiwe *et al*., 2023).

In light of this, exploring natural compounds with potential protective properties becomes imperative. Vegetables play a crucial role in promoting human health as they serve as a source of antioxidants with minimal adverse effects on physiological systems. Broccoli, scientifically known as *Brassica oleracea* L. *var. Italica,* is particularly noteworthy for its rich content of polyphenols, providing significant benefits to the body. Natural antioxidants found in broccoli, such as beta-carotene, along with vitamins C and E, play a direct and/or indirect role in reducing free radicals. Additionally, broccoli contains essential minerals like iron, selenium, calcium, manganese, phosphorus, zinc and potassium, contributing to the regulation of the body's ionic balance (Raeeszadeh *et al*., 2022). Recognizing the significance of addressing lead acetate toxicity and the insufficient research focus on this matter, the objective of this study was to explore the impact of broccoli extract on mitigating changes induced by lead acetate toxicity in the blood, as well as assessing the biochemical parameters in kidneys of pre-pubertal Wistar rats.

Understanding the interplay between lead toxicity, broccoli extract and kidney function in a pre-pubertal rat model holds implications for potential preventive strategies and therapeutic interventions in the context of heavy metal-induced renal toxicity. The findings of this study may offer a foundation for further exploration of natural compounds as adjunctive therapies in mitigating the adverse effects of environmental toxins on renal health.

MATERIALS AND METHODS

Chemicals

The chemicals and reagents utilized in this investigation were of high purity and analytical grade, obtained from Sigma-Aldrich Chemical Company (USA) and Sisco Research Laboratories (India). In particular, biochemical assay kits were sourced from Spinreact, Spain.

Animals

The present investigation was conducted with 30 male pre-pubertal Wistar rats (21 days of age), each weighing between 40-60 grams. The rats were housed at the Animals House of SRM Medical College Hospital and Research Center at 23±2°C with 12-hour light/dark cycles after their purchase from the Tamil Nadu Veterinary Animal Sciences University, Chennai. The animals were provided with a standard diet and unrestricted access to water. Prior to the commencement of experiments, the rats were accommodated in the testing environment for one week to ensure acclimatization. Throughout the study period (22 days), the general physical health of the rats was observed. The National Institute for Laboratory Animal Care's Guide for the Care and Use of Laboratory Animals, as well as the university's ethical rules, were followed throughout the whole procedure and every stage of the study (SAF/IAEC/280622/020).

Experimental design

The experimental rats were randomly assigned into five groups of 30 animals: each group consisting of 6 animals. For every group, the same standard diet was maintained for twenty-two days.

Groups	Animal treatment
Group I	Normal control rats, which received normal saline for 22 days.
Group II	Sub chronic intoxication were induced by oral administration of lead acetate 12 mg/day Kg body weight.
Group III	Lead acetate toxicity induced rats treated with broccoli extract orally (300 mg/kg body weight for 22 days).
Group IV	Lead acetate toxicity induced rats treated with vitamin E (50 mg/kg body weight) orally for 22 days.
Group V	Control rats treated with broccoli extract at 300 mg/kg body weight for 22 days.

After twenty-two days, the rats were administered general anesthesia with ketamine and xylazine and blood samples were collected by intra cardiac puncture to measure biochemical variables. As soon as the rats were killed, the kidney was surgically removed and preserved in 10% formaldehyde-filled container to prepare histopathology slides.

Biochemical estimation

Kidney function test

The evaluation of kidney function markers, such as urea, uric acid, creatinine and BUN, utilized biochemical assay kits acquired from Spinreact, Spain. The results of these assessments were quantified and reported in milligrams per deciliter (mg/dL).

Histopathological evaluation

For histological assessment, kidney segments from each group underwent fixation in 10% neutral buffered formalin for 24 hours, followed by embedding in paraffin and sectioning. Subsequently, semi-thin sections measuring 4-6 μm thick were prepared using an LKB ultra microtome. Hematoxylin and Eosin (H and E) staining was carried out using standard procedures outlined by Gabe (1968) then observed under a light microscope and documented using photography.

RESULTS AND DISCUSSION

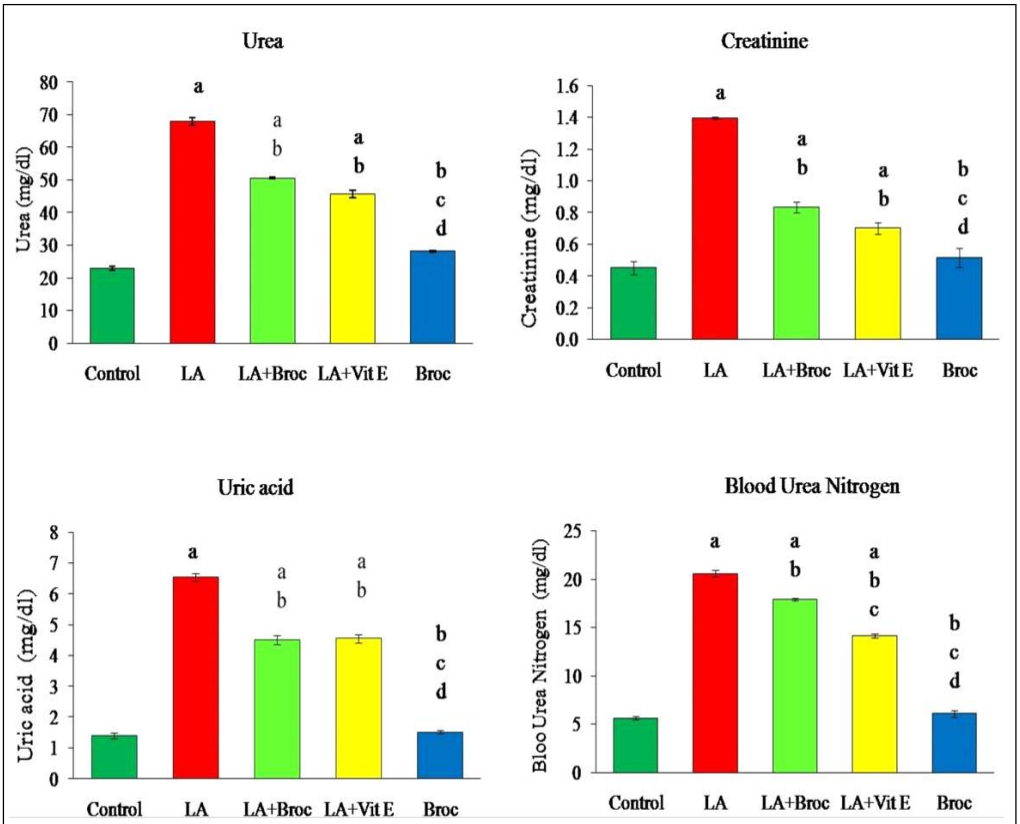
Broccoli extract mitigates the elevated levels of urea, creatinine, uric acid and BUN induced by lead acetate toxicity in pre-pubertal rats

The findings depicted in Fig 1 revealed a notable rise in urea, uric acid, creatinine and BUN levels in the lead acetate-treated group compared to the control group, indicating impaired renal function due to lead acetate toxicity. However, administering broccoli alongside lead acetate treatment demonstrated a reduction in these adverse effects, to the results observed in the Vitamin E treated group. This suggests a potential mitigating effect of broccoli on lead acetate-induced renal toxicity, as evidenced by statistically significant reductions in the mentioned parameters.

Broccoli extract normalizes changes in histoarchitecture of the kidney in lead acetate toxicity-induced pre-pubertal rats

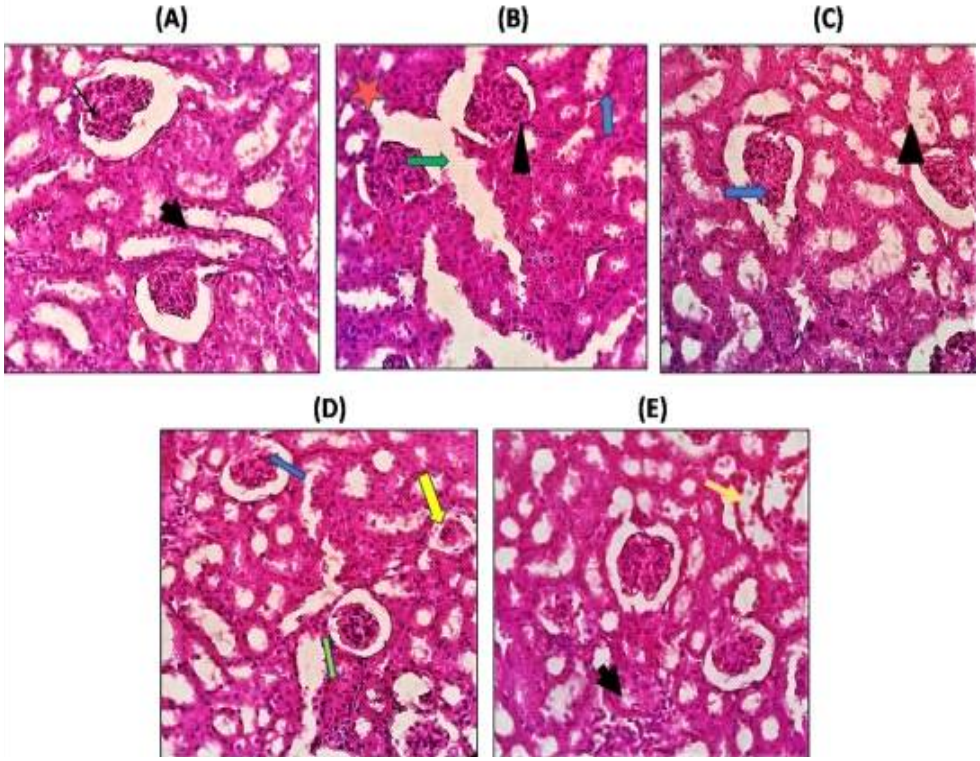
The histopathology of the kidney is depicted in Fig 2. The control group displayed typical renal architecture with dilated tubules and glomeruli at the cortex's periphery. Conversely, the lead acetate group exhibited degeneration and necrosis of renal parenchymal cells, along with significant inflammatory cell infiltration whereas the broccoli extract-treated group revealed degenerated cortical tubules and mild cellular infiltration. Experimental pre-pubertal rats treated with Vitamin E displayed normal glomerular tuft, mild cellular infiltration and renal tubular inflammation. The Broccoli extract treated control group showed a normal renal structure with dilated tubules and glomeruli at the cortex's periphery.

The toxic impact of lead acetate on renal function has been the subject of thorough investigation owing to the pervasive exposure to lead from diverse environmental sources. Lead toxicity is a significant public health concern as it can adversely affect various organs, with the kidneys being particularly vulnerable (Dolati *et al*., 2021). In this study, the primary objective is to explore potential protective mechanisms offered by Broccoli extracts against lead acetate-induced renal toxicity in experimental animals. Broccoli is selected as a potential protective agent due to its known antioxidant properties (Flockhart *et al*., 2023),



The values are expressed as mean±SEM (n= 6 animals each) in mg/dl. Data were analyzed by one way ANOVA and multiple comparisons between groups were performed by Tukey's test. LA-Leadacetate; LA+Broc- Lead acetate treated with Broccoli; LA+Vit E- Lead acetate treated with Vitamn E; Bro- Broccoli alone treated (Positive control). a - compared with control; b - compared with Lead acetate toxicity; c- compared with Lead toxicitytreated with Broccoli extract; d-compared with Lead toxicitytreated with Vitamin E. Statistically significant was achieved at P<0.001.

Fig 1: Effect of broccoli extract on urea, uric acid, creatinine and bun levels in lead acetate toxicity induced pre-pubertal rats.



A- Comprised normal prepubertal rats serving as the control, exhibiting typical dilated tubules and glomeruli at the cortex's periphery. B- consisting of Lead Acetate toxicity-induced prepubertal rats, displayed degeneration and necrosis of renal parenchymal cells, along with significant infiltration of inflammatory cells. C- Lead toxicity induced rats treated with Broccoli extract, exhibited degenerated cortical tubules with mild cellular infiltration in the renal parenchyma. D- Lead toxicity induced rats treated with Vitamin E, demonstrated a normal glomerular tuft, mild cellular infiltration and renal tubular inflammation. E- comprising Broccoli extract-treated control prepubertal rats, exhibited normal dilated tubules and glomeruli at the cortex's periphery.

Fig 2: Histopathology of kidney (40X magnification).

which could counteract the oxidative stress implicated in lead-induced nephrotoxicity.

The experimental design involves a meticulous examination of blood biomarkers known to be indicative of renal function. Blood urea, uric acid, creatinine and blood urea nitrogen (BUN) levels are considered as reliable indicators, collectively offering insights into the overall health and performance of the kidneys (Miao *et al*., 2022). Urea, originating from liver-driven protein metabolism, is indicative of renal filtration efficiency, with elevated levels suggesting impaired kidney function. Uric acid, a byproduct of purine breakdown, provides insights into both kidney health and potential metabolic imbalances, with increased levels associated with conditions like gout. Creatinine, derived from muscle metabolism, is a key indicator of renal function and elevated blood levels indicate compromised kidney clearance. BUN, representing the kidneys′ urea excretion ability, indirectly measures nitrogen content; elevated levels can indicate impaired renal function, though external factors like diet and hydration can influence results. Regular monitoring of these biomarkers is crucial for the early detection of kidney disorders, enabling timely interventions and the maintenance of optimal renal health (Sanders *et al*., 2019; Gounden *et al*., 2023).

This study aimed to explore the potential protective effects of broccoli against lead acetate-induced renal toxicity in experimental animals. It involved a thorough analysis of blood urea, uric acid, creatinine and blood urea nitrogen (BUN) levels to assess renal function. The results highlight the significant impact of lead acetate on renal function and suggest potential protective effects of broccoli and vitamin E in experimental animals. Elevated levels of urea, uric acid, creatinine and BUN in the lead acetate-treated group emphasize the severity of lead-induced nephrotoxicity. The concurrent administration of Broccoli extract significantly attenuated these elevations equal to that of the standard drug Vitamin E, suggesting a promising role in mitigating renal damage when compared with toxicity-induced groups. Importantly, these findings align with existing literature on lead-induced nephrotoxicity and emphasize the potential antioxidant properties of Broccoli (Raeeszadeh *et al*., 2020). The observed reductions in urea, uric acid, creatinine and

BUN levels with these interventions contribute valuable insights into their renoprotective effects.

Additionally, the histopathological examination confirmed normal renal architecture in the control group, while the lead acetate group exhibited severe renal damage, including degeneration, necrosis and inflammatory cell infiltration. Broccoli treatment partially mitigated these effects in lead acetate toxicity induced pre-pubertal rats, showing degenerated tubules and mild cellular infiltration. Vitamin E treatment demonstrated improvement with a normal glomerular tuft, mild cellular infiltration and tubular inflammation in lead toxicity induced pre-pubertal rats. Notably, the Broccoli-treated group maintained normal renal architecture, consistent with biochemical results that coincide with previous studies (Raeeszadeh *et al*., 2020; Raeeszadeh *et al*., 2022), highlighting the potential protective effects of Broccoli, near to that of Vitamin E against lead acetate-induced nephrotoxicity. Further investigation is essential to uncover the molecular mechanisms underlying these protective effects.

CONCLUSION

In conclusion, this study highlights the harmful effects of lead acetate on renal function and the potential protective role of broccoli extract in pre-pubertal rats. Elevated blood biomarkers and severe histopathological changes indicate significant renal damage from lead exposure. However, broccoli extract administration shows promising renoprotective effects similar to vitamin E, suggesting its potential as a natural antioxidant therapy for lead-induced nephrotoxicity. Further research is needed to understand the underlying mechanisms better.

Author contributions

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Conflict of interest

The authors declare that they have no potential conflicts of interest to disclose.

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