**Aqueous Extract of Garcinia kola: A Potential Remedy for Lead-Induced Kidney Damage in Wistar Rats**

**ABSTRACT**

Lead exposure is a pervasive environmental hazard known for its detrimental effects on renal function. Garcinia kola is traditionally valued for its medicinal properties, and its extract is believed to protect organs from toxic insults by mitigating oxidative stress and cellular damage. This study investigates the nephroprotective effects of *Garcinia kola* (bitter kola) aqueous extract against lead-induced kidney damage in Wistar rats. Twenty-five male Wistar rats were divided into five groups: a control group, a lead-only group (60 mg/kg lead acetate), and three treated groups receiving lead followed by *Garcinia kola* extract at doses of 150 mg/kg, 300 mg/kg, and 600 mg/kg for 21 days. Biochemical analyses were conducted to assess serum urea, creatinine, and uric acid levels, alongside morphological assessments of kidney tissues. Results indicated that lead acetate administration significantly elevated serum creatinine levels (*P* = .001) and caused observable morphological changes, indicating renal impairment. Conversely, treatment with *Garcinia kola* extract resulted in a dose-dependent restoration of kidney function, with groups receiving 300 mg/kg and 600 mg/kg showing significant reductions in creatinine (*P* = .001, P = .011) and improvements in overall health metrics. These findings suggest that *Garcinia kola* extract effectively mitigates lead-induced renal damage, likely due to its antioxidant properties. This study highlights the potential of *Garcinia kola* as a therapeutic agent for protecting against heavy metal toxicity, warranting further investigation into its mechanisms of action and long-term efficacy in clinical settings.

**Keywords:** Heavy metal toxicity, Lead toxicity, Morphological kidney assessment, Nephroprotection, Phytotherapy.

1. **INTRODUCTION**

Lead, a ubiquitous heavy metal, poses severe environmental and health risks due to its non-biodegradable nature and persistent use in industrial processes [1,2]. Despite measures to reduce its exposure, lead remains a major toxicant, impacting multiple organ systems in humans and animals [3]. Chronic exposure to lead primarily affects the kidneys, where it induces oxidative stress, inflammation, and cellular damage that may lead to nephropathy or even renal failure over prolonged periods [3,4]. Studies indicate that lead exposure disrupts cellular function in renal tissue by generating reactive oxygen species (ROS), which impairs mitochondrial function and causes apoptosis of renal cells [5]. Given the high vulnerability of kidney tissue to oxidative stress, mitigating lead-induced renal damage has become an area of active research.

Kidneys play a crucial role in filtering blood, excreting toxins, and maintaining homeostasis, and they are particularly sensitive to toxic exposures, including heavy metals like lead. Lead-induced kidney damage can result in impaired glomerular function, tubular necrosis, and altered electrolyte balance, compromising renal physiology. Current therapeutic strategies often involve chelating agents like ethylenediaminetetraacetic acid (EDTA) and dimercaptosuccinic acid (DMSA). However, these treatments are associated with limitations, including potential adverse effects and limited efficacy in chronic cases [6,7]. Consequently, exploring natural compounds that offer protective or therapeutic benefits against lead-induced renal damage has garnered considerable scientific interest.

In recent years, Garcinia kola, commonly known as bitter kola, has attracted attention due to its antioxidant, anti-inflammatory, and hepatoprotective properties. Indigenous to West and Central Africa, Garcinia kola has been traditionally utilized for medicinal purposes, particularly for its potential effects on liver and kidney health. The seeds of Garcinia kola contain bioactive compounds such as flavonoids, xanthones, and biflavonoids, which are known to exhibit potent antioxidant activities by neutralizing free radicals and reducing oxidative stress [8,9]. Moreover, biflavonoids in Garcinia kola have been shown to attenuate inflammation, a key mechanism in renal pathologies induced by toxic insults [10].

Emerging evidence suggests that natural antioxidants, including those found in Garcinia kola, could mitigate oxidative damage in renal tissue, providing a basis for investigating their effects in cases of lead toxicity. Animal studies have demonstrated that extracts from Garcinia kola possess nephroprotective properties, likely due to the scavenging of ROS and modulation of inflammatory pathways [11]. This suggests that aqueous extracts of Garcinia kola may have the potential to counteract lead-induced oxidative damage in renal tissues, thereby preserving kidney function.

Given this background, this study aims to evaluate the therapeutic effects of aqueous extracts of Garcinia kola on lead-induced renal damage in Wistar rats. By focusing on biochemical and histopathological markers of kidney function and oxidative stress, this research seeks to contribute to the growing body of knowledge on natural remedies for heavy metal toxicity and offer a potential alternative approach to preventing lead-induced kidney damage.

1. **MATERIALS AND METHODS**

**2.1 Materials**

**2.1.1 Animals**

This study involved 25 male adult Wistar rats, each weighing between 135 and 197 grams. The animals were sourced from the Department of Physiology’s animal farm at Nnamdi Azikiwe University and were given one week to acclimate, with unrestricted access to food and water. A veterinarian confirmed the animals’ health before and after transport to the research facility. At the facility, the rats were housed in spacious, comfortable cages under a 12-hour light/dark cycle, with their health carefully monitored throughout the study. All procedures adhered strictly to the ethical guidelines set by the Animal Research Ethics Committee of Nnamdi Azikiwe University.

**2.1.2 Purchase of Feed and *Garcinia kola* seeds**

*Garcinia kola* seeds were obtained from Nkwo Market in Nnewi, Anambra State, and were identified and authenticated by a botanist from the Department of Botany at Nnamdi Azikiwe University. The rats were fed Top Feeds Grower’s Mash Super-Deluxe animal feed, manufactured by Eastern Premier Feed Mills Ltd, a branch of Premier Feeds Mills Co. Ltd, located in Plateau State, Nigeria.

**2.2 Methods**

**2.2.1 Determination of LD50**

Toxicity assessment was employed to establish the median toxicity of lead acetate to the animals. The Organization for Economic Co-operation and Development (OECD) Guidelines for the Testing of Chemicals provide standardized methods for determining acute and chronic toxicity, including lethal dose determination, which can help define the drug's safe starting dose. The study employed OECD 425 – the Up-and-Down Procedure. This procedure sequentially administers doses based on the outcome of previous administrations, allowing for a more refined LD50 estimate with minimal animal use [12].

**2.2.2 Preparation of *Garcinia kola* for oral administration**

The process of preparing *Garcinia kola* seeds for oral administration to Wistar rats involved cleaning, drying, grinding, and dosing according to established protocols in experimental pharmacology and toxicology studies [10, 13-16].

Fresh *Garcinia kola* seeds were collected and cleaned thoroughly with distilled water to remove any dirt or contaminants on the surface. The seeds were then air-dried under shade at room temperature until they are completely dry. This helped to prevent fungal growth and reduced moisture content, making the seeds easier to grind. The dried seeds were ground into a fine powder using a laboratory mill or grinder. The powder form increased the surface area, allowing for more consistent dosing and easier administration to rats. The powder was suspended in distilled water to ensure uniform dosing. The prepared suspension was administered orally.

**2.2.3 Experimental Protocol**

The rats were weighed before the start of the experiment using a weighing scale with a 6000g capacity (model WT6000GT, manufactured by Want Balance Instrument Company Limited, China). Throughout the acclimatization and experimental periods, the rats were provided ad libitum with standard pelleted feed and clean tap water. The cages and environment were cleaned and disinfected daily.

The rats were divided into five groups, each containing five rats, and housed in four large, mesh cages. All animals received food and water. Group A served as the negative (baseline) control group, receiving only feed and water with no lead or treatment. Group B served as the positive control group, receiving 60 mg/kg of lead acetate for 14 days to induce toxicity without any treatment, thus establishing the effect of lead exposure alone. Groups C, D, and E were treatment groups, all receiving 60 mg/kg of lead acetate for 14 days to induce toxicity, followed by oral administration of aqueous *Garcinia kola* extract for 21 days at doses of 150 mg/kg, 300 mg/kg, and 600 mg/kg, respectively. Lead administration for groups B to E lasted 14 days, while the extract was given orally to groups C to E for 21 days.

**2.2.4 Animal Euthanasia**

The euthanasia of the Wistar rats adhered strictly to Nnamdi Azikiwe University animal welfare regulations, ethical considerations, and guidelines which conform with established protocols in experimental studies involving Wistar rats [17].

Animals were euthanized using inhalant anaesthesia overdose (CO₂ gas). The gas flow rate displaced 10–30% of the chamber volume per minute to avoid distress. The animal was monitored for unconsciousness, which was followed by euthanasia confirmation after complete cessation of respiratory and cardiac function. Euthanasia was confirmed by assessing the absence of heartbeat, respiration, and reflexes (e.g., corneal reflexes). The carcasses of the Wistar rats were disposed of following institutional biosafety protocols.

**2.2.5 Blood collection**

Blood samples were collected via ocular puncture into plastic plain tubes. The blood samples were allowed to stand for 30 mins to ensure complete clotting. The clotted blood sample was centrifuged (using 800D Electric Centrifuge Machine with 4000RPM W/ 6X20Ml Rotor capacity) at 2500rpm (rotary per minutes) for 10 min and clear serum samples were aspirated off and stored frozen at -20C until required for biochemical analysis.

**2.2.6 Kidney Function Test**

The biochemical parameters analysed in the serum samples included urea, creatinine, and uric acid. These parameters were selected because they are widely accepted indicators of renal function. Urea and creatinine are standard markers of glomerular filtration and overall kidney performance, with elevated levels indicating impaired renal clearance and nephron dysfunction. Creatinine is a reliable marker of glomerular filtration rate (GFR), while urea reflects both renal and hepatic contributions to nitrogen waste elimination. Uric acid was included because it serves as an indicator of oxidative stress and renal tubular handling of purine metabolism, which are often disrupted in lead-induced nephrotoxicity. The combined analysis of these biomarkers provides a more comprehensive evaluation of renal functional integrity in toxicological models. The kidney function test was carried out according to established protocols in rat experimental studies [18,19].

**2.3 Statistical Analysis**

Data in this study were analysed using IBM’s Statistical Package for Social Sciences (SPSS), version 25, with a 95% confidence level for hypothesis testing. Both descriptive and inferential analyses were performed, with results presented as mean and standard error of the mean (SEM). Serum urea, serum creatinine, serum uric acid, and organ relative weight were evaluated using one-way ANOVA followed by a post hoc LSD (least significant difference) test, while body weight was assessed using the student’s independent t-test. Statistical significance was set at *P* ≤ .05.

**2.4 Duration of the study**

The study spanned a total of 11 weeks, with a two-week acclimatization period for the rats, followed by a five-week experimental phase. Data analysis was conducted over the final four weeks.

1. **RESULTS AND DISCUSSION**

**3.1 Morphological assessment**

During the acclimatization period, all rats appeared healthy, with smooth, well-aligned fur, pink eyes, normal skin tone, and increased body size. However, after 14 days of Lead acetate administration, the rats exhibited raised fur and a reduction in body weight. Following the administration of *Garcinia kola*, their fur returned to a smooth appearance, and the animals began to regain weight.

At the end of the experiment, dissection of the kidneys in all groups revealed normal morphology, characterized by a typical brownish-red coloration and bean-like shape, with no observable discoloration.

The analysis of body weight changes (difference between final and initial body weights) in Wistar rats revealed no significant differences in Groups A, D, and E, while Groups B and C exhibited statistically significant changes (Table 1).

The assessment of relative kidney weight indicated a statistically significant difference between the control group and Group B, whereas no significant differences were observed between the control group and Groups C through E (Table 2).

The results of this study indicate that exposure to lead acetate has significant detrimental effects on Wistar rats, which were partially mitigated by treatment with *Garcinia kola* extract. Notably, rats in group B (exposed only to lead acetate) exhibited signs of lead toxicity, as evidenced by their reduced body weight, altered fur appearance, and biochemical disturbances such as elevated creatinine and altered urea and uric acid levels. These findings align with prior research indicating that lead exposure can cause oxidative stress and impair renal function, leading to increased creatinine and urea levels, and disturbances in other metabolic processes [20]. The reduction in body weight and changes in fur observed here are also consistent with typical lead toxicity symptoms in animal models [21].

The administration of *Garcinia kola* extract in groups C, D, and E appeared to mitigate many of these adverse effects. Group C, which received the lowest dose of *Garcinia kola* (150 mg/kg), demonstrated some improvements but still showed statistically significant changes in body weight and less pronounced reductions in urea levels compared to the higher-dose groups (D and E). This suggests a dose-dependent effect, where higher doses of *Garcinia kola* (300 mg/kg and 600 mg/kg) more effectively reversed the biochemical alterations induced by lead exposure. The extract's antioxidant properties, as shown in previous studies, contributed to these protective effects by counteracting lead-induced oxidative stress and restoring renal function [9,22].

**3.2 Result of kidney function test**

The analysis revealed a statistically significant increase in creatinine levels in group B compared to group A (*P* = .001), while groups C, D, and E demonstrated a significant reduction in creatinine levels relative to group B (*P* = .001, *P* = .001, and *P* = .011, respectively) (Table 3).

In terms of urea levels, group B exhibited a non-significant increase compared to group A (*P* = .101). Group C showed a non-significant decrease (*P* = .067), whereas groups D and E demonstrated a statistically significant reduction in urea levels compared to group B (*P* = .035 and *P* = .047, respectively) (Table 3).

For uric acid, group B showed a statistically significant decrease relative to group A (*P* = .001). In contrast, groups C, D, and E had a significant increase in uric acid levels compared to group B, each with *P* = .001 (Table 3).

The significant reduction in creatinine levels in groups C, D, and E compared to group B suggests that *Garcinia kola* extract helped to restore kidney function. This outcome supports prior findings that antioxidant-rich natural extracts can lower creatinine and support kidney health after exposure to toxins [23]. Similarly, while urea levels were not significantly different in group C compared to group B, groups D and E showed significant decreases, further supporting a dose-dependent therapeutic effect.

The observed changes in uric acid levels reveal another dimension of the *Garcinia kola* extract’s protective effects. While group B experienced a significant decline in uric acid compared to the control, groups C, D, and E demonstrated significantly higher uric acid levels post-treatment, aligning them closer to the control group. These changes might reflect *Garcinia kola's* role in normalizing purine metabolism, an effect noted in studies of plant-based antioxidants in toxin-induced metabolic dysregulation [24].

**Mechanism of Garcinia kola’s Nephroprotective Activity**

The protective role of *Garcinia kola* in lead-induced nephrotoxicity is theorized to involve multiple biochemical pathways. Lead generates reactive oxygen species (ROS), which cause lipid peroxidation, DNA damage, and mitochondrial dysfunction in renal cells [19]. The biflavonoids and other polyphenolic compounds in *Garcinia kola* act as potent antioxidants by donating hydrogen atoms to neutralize ROS, upregulating endogenous antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), and stabilizing cellular membranes [9]. Additionally, *Garcinia kola* may modulate nuclear factor erythroid 2–related factor 2 (Nrf2) signalling, a key regulator of antioxidant response, thereby enhancing cellular resilience against oxidative damage [25]. It may also inhibit pro-inflammatory cytokines like TNF-α and IL-6 through downregulation of NF-κB signalling, reducing inflammation-driven tubular injury and fibrosis [9,10]. These mechanisms collectively help preserve nephron integrity and promote renal repair following toxic insults.

1. **CONCLUSIONS**

This study underscores the potential of *Garcinia kola* seed extract to mitigate the toxic effects of lead acetate on renal function and general health in Wistar rats. The findings demonstrate that *Garcinia kola*, particularly at higher doses, can significantly reverse lead-induced alterations in body weight, kidney weight, creatinine, urea, and uric acid levels. The improvement in body weight and fur condition further supports its restorative impact on health following lead exposure. Thus, *Garcinia kola* seed extract may serve as a therapeutic option for managing lead toxicity, possibly due to its antioxidant properties, which protect renal and metabolic functions.

Based on these findings, further studies are recommended to explore the specific mechanisms through which *Garcinia kola* exerts its protective effects, particularly in modulating oxidative stress pathways. Additionally, it would be beneficial to conduct long-term studies to evaluate the efficacy and safety of *Garcinia kola* in chronic lead exposure models. Clinical trials may also be considered to assess the potential applicability of *Garcinia kola* as a supplement for individuals at risk of lead exposure. Standardized dosing guidelines should be developed to optimize therapeutic outcomes, ensuring effective doses while minimizing potential side effects.

**Competing interests**

Authors have declared that no competing interests exist.

**Authors’ contribution**

This work was carried out in collaboration of all authors; and all authors read and approved the final manuscript. Author Darlington Nnamdi Onyejike (DNO) designed the study, carried out the data analysis and wrote the first draft of the manuscript. Author Uju Blessing Nwafor (UBN) carried out the experiment, managed the animals, managed the literature searches and curated the data. Author Somadina Nnamdi Okeke (SNO) conceptualized the study, wrote the experimental protocol, supervised the experiment, reviewed the draft.

**Consent**

Authors enlisted in this manuscript have given full consent for this draft article to be submitted to the Asian Journal of Current Research.

**Ethical approval**

The ethical approval was obtained from the Nnamdi Azikiwe University Animal Research Ethics Committee. The reference number is NAU/AREC/2023/580 dated 5th of August 2023.

**Disclaimer (Artificial intelligence)**

Authors hereby declare that generative AI technology such as Large Language Models has been used during the editing of the manuscript to improve language and readability. ChatGPT (GPT-4o mini) was used.

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**TABLES**

**Table 1: Result of body Weight Analysis**

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| Groups | Initial weight (g)  MEAN±SEM | Final weight (g)  MEAN±SEM | BWC (g) | *P*-value | T-value |
| Group A | 134.67±11.89 | 212.67±7.45 | 78.00 | 0.056 a | -4.044 |
| Group B | 183.00±6.63 | 167.00±7.04 | -16.00 | 0.020\* | 3.761 |
| Group C | 189.20±3.30 | 219.20±5.99 | 30.00 | 0.011\* | -4.497 |
| Group D | 185.00±10.11 | 194.40±39.37 | 9.40 | 0.665 a | 0.466 |
| Group E | 189.80±6.65 | 191.20±6.65 | 1.40 | 0.910 a | -0.121 |

Data was analyzed using paired t-test and values were considered significant at *P ≤ .05*.

SEM: Standard error of mean, BWC: Body weight change, \*: significant, a: non-significant.

**Table 2: Result of Relative Weight of the Kidney**

|  |  |  |
| --- | --- | --- |
| Groups | Relative kidney weight (g) | *P*-value |
|  | MEAN±SEM |  |
| Group A | 0.27±0.04 |  |
| Group B | 0.40±0.04\* | 0.023 |
| Group C | 0.28±0.02 \* | 0.018 |
| Group D | 0.37±0.03a | 0.484 |
| Group E | 0.32±0.02a | 0.099 |
| F-value | 3.03 |  |

Data was analyzed using ANOVA followed by post Hoc LSD comparison and values were considered significant at *P ≤ .05*.

SEM: Standard error of mean, \*: significant, a: non-significant.

**Table 3: Result of kidney function test**

|  |  |  |  |
| --- | --- | --- | --- |
| Groups | Creatinine level (mg/dl) | Urea level (mg/dl) | Uric acid (mg/dl) |
|  | MEAN±SEM | MEAN±SEM | MEAN±SEM |
| Group A | 0.69±0.02 | 111.64±5.66 | 8.43±6.26 |
| Group B | 3.18±0.49\* | 129.78±2.34 a | 4.95±1.03\* |
| Group C | 1.09±0.29\* | 109.16±8.71 a | 6.46±0.94 \* |
| Group D | 0.77±0.10\* | 105.23±8.76\* | 9.53±0.05 \* |
| Group E | 0.82±0.15\* | 107.04±7.85\* | 9.82±0.04 \* |
| F-value | 14.94 | 1.95 | 10.25 |

Data was analyzed using ANOVA followed by post Hoc LSD comparison and values were considered significant at *P ≤ .05*.

SEM: Standard error of mean, \*: significant, a: non-significant.