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Biochemical and histopathological effects of *Moringa oleifera* aqueous leaf extract on the kidney function in male Wistar rats

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Abstract

Moringa oleifera, a plant known for its nutritional and medicinal properties, has gained popularity in traditional and modern medicine. This study aimed to evaluate the effects of *Moringa oleifera* leaf extract on the histological and biochemical components of the kidney in male Wistar rats (*Rattus norvegicus*), hypothesizing that the extract may induce organ toxicity. Forty-eight rats were divided into four groups, receiving varying doses of the extract (1000, 2000, and 3000 mg/kg) for three weeks. Biochemical parameters, including urea and creatinine, were assessed, alongside histological examinations of kidney tissues. Statistical analysis was performed using Student's t-test, with significance set at $p < 0.05$. Results indicated no significant differences in biochemical parameters across all groups compared to controls ($p > 0.05$), suggesting that *Moringa oleifera* leaf extract does not adversely affect kidney function. Histological evaluations revealed normal architecture in all groups, with no visible lesions or damage, supporting the biochemical findings and the percentage yield of the extract was determined to be 11.7%. However, the observed hyperplasia in the renal corpuscle at the 22nd day indicates a regenerative response, which may reflect the extract's potential to promote cellular repair and regeneration. The results suggest that *Moringa oleifera* may be a valuable addition to dietary and therapeutic regimens, particularly in regions where it is traditionally used for its health benefits. Future studies should explore the mechanisms underlying the regenerative effects observed and further investigate the extract's potential therapeutic applications in human health.

Keywords: *Moringa oleifera*; Kidney function; Histology; Biochemical analysis; Wistar rats

1. Introduction

Moringa oleifera, commonly known as the drumstick tree, is a plant of significant socio-economic importance due to its extensive nutritional, pharmacological, and industrial applications (1) Traditionally utilized in various cultures for its medicinal properties, Moringa has garnered attention in modern research for its potential therapeutic benefits, including its role in treating a range of ailments such as inflammation, diabetes, and infections (2,3). Despite its widespread use, there is a notable gap in scientific understanding regarding the safety and effects of *Moringa oleifera* leaf extract on vital organs, particularly the kidneys, which are crucial for waste excretion (4). The primary objective of this study is to evaluate the histological and biochemical effects of *Moringa oleifera* leaf extract on the kidneys of Wistar rats. This investigation is driven by the hypothesis that the leaf extract may exert both beneficial and potentially harmful

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effects on this organ, depending on dosage and duration of administration. Previous studies have indicated that the chemical composition of Moringa extracts can vary significantly based on geographical factors, which may influence their pharmacological effects (2). Therefore, this study aims to provide localized data on the extract's safety profile in our specific context, particularly about its impact on kidney function.

Understanding the impact of *Moringa oleifera* on kidney function is essential for guiding its use in traditional medicine and ensuring the safety of consumers. By employing histological and biochemical analyses, this research seeks to contribute valuable insights into the organ-specific effects of *Moringa oleifera*, thereby informing both practitioners and users about its safe application in herbal therapies. The findings from this study are expected to enhance the scientific basis for the use of Moringa in herbal medicine and promote safer practices among traditional herbal practitioners.

2. Materials and methods

2.1. Study Design

This study employed a randomized controlled experimental design to evaluate the effects of *Moringa oleifera* leaf extract on the histological and biochemical parameters of the kidney in Wistar rats (*Rattus norvegicus*). The study was conducted over a period of three weeks, with assessments made at multiple time points to determine both acute and chronic effects of the extract.

2.2. Study Location

The study was conducted in the Department of Medical Laboratory Science Teaching Laboratory, Imo State University, Owerri, Imo State, Nigeria.

2.3. Procurement of Plant Material

Fresh leaves of *Moringa oleifera* were collected from the botanical garden of Imo State University, Owerri, Nigeria, and authenticated by a plant botanist.

2.4. Plant Extraction Preparation

The leaves were washed, dried, and ground into a powder. The extraction was performed using distilled water, and the resulting extract was concentrated using a rotary evaporator. The yield of the extract was calculated to be 11.7%.

2.5. Determination of LD50 of *Moringa oleifera* Leaf Extract Lorke, (5)

The LD50 of *Moringa oleifera* leaf extract was determined using Lorke's method (5) whereby no mortality or behavioral change was observed after 24 hours even at the highest dose of 5900mg/kg of the leaf extract as observed in the phase 2 treatment phase done in Lorke's method of determining LD50 of a substance.

2.6. Experimental Animals

Forty-eight (48) healthy male Wistar rats (200-250g) were obtained from the Animal House of the College of Medicine and Health Sciences, Imo State University. The rats were acclimatized for one week before the experiment.

2.7. Setting

The research was conducted at the Animal Farm of Imo State University, Owerri, Nigeria. The study utilized the facilities available for animal housing, extraction, and biochemical analysis.

2.8. Sampling Techniques

The rats were randomly assigned to four groups (A, B, C, and D) with 12 rats in each group. Group A received 1000 mg/kg of the extract, Group B received 2000 mg/kg, Group C received 3000 mg/kg, and Group D served as the control group with no treatment. Randomization was achieved using a simple random sampling method.

2.9. Procedure Interventions

- **Preparation of Extract**: Fresh *Moringa oleifera* leaves (5 kg) were collected, washed, dried, and ground into a fine powder. The powder was then extracted using distilled water at 60°C for 48 hours. The extract was filtered and concentrated using a rotary evaporator to yield a semisolid residue.

- **Animal Acclimatization**: The rats were acclimatized for one week in a controlled environment with free access to sterilized tap water and dry rat pellets.
- **Administration of Extract**: The extract was administered orally to the rats once daily for three weeks using a cannula attached to a graduated syringe. The control group received only water and rat pellets.
- **Sample Collection:** Blood samples were collected via cardiac puncture at the end of the 8th, 15th, and 22nd days of extract administration for biochemical analysis. The rats were then sacrificed, and the kidneys were dissected and fixed in neutral buffered formalin for histological examination.
- **Histological Processing**: The kidney tissues were processed using standard histological techniques, including dehydration in graded alcohols, clearing in xylene, and embedding in paraffin wax. Sections were cut at 5 microns and stained with Hematoxylin and Eosin.
- **Biochemical Analysis**: The kidney function test, such as Urea and creatinine, was carried out on a collected blood sample from the experimental rats.

2.10. Statistical Analysis

Data were analyzed using descriptive statistics, and results were expressed as mean ± standard deviation. Comparisons between groups were performed using Student's t-test, with a significance level set at $p < 0.05$. Statistical analyses were conducted using appropriate software.

3. Results

3.1. General Observations

The mean body weights of the rats were recorded at different time points during the study. No significant changes in body weight were observed among the treatment groups compared to the control.

3.2. Histological Findings

Histological examination of kidney tissues from the control group revealed normal architecture. Similarly, tissues from all treatment groups (1000 mg/kg, 2000 mg/kg, and 3000 mg/kg) exhibited no pathological lesions in all groups at different doses and at different duration except for animals that were given high dose of *Moringa oleifera* leaf extract at 3000mg/kg at the 21st day (three weeks) of experiment.

3.3. Histological Findings of Control and Experimental Groups A, B and C

3.3.1. Control Group

Histological sections of kidneys from control group showed normal histological features which served as a basis for comparing with experiment groups.

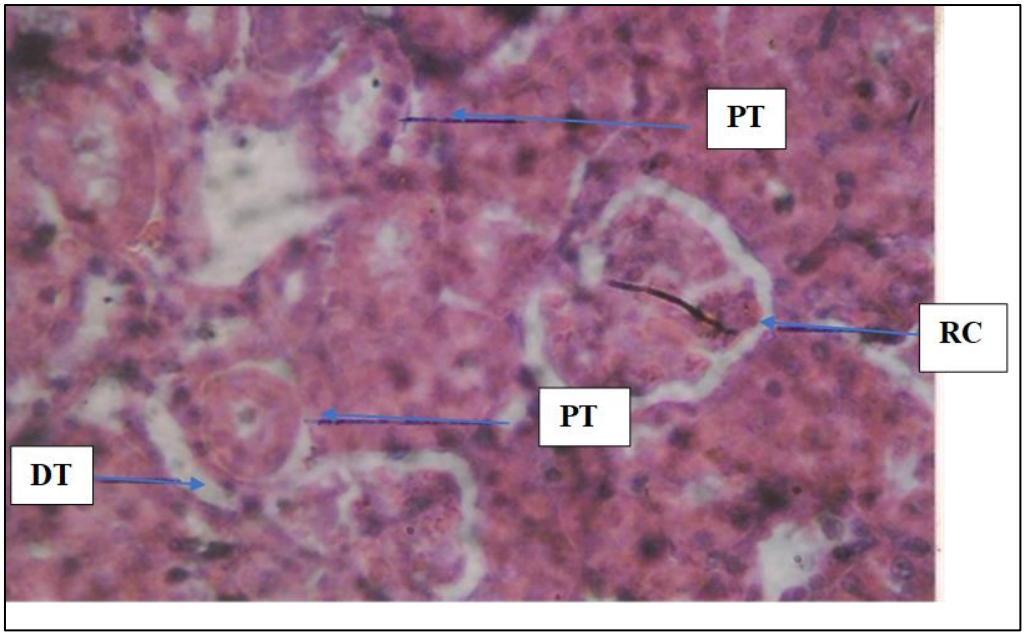

Figure 1 Group D. Normal Control given rat pellet and water only at day 21 of the experiment. Shows normal proximal convoluted tubules (PT), normal glomerulus (NG), renal corpuscles (RC), and distal convoluted tubule(DT), all showing normal histoarchitecture of the kidney of Wistar rat. Magnification. 400 x. H&E stain

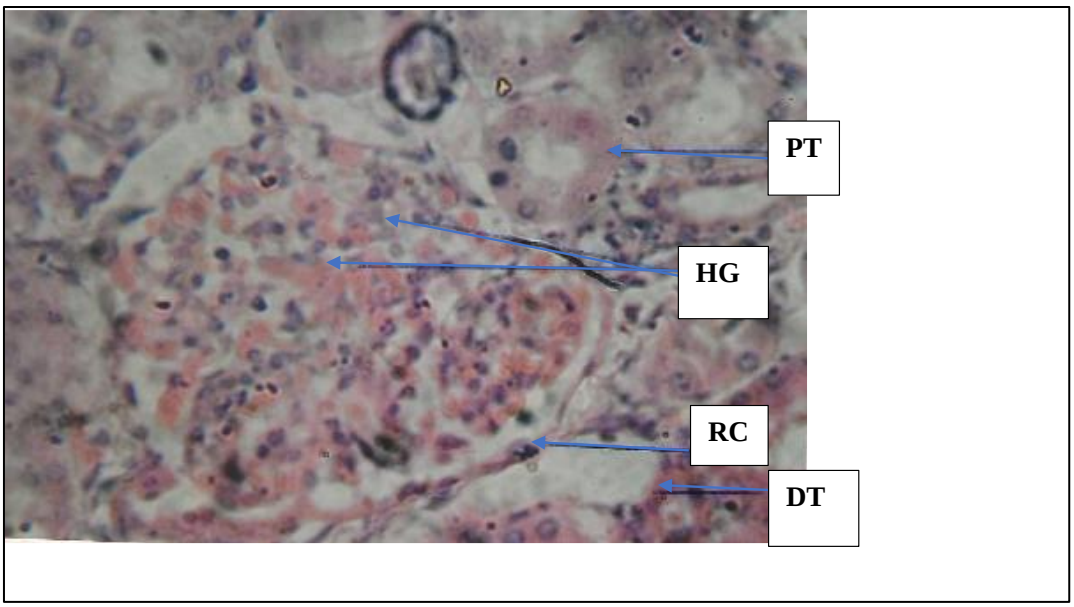

Figure 2 Group C given 3000mg/kg *Moringa oleifera* leaf extract. Wistar rat sacrificed at 21st day of the experiment. Histopathological findings show severe hyperplastic glomeruli (HG) in the renal corpuscles (RC), normal cuboidal epithelium lining the proximal convoluted tubules (PT), and distal convoluted tubules (DT) showing normal histoarchitecture. Magnification. 400 x. H&E stain

3.4. Biochemical Analysis

Biochemical assays showed no significant differences in kidney function parameters among the treatment groups compared to the control group (P>0.05). This includes measurements of urea, creatinine. The results are summarized as thus:

Group A: No significant changes were observed in urea (32.0 mg/dl), creatinine (0.50 mg/dl), compared to the control group (P > 0.05).

Group B: Similar findings were noted, with urea (31.8 mg/dl), creatinine (0.56 mg/dl), showing no significant differences ($P > 0.05$).Again, no significant differences were found in the biochemical parameters, with urea (31.8 mg/dl), creatinine (0.58 mg/dl), compared to the control group ($P > 0.05$).

3.5. Time-Dependent Effects

To assess whether the effects of Moringa oleifera leaf extract were time-dependent, comparisons were made among the three different durations (1 week, 2 weeks, and 3 weeks) for each group. No significant differences were observed across the time points for any of the biochemical parameters in Groups A, B, and C ($P > 0.05$).

Summarily, the administration of *Moringa oleifera* leaf extract at varying doses did not result in significant changes in body weight, histological architecture of the kidneys, or biochemical markers of organ function. The extract appears to be safe for consumption, with no evidence of organ toxicity observed in the study.

4. Discussion

The present study aimed to evaluate the effects of *Moringa oleifera* leaf extract on the histological and biochemical components of the kidney in Wistar rats. The findings provide valuable insights into the safety and potential therapeutic applications of *Moringa oleifera*, a plant recognized for its rich nutritional and medicinal properties. The findings also indicates that the administration of *Moringa oleifera* leaf extract does not produce significant organ toxicity, as evidenced by the absence of pathological lesions in histological sections and stable biochemical parameters across different dosages and time frames. This discussion will contextualize these findings within the existing literature, highlight their significance, address limitations, and propose recommendations for future research.

This study observed significant variations in body weight among the different groups of rats following the administration of *Moringa oleifera* leaf extract. Notably, Group B, which received a moderate dose of 2000 mg/kg, exhibited a significant decrease in weight compared to the control group, while Groups A (1000 mg/kg) and C (3000 mg/kg) showed weight increases. This observation aligns with the findings of Okorochi *et al.* (6), who reported significant differences in weight gain among control groups. The weight loss in Group B may suggest a dose-dependent effect of the extract, potentially indicating metabolic alterations or a stress response to the extract at this dosage. Conversely, the weight gain in Groups A and C may imply that lower and higher doses could stimulate appetite or metabolic processes favorably, as suggested by Ayotunde, (7) who noted weight gain in animals treated with *Moringa oleifera*.

Interestingly, the weight loss observed in all experimental groups at the 15th and 22nd days of administration could indicate a time-dependent effect, where the initial positive response to the extract is followed by a decline in weight. This finding contrasts with the study by Ayotunde, (7) which reported adverse effects associated with high doses of *Moringa oleifera*, suggesting that the effects of the extract may vary based on dosage and duration of administration. Furthermore, histological examination of the kidney tissues revealed no significant pathological lesions across all experimental groups at various time points (8th, 15th, and 22nd days). The absence of visible lesions in suggests that *Moringa oleifera* leaf extract does not induce toxicity at the administered doses, corroborating findings from previous studies (6,7). The observed hyperplasia in the renal corpuscle at the 22nd day indicates a regenerative response, which may reflect the extract's potential to promote cellular repair and regeneration, a finding that aligns with the work of (8,9). In addition, the findings of this study indicate that *Moringa oleifera* leaf extract does not produce significant histological or biochemical alterations in the kidneys of Wistar rats at the tested doses. These results align with previous studies suggesting the safety of *Moringa oleifera* extracts (1). The lack of observed toxicity supports the traditional use of *Moringa oleifera* in herbal medicine, although further studies are warranted to explore its long-term effects and potential therapeutic applications. The histological results are particularly significant as they provide a basis for the safety of *Moringa oleifera* in traditional and modern medicinal practices. The lack of pathological changes in the kidney tissues suggests that the extract can be consumed without adverse effects on these vital organs, supporting its use in herbal medicine.

In addition, biochemical assessments of kidney function markers (Urea and Creatinine) showed no significant changes across the experimental groups compared to the control. This is indicative of preserved kidney function, suggesting that *Moringa oleifera* leaf extract does not adversely affect these organs at the tested doses. The stability of biochemical parameters throughout the study duration indicates that the extract's effects are not time-dependent, as supported by the findings of Ezeamuzie *et al.*((10), which also demonstrated the safety of *Moringa oleifera* in biochemical assays.

Furthermore, the study's findings suggest that the effects of *Moringa oleifera* leaf extract are not significantly time-dependent, as no adverse effects were observed across different durations of administration. This is a critical observation, as it implies that the extract can be safely administered over extended periods without compromising organ function. Furthermore, the lack of significant differences in biochemical parameters among the various dosages (1000 mg/kg, 2000 mg/kg, and 3000 mg/kg) indicates that the extract's safety profile remains consistent regardless of the dose, which is an essential consideration for its potential therapeutic applications.

The results challenge the notion that higher doses of herbal extracts are always more effective or beneficial, as seen in Group B's weight loss despite being administered a moderate dose. This finding underscores the importance of dosage optimization in herbal medicine, as excessive amounts may lead to unintended consequences. Nevertheless, the significance of these findings lies in their implications for the use of *Moringa oleifera* as a dietary supplement and potential therapeutic agent. The absence of organ toxicity suggests that *Moringa oleifera* can be safely consumed within the tested dosage range, which is particularly relevant in regions where this plant is traditionally used for its nutritional and medicinal properties. The findings also support the notion that *Moringa oleifera* may serve as a functional food with health benefits, including potential nephroprotective effects, as suggested by the regenerative changes observed in the renal corpuscle. Furthermore, the study contributes to the growing body of literature on the pharmacological properties of *Moringa oleifera*, reinforcing its status as a "superfood" with a rich profile of vitamins, minerals, and bioactive compounds (1,11). This research underscores the need for further exploration of the mechanisms underlying the protective effects of *Moringa oleifera* on kidney function. In addition to our findings, despite the promising findings, this study has several limitations. Firstly, the sample size was relatively small, which may limit the generalizability of the results. Additionally, the study focused solely on male albino Wistar rats, and the effects of *Moringa oleifera* may differ in female rats or other animal models. Furthermore, the study did not explore the long-term effects of *Moringa oleifera* consumption, which is crucial for understanding its safety and efficacy as a dietary supplement. Another limitation is the lack of mechanistic studies to elucidate the pathways through which *Moringa oleifera* exerts its protective effects. Future studies should aim to investigate the molecular mechanisms involved in the nephroprotective effects of *Moringa oleifera*, including its antioxidant properties and interactions with metabolic pathways.

Summarily, the findings of this research on the effects of *Moringa oleifera* leaf extract on the histological and biochemical components of the kidney in Wistar rats lead to several important conclusions. Firstly, the study demonstrated that *Moringa oleifera* leaf extract does not exhibit significant organ toxicity at the administered doses of 1000 mg/kg, 2000 mg/kg, and 3000 mg/kg, as evidenced by the absence of pathological lesions in kidney tissues across all treatment groups. Biochemical analyses further corroborated these findings, showing no significant alterations in kidney function markers (urea and creatinine) when compared to control groups. Moreover, the research indicated that the effects of *Moringa oleifera* leaf extract are not time-dependent, as there were no significant changes in the biochemical parameters across the different time points (1 week, 2 weeks, and 3 weeks) of administration. This suggests that the extract can be safely consumed without concern for cumulative toxicity over time. The relevance of these findings is substantial, particularly in the context of traditional medicine and dietary supplementation. *Moringa oleifera* is widely recognized for its nutritional and medicinal properties, and this study provides scientific validation of its safety for consumption. The lack of adverse effects on vital organs such as the kidneys supports its potential use as a natural remedy and dietary supplement, particularly in regions where it is traditionally used for its health benefits.

5. Conclusion

In conclusion, *Moringa oleifera* leaf extract can be recommended for consumption within the tested dosage range, as it appears to be a safe and beneficial addition to diets, with implications for enhancing nutritional intake and promoting health without the risk of organ toxicity. Further research could explore the long-term effects and potential therapeutic applications of this versatile plant.

Recommendations for future research

Based on the findings and limitations of this study, several recommendations for future research can be made:

- **Long-term Studies:** Conduct long-term studies to assess the chronic effects of *Moringa oleifera* leaf extract on kidney function, including potential cumulative effects over extended periods.
- **Diverse Animal Models:** Investigate the effects of *Moringa oleifera* in various animal models, including both sexes and different species, to provide a comprehensive understanding of its safety and efficacy.
- **Mechanistic Studies**: Explore the underlying mechanisms of action of *Moringa oleifera*, particularly its antioxidant and anti-inflammatory properties, to better understand how it protects against organ toxicity.

- **Human Clinical Trials**: Initiate clinical trials to evaluate the safety and efficacy of *Moringa oleifera* in human populations, particularly in those with pre-existing kidney conditions.
- **Standardization of Extracts**: Standardize the extraction methods and dosages used in studies to facilitate comparisons across research and ensure consistent results.

In conclusion, the current study provides valuable insights into the safety profile of *Moringa oleifera* leaf extract, highlighting its potential as a beneficial dietary supplement. Further research is warranted to explore its therapeutic applications and elucidate the mechanisms behind its protective effects on kidney health.

Compliance with ethical standards

Disclosure of conflict of interest

The authors have not declared any conflict of interest.

Statement of ethical approval

The Institutional Animal Care and Use Committee of Imo State University obtained the study's ethical approval. All procedures involving animals were conducted by the guidelines for the care and use of laboratory animals. Informed consent was obtained for the use of animals in this research.

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