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21 DAYS EFFECTS OF ETHANOL ROOT EXTRACT OF *Moringa oleifera* Lam. ON KIDNEY AND LIVER FUNCTIONS IN WISTAR RATS

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ABSTRACT

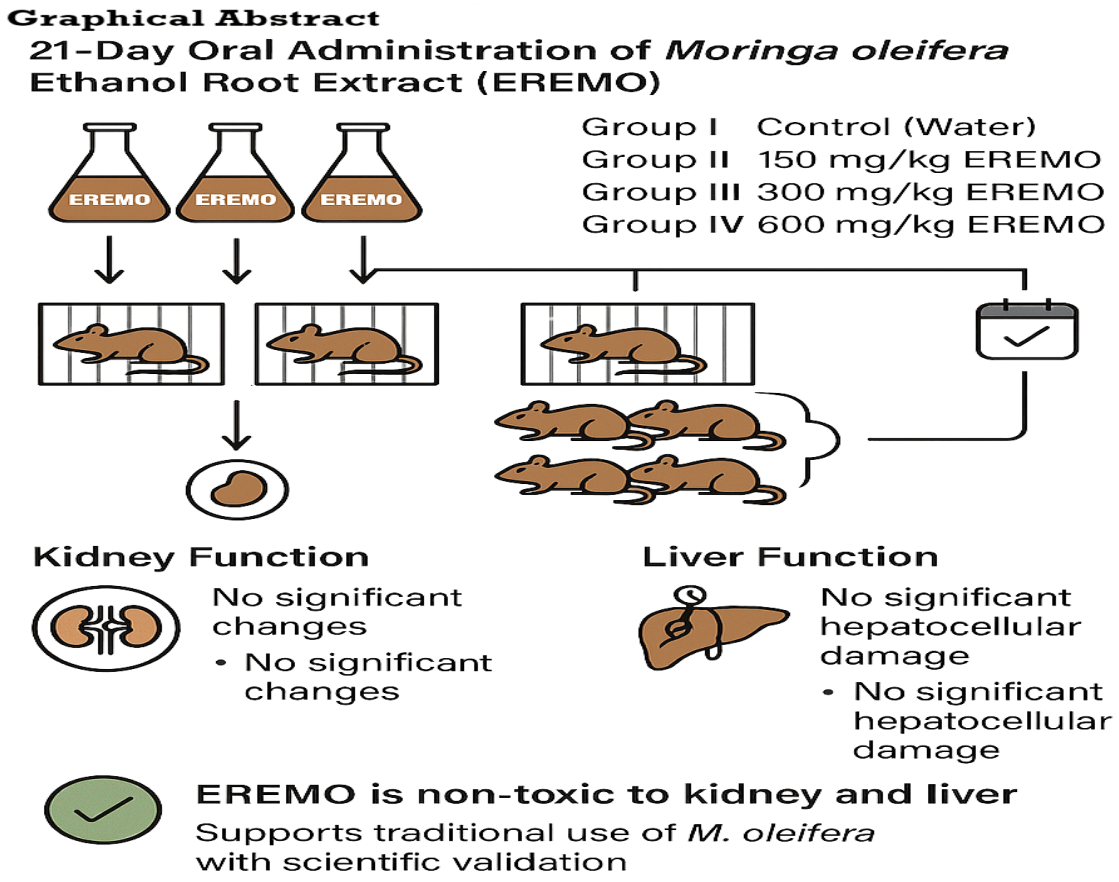
Moringa oleifera Lam. has been widely used in traditional medicine to manage a variety of ailments. Despite its reported medicinal benefits, concerns remain regarding its potential toxicity when used excessively or improperly. This study evaluated the subacute effects of ethanol root extract of *M. oleifera* (EREMO) on liver and kidney function in Wistar rats over a 21-day period.

A total of 600 g of powdered *M. oleifera* root was extracted using 99% ethanol. Sixteen adult Wistar rats were randomly divided into four groups (n = 4). Group I received distilled water (control), while Groups II, III, and IV were administered 150 mg/kg, 300 mg/kg, and 600 mg/kg of EREMO, respectively, via oral gavage for 21 days. Blood samples were collected after the experimental period for biochemical analyses.

Results showed no statistically significant differences ($P > 0.05$) in serum electrolyte concentrations (Na^+, K^+, Cl^-, HCO_3^-), urea, or creatinine levels among treated groups compared to the control. Similarly, liver enzyme activities (ALP, AST, ALT) and total and conjugated bilirubin levels did not differ significantly between control and treatment groups.

These findings indicate that EREMO did not exert nephrotoxic or hepatotoxic effects under the tested conditions. The outcomes support previous biosafety reports regarding *M. oleifera* extracts and suggest that the ethanol root extract is well-tolerated at doses up to 600 mg/kg over a 21-day period. Further studies are recommended to evaluate long-term safety and explore its therapeutic potential.

Keywords: *Moringa oleifera*; 21 days effects; ethanol root extract; Wistar rats; liver; kidney.



1.0 INTRODUCTION

Moringa oleifera Lam., a member of the family Moringaceae, is a fast-growing, drought-resistant tree widely cultivated across tropical and subtropical regions [2]. It is a deciduous, perennial, and dicotyledonous plant [19], native to the sub-Himalayan regions of northern India [24], and has gained significant agricultural and therapeutic importance in several regions, including Africa [11].

Often termed the "miracle tree," *M. oleifera* is notable for its broad pharmacological and nutritional utility. Virtually every part of the plant—root, bark, gum, leaves, fruits (pods), flowers, seeds, and seed oil—is used in indigenous medicine for treating various ailments, offering both therapeutic and nutritional value [2, 4].

Numerous pharmacological activities have been attributed to different parts and extracts of *M. oleifera*. These include antioxidant [16], antibacterial [15], antidiabetic [9], anticancer [14, 20], immunomodulatory [22], antiallergic [12], anti-inflammatory [6], antifungal [7], and hepatoprotective [5] effects. Despite these well-documented benefits, herbal medicines may also elicit adverse effects due to inappropriate usage, dose miscalculations, or long-term consumption. Therefore, preclinical toxicological assessments are essential to verify the safety of plant-derived therapies [3].

This study investigates the sub-acute (21-day) toxicity of ethanol root extract of *M. oleifera* (EREMO) by assessing its impact on hepatic and renal function biomarkers in male Wistar rats.

2.0 MATERIALS AND METHODS

Experimental Materials

The following materials were utilized in the experiment: wire-meshed plastic cages, gloves, nose masks, plastic buckets, cotton wool, masking tape, digital weighing balance, beaker, fine-mesh strainer, syringes, surgical scissors, hot air oven, measuring cylinder, mortar and pestle, oral gastric tube, glass jars, test kits, Gentian violet, chloroform, formalin, and 99.9% absolute ethanol.

Collection and Identification of Plant Materials

Fresh roots of *M. oleifera* were harvested from mature trees located in Obe quarters, Benin City, Edo State, Nigeria. Botanical identification and authentication were carried out at the Herbarium Unit, Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin. The specimen was

assigned a voucher number (UBH-M340), and identification was performed by Professor Akinnibosun Henry Adewale, a plant ecologist/taxonomist.

Extract Preparation

The collected roots were thoroughly washed, air-dried under shade, and pulverized into fine powder. A 600 g sample was macerated in 99.9% ethanol for 72 hours in a tightly sealed glass jar. The resulting extract was filtered using Whatman No. 1 filter paper and evaporated to a semi-solid paste using a hot air oven.

Preparation of Doses

Working solutions of EREMO at concentrations of 150, 300, and 600 mg/kg were prepared by dissolving measured quantities of the paste in appropriate volumes of distilled water.

Experimental Animals

Sixteen (16) male Wistar rats weighing between 157 g and 292 g were procured from the Animal Unit, Department of Biochemistry, Faculty of Life Sciences, University of Benin. They were housed in standard laboratory conditions with adequate ventilation, fed commercial grower mash, and provided water ad libitum. After a 7-day acclimatization period, the experiment commenced. Ethical clearance was obtained from the Phytomedicine Unit, Department of Plant Biology and Biotechnology, University of Benin (Approval No: LS28174).

Experimental Design

The animals were randomly distributed into four groups of four rats each. Group I (control) received only distilled water, while Groups II, III, and IV were orally administered 150, 300, and 600 mg/kg of EREMO daily, respectively, for 21 consecutive days using an oral cannula. At the end of the treatment period, rats were sacrificed under mild chloroform anesthesia, and blood samples were collected via cardiac puncture for biochemical analysis.

Biochemical Analysis

Blood samples were allowed to clot and centrifuged at 3,000 rpm for 10 minutes to obtain serum. Biochemical parameters indicative of kidney and liver function—including electrolytes, urea, creatinine, liver enzymes (ALP, AST, ALT), and bilirubin levels—were evaluated following established protocols [23].

Statistical Analysis

All data were analyzed using SPSS version 16.0. Results were expressed as Mean ± Standard Error of the Mean (SEM). Analysis of variance (ANOVA) followed by Tukey's post hoc test was used to determine statistically significant differences among groups. Significance was accepted at $P < 0.05$.

3.0 RESULTS AND DISCUSSION

Effect of EREMO on Kidney Function Parameters

Table 1 below presents the effect of 21-day oral administration of ethanol root extract of *Moringa oleifera* (EREMO) on renal function markers in Wistar rats. The measured parameters include serum electrolytes—sodium (Na^+), potassium (K^+), chloride (Cl^-), and bicarbonate (HCO_3^-)—as well as nitrogenous waste products—urea and creatinine. Across all treatment groups (150, 300, and 600 mg/kg body weight), there were **no statistically significant differences** ($P > 0.05$) in comparison with the control group.

Table 1: Effect of EREMO on the kidney function parameters of Wistar rats

Parameters	Control (Distilled H_2O)	EREMO 150 mg/kg	EREMO 300 mg/kg	EREMO 600 mg/kg
Na^+	139.33 ± 1.20[a]	140.67 ± 0.88[a]	140.33 ± 0.88[a]	142.00 ± 1.53[a]
K^+	6.33 ± 0.52[a]	6.67 ± 0.64[a]	6.33 ± 0.72[a]	5.80 ± 0.32[a]
Cl^-	101.33 ± 0.67[a]	103.00 ± 0.58[a]	101.67 ± 1.20[a]	102.00 ± 0.58[a]
HCO_3^-	22.00 ± 1.73[a]	20.00 ± 2.65[a]	23.00 ± 1.00[a]	23.00 ± 0.00[a]
Urea (mg/dL)	60.00 ± 3.61[a]	55.00 ± 2.89[a]	52.67 ± 2.73[a]	54.33 ± 4.70[a]
Creatinine (mg/dL)	1.37 ± 0.24[a]	1.23 ± 0.15[a]	1.40 ± 0.29[a]	1.37 ± 0.26[a]

Means with similar superscripts across a row are not significantly different ($P > 0.05$) based on Tukey's post-hoc mean separation. Values are expressed as Mean ± SEM.

The findings demonstrate that EREMO did not disrupt the electrolyte balance nor impair renal filtration capacity, as evidenced by unchanged levels of urea and creatinine. These findings are in alignment with the study by [17], which revealed no significant alteration in biochemical indices following EREMO administration in

female Wistar rats. Similarly, [8] reported no significant changes in serum bilirubin, creatinine, or urea levels after administering *Moringa oleifera* extracts, affirming the non-toxic nature of the plant at therapeutic doses.

The integrity of renal function is critical, and biochemical markers such as urea, creatinine, and electrolytes are important indicators of nephrotoxicity. Hence, the lack of significant deviations in these parameters suggests that EREMO, at doses up to 600 mg/kg body weight, does not exert any nephrotoxic effect after 21 days of sub-acute exposure. This finding is corroborated by [1], who previously reported that ethanol extracts of *Moringa oleifera* leaves showed no signs of acute or sub-acute toxicity, likely due to the absence of harmful alkaloids and the presence of antioxidant phytochemicals.

Serum biochemical assays are often employed as non-invasive tools for early detection of organ-specific toxicities [21]. Given the stability of kidney function markers observed in this study, it can be concluded that EREMO poses no risk to renal health under the study's dosing and duration regimen.

Effect of EREMO on Liver Function Parameters

Table 2 shows the liver function test results in Wistar rats treated with EREMO for 21 days. The hepatic biomarkers assessed include Alkaline Phosphatase (ALP), Alanine Aminotransferase (ALT), Aspartate Aminotransferase (AST), Total Bilirubin (TB), and Conjugated Bilirubin (CB). No significant differences ($P > 0.05$) were observed between the EREMO-treated and the control groups.

Table 2: Effect of EREMO on the liver function parameters of Wistar rats

Parameters	Control (Distilled H_2O)	EREMO 150 mg/kg	EREMO 300 mg/kg	EREMO 600 mg/kg
ALP (IU/L)	157.00 ± 9.45^{a}	180.00 ± 11.68^{a}	176.67 ± 22.84^{a}	149.00 ± 9.87^{a}
AST (IU/L)	61.33 ± 9.67^{ab}	91.00 ± 6.08^{ab}	99.00 ± 14.73^{b}	52.33 ± 7.54^{a}
ALT (IU/L)	29.00 ± 4.00^{a}	31.67 ± 2.03^{a}	43.67 ± 6.89^{a}	35.67 ± 4.37^{a}
TB (mg/dL)	0.50 ± 0.06^{a}	1.00 ± 0.20^{a}	0.67 ± 0.22^{a}	0.37 ± 0.33^{a}
CB (mg/dL)	0.17 ± 0.03^{a}	0.23 ± 0.03^{a}	0.20 ± 0.00^{a}	0.13 ± 0.03^{a}

Means with similar superscripts across a row are not significantly different ($P > 0.05$) based on Tukey's post-hoc mean separation. Values are expressed as Mean ± SEM.

The liver is an essential metabolic organ involved in detoxification, protein synthesis, glucose homeostasis, and red blood cell regulation [10]. Any elevation in liver enzyme levels—such as AST, ALT, or ALP—typically reflects hepatic injury or dysfunction. However, the current results indicate that EREMO does not significantly alter the levels of these enzymes, thereby signifying an absence of hepatocellular damage.

This is consistent with the findings of [23], who reported that methanol root extract of *Moringa oleifera* did not significantly affect liver enzyme levels in non-diabetic rats. Furthermore, unchanged bilirubin levels (both total and conjugated) in this study suggest that hepatic excretory function remains unaltered. These findings are also supported by [8], who concluded that *Moringa oleifera* extracts do not induce hepatotoxicity at conventional doses.

It is well established that elevated serum AST, ALT, ALP, and bilirubin can be indicators of liver diseases, muscular damage, hemolytic anaemia, or chronic alcohol consumption [13]. While [18] previously observed significant reductions ($P < 0.05$) in liver enzymes following treatment with *Moringa oleifera* seeds and leaves—suggesting their potential in treating hepatic disorders—the absence of such reductions in the current study implies that EREMO may not possess the same therapeutic effects. Thus, while EREMO appears safe and non-toxic to hepatic tissues, it may not be effective as a remedy for pre-existing hepatic abnormalities.

4.0. Conclusion

In summary, the findings from this 21-day sub-acute toxicity study indicate that oral administration of EREMO does not induce adverse effects on the kidney or liver of Wistar rats. The lack of significant changes in serum biochemical markers across all dose groups supports the conclusion that EREMO is **non-toxic** at the tested concentrations. These results substantiate earlier reports on the safety profile of *Moringa oleifera* extracts [1, 8, 17, 21, 23], highlighting the plant's potential for safe therapeutic application, albeit with limited evidence for curative effects on hepatic dysfunctions.

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