



Phytochemical Composition and Effect of Methanol Leaf Extract of *Eremomastax speciosa* on Liver and Kidney Function in Wistar Rats

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Abstract

This study evaluated the phytochemical composition and effects of methanol leaf extract of *Eremomastax speciosa* on selected liver and kidney function parameters in Wistar rats. Pulverized leaves were macerated in 99.8% methanol for 72 hours, filtered and concentrated via rotary evaporation at 40°C. The extracts were obtained using methanol. The phytochemical screening of the extracts was carried out using standard qualitative procedures. For the *in vivo* study, 16 adult female Wistar rats were divided into four groups (n = 4). Control group received normal saline, while groups 1 – 3 received incremental daily oral doses (500, 1000, and 1500 mg/kg) of the extract for 15 days. After the administration of the extract for fifteen (15) days, the rats were fasted overnight, and then sacrificed by anaesthetizing with chloroform. Blood was collected by cardiac puncture to obtain haemolysis free blood samples with the aid of syringes and needles and transferred into pre-labelled, sterile plain sample bottles. Phytochemical screening confirmed the presence of flavonoids, tannins, saponins, alkaloids, and cardiac glycosides. The extraction process yielded 82.02g of crude extract (17.45% w/w). There was no significant difference (P>0.05) in AST and ALP levels but high dosages of *Eremomastax speciosa* extracts significantly increase serum ALT concentration. Elevated total bilirubin was observed at moderate and high dosages while elevated direct bilirubin was recorded at high dosage. Medium dosage of extract significantly increased serum chlorine (Cl⁻) and bicarbonate (HCO₃⁻) concentrations. Serum creatinine was observed to drop significantly at low and moderate dosages. Significant increase in urea levels was observed at low and moderate dosages. The findings established the presence of phytochemical constituents and absence of observed acute toxicity at the tested limited dosage. Therefore, there is need for further pharmacological, toxicological, long term toxicity assessments and detailed histopathological examination.

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1.0 Introduction

Plants are the most common source of traditional medicines and their extracts have continued to gain recognition as a source of therapeutic agents in many parts of the world. They have been used individually and in combination in the treatment of different ailments by traditional medical healers for a very long time (Udofia et al., 2023b). The medicinal alkaloid, flavonoid and terpenoid content of plants makes plants the richest source of pharmaceuticals for conventional medical systems, nutraceuticals, food supplements, pharmaceutical intermediates, and chemical entities for synthetic drugs (Iba et al., 2022).

Liver function is critical to maintaining the body's metabolic processes, detoxification, and synthesis of important biomolecules (Offor et al., 2015). Disruptions in liver and kidney function are often precursors to a range of chronic conditions such as fatty liver disease, diabetes, and cardiovascular disorders (Odeja et al., 2019; Udofia et al., 2023a). Therefore, the exploration of natural substances with potential hepatoprotective has gained significant attention in pharmacological research (Oyagbemi & Odetola, 2010; Uffia et al., 2024).

The increasing prevalence of liver-related diseases and metabolic disorders highlights the need for safe and effective treatment options. While synthetic drugs are available, their associated side effects and cost often limit their widespread use. Natural products, such as plant extracts, offer a promising alternative due to their accessibility and perceived safety (Umoh et al., 2020; Uffia, et al., 2021). Among the several plants identified with such potential is *Eremomastax speciosa*. *Eremomastax speciosa* is a plant that is commonly used in traditional medicine in West Africa. The plant is known to possess several medicinal properties, including

anti-inflammatory, analgesic, antimicrobial, antifungal, and antioxidant activities (Siwe et al., 2015). Mboso et al., (2013) noted that *Eremomastax speciosa* in combination with other herbs have been in use by traditionalist in the local regions of Cross River and Akwa Ibom States to treat male and women with fertility problems. It is known commonly as “golden seal”; “African blood tonic plant”; local name, Edem Ididout, Ndana-edem (Erhabo et al., 2012; Onoja et al., 2017).

These plants have a variety of properties and various biological components that can be used to treat various diseases. However, harmful effects of plants are common clinical occurrence. Medicinal plants in traditional clinical practices are as of now not appropriately checked, resulting in an expanded danger of toxicity because of the wrong administration, particularly of those plants that are dangerous at low doses (Uffia et al., 2025; Udo et al., 2023).

Observations over time have shown that majority of researchers have concentrated their studies on the advantages that various plants have for biological system without having a proper understanding of the effects that each level of administration of the various doses of consumption of this extracted plant liquor have on their different organs such as the heart, kidney and the liver (Okokon et al., 2013; Uffia et al., 2013; Udofia et al., 2026).

The research focused on evaluation of the phytochemical composition and effects of methanol leaf extract of *Eremomastax speciosa* on selected liver and kidney function parameters in Wistar rats. This study bridges this gap by evaluating the extract's toxicological markers alongside a comprehensive liver and kidney function analysis.

2.0 Materials and Methods

2.1 Plant collection and identification

Eremomastax speciosa was sourced from a local bush in Ikot Ekpene Local Government Area, Akwa Ibom State, Nigeria. Authentication was performed by Dr. Imo Johnny, a taxonomist at the Department of Pharmacognosy and Natural Medicine, University of Uyo and was deposited at the Department of pharmacy Herbarium, University of Uyo with the Voucher number UUPH 1(b).

2.2 Plant Extraction

Freshly harvested leaves of *Eremomastax speciosa* were washed properly to remove dirt and other contaminants, air dried and pulverized. The powdered plant sample was weighed 750 grams. The 750 grams powdered plant was then measured into a 10 litre extraction vessel after which 500 mL of 99.8% assay methanol was added. The mixture in the jar was allowed to settle and kept at room temperature (25 - 32°C) for 72 hours with periodic agitation. The mixture was then filtered and concentrated in vacuum at 40°C using rotary evaporator (Phrompittayarat et al., 2007; Udofia et al., 2026). The process yielded 82.02g of crude methanol extract (17.45% w/w yield). The extract was refrigerated at -4°C for further use.

2.3 Phytochemical Screening

Phytochemical screening was performed to identify bioactive chemical elements such as alkaloids, saponins, tannins, flavonoids, cardiac glycosides, and anthraquinones. The screening was done on each of the extract according to the standard methods. (Sofowora, 1993; AOAC., 2005).

2.4 Acute Toxicity Study

Acute toxicity test was carried out based on OECD/OCDE 423 guideline. Female swiss mice were randomly divided into group of three (3) mice each, they were starved of food but allowed access to water ad libitum 4 hours before the experiment. The ethanol extract was administered orally to three (3) mice at a dose of 2000 mg/kg on the first day. When the mice

were observed to exhibit no signs of toxicity after 24 hours, another 3 mice were administered with 5000 mg/kg of ethanol extract orally. The mice were observed for physical signs of toxicity and death with 24 hours. The LD₅₀ was extrapolated for the OECD 423 guideline annex 2d.

ANNEX 2d: Test Procedure with a Starting Dose of 2000 mg/kg Body Weight

Figure 1 shows the flowchart illustrating the stepwise "Acute Toxic Class" method (OECD Test Guideline 423) for estimating median lethal dose (LD₅₀). Observed mortality at each dose determines whether to increase, repeat, or reduce the dose in the next step (OECD, 2001).

2.5 Animal Treatment and Grouping

A total of 16 adult female Wistar rats weighing 100-160g were used. They were obtained from the animal house of the Department of Biochemistry, Faculty of Basic Medical Sciences, University of Port-Harcourt, Nigeria and brought to the Department of Pharmacy, Faculty of Basic Medical Sciences, University of Uyo, Nigeria where the study was done. The animals were randomly divided into four groups of four (4) rats each. They were housed in a ventilated room in wooden cages with wire mesh top and maintained under standard conditions of humidity (50±5%) and temperature (28±2°C) and 12 hours light / dark cycle and acclimatized for two weeks. The animals were fed with grower's pellet feed (Grand, Bendel feeds, Edo state) and water ad-libitum throughout the experimental period. All experimental animals were handled in accordance with the standard guide for the care and use of laboratory animals (Olfert *et al.*, 1993).

2.6 Experimental Design

The animals were divided into four (4) groups of four (4) rats per group and labelled (1-4).

- Group 1: Control (10 mL normal saline).
- Groups 2-4: 500, 1000, and 1500 mg/kg of extract respectively.

Doses were administered daily via oral cannula for 15 days. Administration was as shown on Table 1.

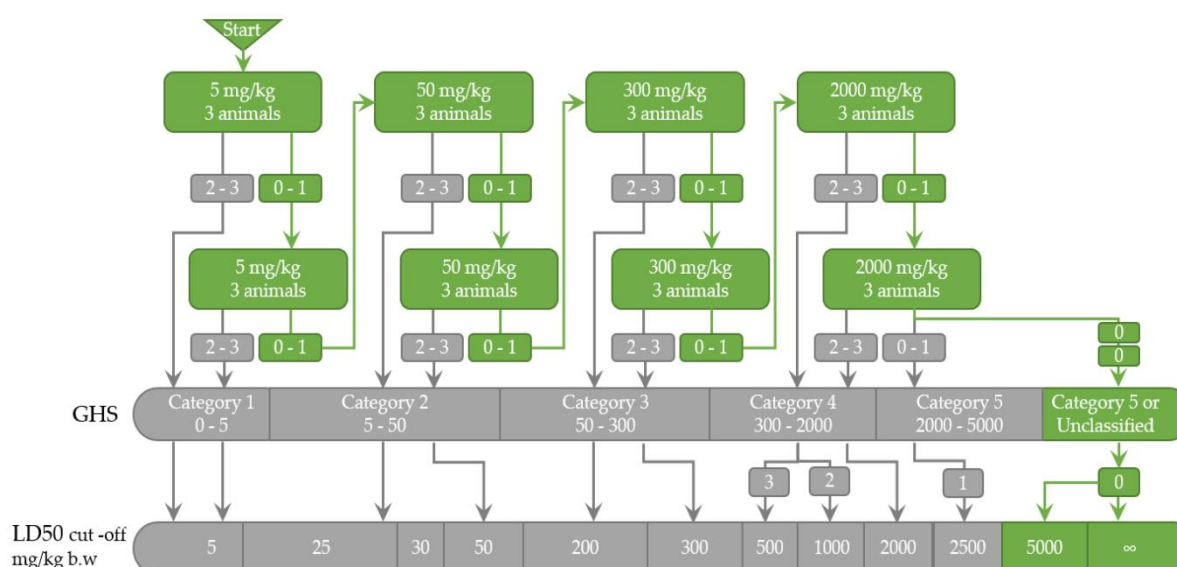


Figure 1: Flowchart of the OECD 423 acute toxicity test protocol

Table 1: Experimental Design

Control Groups	No of Rats	Treatment	Dosage
I (Normal Control)	4	Normal saline	10 mL/kg
II (Low Dose)	4	Extract of <i>Eremomastax speciosa</i>	500 mg/kg
III (Medium Dose)	4	Extract of <i>Eremomastax speciosa</i>	1000 mg/kg
IV (High Dose)	4	Extract of <i>Eremomastax speciosa</i>	1500 mg/kg

2.7 Liver Function and Kidney Function Assessment

After the administration of the extract for fifteen (15) days, the rats were fasted overnight, and then sacrificed by anaesthetizing with chloroform. Blood was called by cardiac puncture to obtain haemolysis free blood samples with the aid of syringes and needles and transferred into pre-labelled, sterile plain sample bottles. Randox laboratories kit was used to assess serum test alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) analysis; Urease-GLDH kit and Jaffe kinetic kit were for urea and creatinine analysis respectively in Microlab 300-Chemistry analyser manufactured by ELITechGroup B.V., Netherland. Electrolyte analyser model ISE 4000 manufactured by SFRI SAS Medical diagnostics, France was used for serum electrolyte and in accordance with methods previously used by Oyagbeme et al., (2013) and Omodamiro et al. (2021).

2.8 Ethical Approval

Approval for the use of the animals for the study was obtained from the Animal Ethics Committee of the Faculty of Pharmacy, University of Uyo, Nigeria. All animal experiments were conducted in accordance with internationally accepted Laboratory Animal Use and Care of Laboratory Animals (1996) as adopted and promulgated by the National Institute of Health (NIH publication No. 85(23), revised (1996), based on Helsinki convention and guidelines and rules of Faculty of Pharmacy, University of Uyo, Nigeria for animal experimentation.

2.9 Statistical Analysis

Statistical analysis was performed using SPSS vs 23.0 and Microsoft excel. Data obtained from the biochemical assays were expressed as mean $\pm$ standard error of mean (SEM) and presented in tables. The significance differences were determined using one-way analysis of variance (ANOVA) while p-value of less than 0.05 ($p < 0.05$) was considered statistically significant.

3.0 Results

3.1 Phytochemical Screening and Yield

The extraction of 750g of dried *Eremomastax speciosa* leaves yielded 82.02g (17.45% w/w) of

crude methanol extract. Qualitative screening confirmed the presence of alkaloids, saponins, tannins, flavonoids, and cardiac glycosides. Anthraquinones were notably absent (Table 2).

Table 2: Results of phytochemical Screening of leaves of *Eremomastax speciosa*

Phytochemical	Test	Observation	Inference
Alkaloids	Dragendorff's reagent	A red precipitate	+
Tannins	Ferric chloride	Blue black precipitate	+
	Salkowski's test	Brown ring formed at interphase	+
	Keller Kiliani test	Brown ring formed at interphase	+
Cardiac glycosides	Lieberman's test	A pink to blue to brown colouration at the interphase	+
	Magnesium metal test	Orange colouration	+
Flavonoids	Sodium hydroxide test	A yellow colouration	+
	Ammonia test	A yellow colouration	+
Anthraquinones	Modified Borntrager's test	No red, violet or pink colouration	–
	Borntrager's test	No red, violet or pink colouration	–
Saponins	Frothing test	Persistent frothing	+

Keys: + present; - absent

3.2 Acute Toxicity Study

The acute toxicity study was conducted to determine the lethal dose (LD₅₀) of the methanol extract of *Eremomastax speciosa*. Initially, a dose of 2000 mg/kg was administered to the test organism. After a 24-hour observation, no signs of toxicity or mortality were observed. Therefore, the dose was increased to 5000 mg/kg. Again, no toxicity or mortality was observed after 24 hours. This result suggests that the methanol extract of *Eremomastax speciosa* has a relatively high safety margin, as no acute toxicity was observed at doses up to 5000 mg/kg. The LD₅₀ for the methanol extract was extrapolated based on the OECD/OCDE 423 guidelines.

3.3 Effect of Methanol Extract of *Eremomastax speciosa* on Liver Enzymes

From the results shown in Tables 3, serum analysis of AST revealed a non-significant

increase ($P > 0.05$) in groups 1, 2 and 3 treated with 500 mg/kg, 1000 mg/kg and 1500 mg/kg body weight of the extract when compared to the normal control. However, ALT recorded a significant ($P < 0.05$) increase in group 3 when compared to groups 1 and normal control. ALP level showed no significant ($P > 0.05$) increase in groups 2 and 3 when compared to the normal control. Furthermore, no significant ($P > 0.05$) decrease was recorded in group 1 when compared to groups 2, 3 and control. Total bilirubin showed a significant ($P > 0.05$) increase in group 3 when compared to the normal control. No significant difference in direct bilirubin was recorded in all treated groups when compared to the normal control.

3.4 Effect of Methanol Extract of *Eremomastax speciosa* on Serum Electrolytes

From the results shown in Table 4, analysis of serum electrolyte levels revealed the K⁺

concentration showed a significant increase ($P < 0.05$) in group 3 when compared to the normal control. Na^+ levels recorded no significant increase in all treated groups when compared to the normal control. However, Cl^- concentration showed a significant ($P < 0.05$) decrease in group 3 when compared to groups 1, 2 and control. Serum HCO_3^- revealed a significant decrease ($P < 0.05$) in group 1 when compared to groups 2, 3 and the control.

3.5 Effect of Methanol Extract of *Eremomastax speciosa* on kidney function

From the results shown in Table 5, other kidney function analysis showed that serum creatinine recorded no significant difference ($P > 0.05$) in all treated groups when compared to the normal control. Urea level in group 1 and 2 revealed a significant increase when compared to the normal control.

Table 3: Effect of Methanol Extract of *Eremomastax speciosa* on Liver Enzymes of Wistar Rats

Groups	AST (U/L)	ALT (U/L)	ALP (U/L)	TBil ($\mu\text{mol/L}$)	DBil (mg/dL)
(Control)	11.25 $\pm$ 12.92 ^a	37.25 $\pm$ 8.12 ^a	79.00 $\pm$ 21.12 ^a	3.65 $\pm$ 1.36 ^a	117 $\pm$ 0.37 ^a
Group 1 (500mg/kg)	18.50 $\pm$ 19.20 ^a	35.25 $\pm$ 18.21 ^a	69.25 $\pm$ 13.33 ^a	3.12 $\pm$ 0.94 ^a	077 $\pm$ 2.85 ^a
Group 2 (1000mg/kg)	99.75 $\pm$ 38.42 ^a	66.75 $\pm$ 6.86 ^a	149.50 $\pm$ 57.51 ^a	5.52 $\pm$ 2.25 ^a	1.55 $\pm$ 0.34 ^a
Group 3 (1 500mg/kg)	74.50 $\pm$ 39.79 ^a	90.25 $\pm$ 11.41 ^b	172.75 $\pm$ 41.53 ^a	20.30 $\pm$ 10.31 ^b	1.42 $\pm$ 1.08 ^a

Note: Values are expressed as Mean $\pm$ SEM. Means with different subscripts along the same row are significantly different. Statistically significant differences are considered at $P < 0.05$. Abbreviations: AST (Aspartate aminotransferase), ALT (Alanine aminotransferase), ALP (Alkaline phosphatase), TBil (Total Bilirubin), DBil (Direct Bilirubin)

Table 4: Effect of Methanol Extract of *Eremomastax speciosa* on Serum Electrolytes of Wistar Rats

Groups	K^+ (mmol/L)	Na^+ (mmol/L)	Cl^- (mmol/L)	HCO_3^- (mmol/L)
(Control)	6.15 $\pm$ 0.34 ^a	146.00 $\pm$ 1.58 ^a	110.75 $\pm$ 1.49 ^a	0.43 $\pm$ 0.86 ^a
Group 1 (500mg/kg)	6.50 $\pm$ 0.17 ^a	147.00 $\pm$ 2.91 ^a	119.25 $\pm$ 2.01 ^a	9.00 $\pm$ 0.40 ^a
Group 2 (1000mg/kg)	6.82 $\pm$ 0.19 ^a	144.25 $\pm$ 1.93 ^a	120.50 $\pm$ 1.50 ^b	1.25 $\pm$ 0.25 ^b
Group 3 (1500mg/kg)	7.37 $\pm$ 0.58 ^b	164.75 $\pm$ 22.45 ^a	107.25 $\pm$ 4.76 ^a	1.25 $\pm$ 0.62 ^b

Note: Values are expressed as Mean $\pm$ SEM. Means with different subscripts along the same row are significantly different. Statistically significant differences are considered at $P < 0.05$. Abbreviations: Potassium (K^+), Sodium (Na^+), Chlorine (Cl^-), Bicarbonate (HCO_3^-)

4.0 Discussion

The analysis of *Eremomastax speciosa* indicated the presence of diverse range of bioactive secondary metabolites, including saponins, tannins, alkaloids, cardiac glycosides, and flavonoids. This suggests that extract has beneficial pharmacological effects. The research affirmed the work of Onoja et al., (2017) which state that *Eremomastax speciosa* exhibits wide

range of biological activities because of the presence of tannins, saponins, alkaloids, flavonoids and cardiac glycosides. These results align with the findings of Udofia et al., (2026), who identified similar compounds as the primary drivers of the plant's therapeutic efficacy. These bioactive compounds are vital and essential to the plant's bioactivity; for instance, flavonoids and tannins are well

recognized for their ability to inhibit oxidative stress (Cichon et al., 2020). According to Mboso et al. (2013), *Eremomastax speciosa* plants may be utilised in conjunction with the vitex plant, which is used to treat female infertility issues,

due to the high concentration of terpenes and flavonoids. Tannins, on the other hand, are found in some herbs and give them their characteristic astringent taste (Ekpo, et al., 2012).

Table 5: Effect of Methanol Extract of *Eremomastax speciosa* on kidney function of Wistar Rats

Groups	Creatinine ($\mu\text{mol/L}$)	Urea(mmol/L)
(Control)	70.75 $\pm$ 22.02 ^a	8.37 $\pm$ 1.26 ^a
Group 1 (500mg/kg)	34.00 $\pm$ 92.99 ^a	11.70 $\pm$ 0.70 ^b
Group 2 (1000mg/kg)	31.25 $\pm$ 71.96 ^a	11.22 $\pm$ 0.47 ^a
Group 3 (1500mg/kg)	67.50 $\pm$ 21.04 ^a	8.97 $\pm$ 1.15 ^a

Note: Values are expressed as Mean $\pm$ SEM. Means with different subscripts along the same row are significantly different. Statistically significant differences are considered at $P < 0.05$

In this study, there was no significant increase in AST and ALP levels serves as indication that there may not be enzyme leakage into blood stream due to liver damage. Significant elevation of the serum ALP and ALT following high-dose *Eremomastax speciosa* administration may indicate compromised hepatobiliary integrity and altered enzyme activity, but definitive evidence of hepatotoxicity would require additional biochemical and histological evaluation. This result was consistent with the findings of Adebayo and Aliyu (2009), who reported similar dose-related hepatocellular effects with *Tithonia diversifolia* leaf extract. This result was also consistent with research of Offor et al., (2015) who found a significant decrease in the activities of alkaline phosphate (ALP) at low dosages when evaluating effect of ethanol leaf-extract of *Annona Muricata* on liver enzymes of albino rats. ALT and AST are cytosolic enzymes released into circulation during hepatocyte damage, and elevated values generally reflect hepatocellular injury. ALP, however, is largely linked to the biliary tract and bone metabolism, and increased levels are often indicative of cholestatic disorders (Odeja et al., 2019).

Ndrepepa (2021) noted that increase in alanine aminotransferase (ALT) is synonymous with inflammatory liver disease, drug toxicity, alcoholic liver diseases, fatty liver disease and

toxic herbs induced damage. This finding is of critical importance because the liver is susceptible to xenobiotic-induced damage because of its crucial function in xenobiotic metabolism, its portal placement within the circulation, and its anatomical and physiological structure (Siwe et al., 2015). Elevated total bilirubin was observed at moderate and high dosages which suggest that extract could induce breakdown of red blood cell, impaired liver conjugation and block bile flow (cholestasis). Elevated direct bilirubin was also recorded at high dosages which suggest that extract could induce hepatocellular injury and cholestatic injury (Oyagbemi et al., 2013; Omodamiro et al., 2021). In this study, Elevated levels of serum potassium (K^+) and bicarbonate (HCO_3^-) was observed at high dosages of extract. Medium dosages of extract significantly increase serum chlorine (Cl^-) and bicarbonate (HCO_3^-) levels which suggests that a moderate increase in dosage may be affecting the electrolytes balance in the test organism. Conversely, Sodium (Na^+) levels did not increase significantly at low, moderate and high dosages, suggesting a dose-dependent hyponatremia. The hyponatremia is caused by excessive loss of water from the bodily fluid. Elevated bicarbonate concentrations at

moderate and high dosages suggest that the extract could induce metabolic alkalosis. These results align with the findings of Nwankpa et al., (2018) who observed similar trend when assessing kidney function indices in male Wistar rats administered ethanol stem extract of *Dennettia tripetala*. Serum creatinine was observed to drop significantly at low and moderate dosages suggesting that the kidneys are compromised by the administration of the extract (Adebayo et al., 2005). Significant increase was observed in urea levels at low and moderate dosages suggesting that extract could increase in urea production due to increased protein breakdown, pre-renal dehydration and reduced kidney perfusion. Serum urea concentration is frequently thought to be a more accurate indicator of renal function than serum creatinine (Ufuoma et al., 2020).

5.0 Conclusion

The study demonstrated that methanol leaf extract of *Eremomastax speciosa* contains several phytochemical constituents, including alkaloids, tannins, saponins, flavonoids and cardiac glycoside, while anthraquinones were not detected. The extract produced no observable signs of acute toxicity at the tested limited dose indicating a favourable safety profile under the test condition; however, selected biochemical parameters varied at some of administered doses. In particular, alterations in liver enzymes and serum electrolytes warrant further investigation to establish their biological significance. This study was limited to a 15-day administration period in a healthy rat model; therefore, the long-term chronic effects of consumption remain unknown. Further studies involving larger sample sizes, detailed histopathological examination, additional renal and hepatic biomarkers, and longer-term toxicity assessment is recommended.

Compliance with Ethical Standards

Declarations: The authors declare that they have no conflict of interest.

Data availability: All data used in this study will be readily available to the public.

Consent for publication: Not Applicable.

Availability of data and materials: The publisher has the right to make the data public.

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Contribution of Authors

Uffia, I. D. designed the experiments, conducted laboratory analyses, interpreted the results and wrote the manuscript; Udofia, O. E. conducted the statistical analyses; John, G. E. and Esen, R. O. contributed reagents and materials and Bassey, U. E. revised the article for technical merits. All authors have read and agreed to the published version of the manuscript.

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