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SUB-ACUTE ORAL TOXICITY OF *Catunaregam nilotica* ROOT BARK EXTRACT IN ALBINO RATS

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Abstract

Catunaregam nilotica is a widely utilized African medicinal plant with diverse ethnopharmacological applications, including the treatment of snakebite envenomation, malaria, and inflammatory disorders. Despite its extensive traditional use, data on its safety following repeated oral exposure remain limited. This study aimed to evaluate the sub-acute oral toxicity profile of the methanol extract of *C. nilotica* root bark in albino rats. A 28-day repeated-dose toxicity study was conducted in accordance with OECD Test Guideline 407. Albino rats (n = 6 per group) were administered the extract orally at doses of 250, 500, and 1000 mg/kg body weight daily. Clinical observations, body weight changes, hematological and biochemical parameters, relative organ weights, and histopathological examinations were assessed. No mortality or treatment-related clinical signs were observed throughout the study. Body weight gain and relative organ weights were comparable to controls. Hematological indices remained within normal physiological ranges. Serum biochemical markers of hepatic (ALT, AST, ALP, albumin, total protein, bilirubin) and renal (urea, creatinine, electrolytes) function showed no significant alterations ($p > 0.05$). Histopathological examination of the liver, kidneys, and heart revealed normal tissue architecture without evidence of toxicity. The No-Observed-Adverse-Effect Level (NOAEL) was established at 1000 mg/kg/day. The methanol extract of

Keywords:
Catunaregam nilotica, Sub-acute toxicity, OECD 407, NOAEL, Medicinal plants, Safety evaluation.

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Catunaregam nilotica root bark is safe under sub-acute exposure conditions and supports its continued traditional use. However, further chronic toxicity studies are recommended.

Introduction

Medicinal plants remain integral to global healthcare systems, serving as primary therapeutic resources for a substantial proportion of the world's population (Latif and Nawaz, 2026). This dependence is particularly pronounced in sub-Saharan Africa, where traditional herbal medicine is deeply embedded in healthcare delivery owing to its accessibility, affordability, and cultural acceptance (Onukansi *et al*., 2025). Consistent with this trend, recent assessments by the World Health Organization not only reaffirm the sustained reliance on plant-based therapies but also underscore the growing scientific and commercial interest in phytomedicine as a reservoir of structurally diverse bioactive compounds for drug discovery (El-Saadony *et al*., 2025). Notwithstanding these benefits, the increasing utilization of herbal preparations has intensified concerns regarding their safety, quality assurance, and evidence-based validation. A fundamental challenge in phytotherapy is the persistent perception that natural products are inherently safe (Aliu *et al*., 2025). This assumption has been increasingly challenged by accumulating evidence indicating that numerous medicinal plants possess inherent toxic potential, particularly under conditions of prolonged or repeated exposure (Mensah *et al*., 2019). Clinical and experimental reports of herb-induced hepatotoxicity, nephrotoxicity, and hematological alterations further emphasize the necessity for rigorous toxicological evaluation of plant-derived products (Ballotin *et al*., 2021). Importantly, many of these adverse effects are attributable to bioactive secondary metabolites which, despite their therapeutic relevance, may exhibit dose-dependent or cumulative toxicity (Reshi *et al*., 2025; Purwaningsih *et al*., 2025). Accordingly, systematic safety assessment is indispensable for bridging the gap between traditional usage and clinical applicability. *Catunaregam nilotica* (Stapf) Tirveng. (Rubiaceae) is a medicinal plant of considerable ethnopharmacological importance across Africa and parts of Asia (Timalsina *et al*., 2021; Hassan *et al*., 2023). It is extensively employed in traditional medicine for the management of snakebite envenomation (Sani *et al*., 2026), as well as for malaria, gastrointestinal disorders, and inflammatory conditions (Yagi and Yagi, 2018). In rural settings, its preparations are frequently administered repeatedly and often without standardized dosing regimens, thereby increasing the likelihood of cumulative toxicity.

Phytochemical investigations have demonstrated that *C. nilotica* contains a diverse spectrum of bioactive constituents, including flavonoids, iridoids, triterpenoids, alkaloids,

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and phenolic compounds (Rather *et al*., 2015). These metabolites have been associated with a broad range of pharmacological activities, including antioxidant, anti-inflammatory, antimicrobial, and antivenom effects (Sorrenti *et al*., 2023). Mechanistically, these compounds may modulate oxidative stress pathways, suppress pro-inflammatory mediators (Solomon *et al*., 2022), and interact with key enzymatic components of snake venom, such as phospholipases A_2 and metalloproteinases (Sani *et al*., 2026). However, the same biochemical reactivity underlying these therapeutic effects may also predispose to off-target toxicity, particularly in metabolically active organs such as the liver and kidneys (Gu and Manautou, 2012). In this context, toxicological evaluation of medicinal plants necessitates a tiered approach encompassing acute, sub-acute, and chronic exposure studies (Alelign *et al*., 2020). While acute toxicity studies provide essential preliminary data on lethality and immediate adverse effects, they are inherently limited in predicting the consequences of repeated exposure (Erhirhie *et al*., 2018). Sub-acute toxicity studies, typically conducted over a 28-day period, are therefore critical for identifying early manifestations of systemic toxicity, target organ susceptibility, and adaptive physiological responses (Kumar *et al*., 2014). Moreover, such studies enable the determination of the No-Observed-Adverse-Effect Level (NOAEL), a pivotal parameter in toxicological risk assessment and dose extrapolation (Agathokleous *et al*., 2021; Olayode *et al*., 2019).

Despite its widespread ethnomedicinal use, the safety profile of *Catunaregam nilotica* under repeated-dose conditions remains inadequately characterized, with existing studies being limited in scope, fragmented, and methodologically heterogeneous. This paucity of standardized toxicological data constitutes a major impediment to its integration into evidence-based medical practice and its prospective development as a phytopharmaceutical agent. Therefore, the present study was designed to systematically evaluate the sub-acute oral toxicity of the methanol extract of *Catunaregam nilotica* root bark in albino rats following repeated administration over 28 days, in accordance with OECD Test Guideline 407.

Plant Material Collection and Authentication

Fresh root bark of *Catunaregam nilotica* (Stapf) Tirveng. (Family Rubiaceae) was collected from Gabake village, Kaura Namoda, Zamfara State, Nigeria. Botanical identification and authentication were performed by taxonomist at the Botany Unit, Department of Science Laboratory Technology, Federal Polytechnic Kaura Namoda. A voucher specimen was prepared and deposited in the institutional herbarium for future reference (Culley, 2013).

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Preparation of Crude Methanol Extract

The collected root bark was washed with distilled water to remove extraneous materials and shade-dried at controlled ambient temperature with adequate ventilation to preserve thermolabile constituents. The dried material was pulverized using a laboratory-grade mechanical grinder and sieved to obtain uniform particle size. A known quantity (1000 g) of powdered material was macerated in analytical-grade methanol (1:5 w/v) for 72 h at room temperature with intermittent agitation. The extract was filtered sequentially through muslin cloth and Whatman No. 1 filter paper. The filtrate was concentrated under reduced pressure using a rotary evaporator at 40 °C and further dried in a water bath (40–45 °C) to obtain a semi-solid crude extract (Wado, Suleman, & Mohammed, 2022). The extract was stored in sterile, airtight containers at 4 °C until further use.

The percentage yield of the crude extract was calculated as: $= \frac{\text{Weight of dried extract}}{Initial\ weight\ of\ powdered\ material} \times 100$

Phytochemical Analysis

The crude extract was subjected to preliminary phytochemical screening using standard qualitative methods (Harborne, 1973; Trease and Evans, 1989; Sofowora, 1993).

Experimental Animals and Ethical Compliance

Healthy adult albino rats (*Rattus norvegicus*), weighing 120–180 g, were obtained from the Animal House Facility of Usmanu Danfodiyo University, Sokoto. Animals were housed in animal house and provided with standard pellet diet and water *ad libitum*. Animals were acclimatized for 7 days prior to experimentation. All procedures were conducted in accordance with the guidelines of the Organisation for Economic Co-operation and Development Test Guideline 407 for repeated-dose 28-day oral toxicity studies and were approved by the Institutional Animal Ethics Committee. To minimize bias, animals were randomly assigned to experimental groups. All outcome assessments were conducted by investigators blinded to group allocation (Organisation for Economic Co-operation and Development, 2025).

Sub-Acute Oral Toxicity Study

Dose selection was based on preliminary acute toxicity studies and existing literature (Sani *et al.,* 2026), ensuring coverage of low, intermediate, and high exposure levels within the safety margin. Three dose levels (250, 500, and 1000 mg/kg body weight) were selected in accordance with OECD recommendations. The sub-acute toxicity study was conducted following the Organisation for Economic Co-operation and Development Test Guideline 407 for repeated-dose 28-day oral toxicity in rodents.

Animals were randomly allocated into four experimental groups (n = 6 per group):

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Group I: Control (1% Tween 80)

Group II: 250 mg/kg body weight extract

Group III: 500 mg/kg body weight extract

Group IV: 1000 mg/kg body weight extract

The extract was freshly reconstituted in 1% Tween 80 and administered orally once daily for 28 consecutive days via oral gavage at a constant dosing volume of 10 mL/kg body weight. Animals were observed daily for clinical signs of toxicity, including changes in skin, fur, eyes, mucous membranes, respiratory function, autonomic activity, and behavioral patterns (e.g., locomotion, grooming, tremors, convulsions), as well as mortality (Organisation for Economic Co-operation and Development, 2025). Observations were recorded using a standardized scoring system to ensure consistency and reduce observer bias. Mortality and morbidity were monitored throughout the study period.

Body Weight and Feed Intake Monitoring

Body weights were recorded at baseline (Day 0) and weekly thereafter using a calibrated digital balance. Feed and water intake were monitored periodically as indicators of metabolic status and treatment-related effects (Shamaki, et al., 2017).

Organ Collection and Relative Organ Weight Determination

On Day 29, animals were fasted overnight and anesthetized using ketamine (80 mg/kg, intraperitoneal) prior to sacrifice. Blood samples were collected via cardiac puncture. Vital organs (liver, kidneys, heart, and spleen) were excised, trimmed of surrounding tissues, rinsed in cold normal saline, blotted dry, and weighed using a precision analytical balance. Relative organ weights (ROWs) were calculated to assess potential organ-specific toxicity using the following formula:

$$\text{Relative organ weight (\%)} = \frac{\text{Organ weight}}{Final\ body\ weight} \times 100$$

This parameter was used to detect possible organ hypertrophy or atrophy associated with repeated exposure (OECD, 2025).

Hematological Analysis

Blood samples collected into EDTA tubes were analyzed immediately using an automated hematology analyzer (Sysmex XP-300, Kobe, Japan) according to the manufacturer's instructions. Parameters evaluated included red blood cell count (RBC), hemoglobin concentration (Hb), packed cell volume (PCV), total white blood cell count (WBC), and platelet count (PLT). (Al-Najdawi, Fararjeh, & Khataibeh, 2025). These indices were selected to evaluate the potential effects of the extract on hematopoietic function, immune status, and hemostatic balance, as alterations in these parameters are commonly

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associated with systemic toxicity and inflammatory responses (Erhabor, Muhammad, Onwuchekwa, & Egenti, 2021).

Serum Biochemical Analysis

Blood samples for biochemical analysis were collected via cardiac puncture into plain (non-anticoagulated) tubes and allowed to clot at room temperature for 20–30 min. The samples were then centrifuged at 3000 rpm for 10 min to obtain clear serum, which was carefully aspirated and stored at 4 °C for immediate analysis (Mendy, Caboux, & Lawlor, 2017). Serum biochemical parameters were determined using commercially available diagnostic reagent kits (Randox Laboratories Ltd., UK) in accordance with the manufacturer's instructions with appropriate calibration and quality control.

Liver Function Indices

The following hepatic biomarkers were evaluated: Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Total protein, Albumin and Total bilirubin. These parameters were assessed as indicators of hepatocellular integrity, biliary function, and hepatic synthetic capacity. Elevations in transaminases (ALT and AST) are commonly associated with hepatocellular injury, while alterations in ALP and bilirubin may reflect cholestatic dysfunction. Total protein and albumin levels were used to evaluate hepatic biosynthetic activity (Vasimahmed, Zubair, & Minter, 2023).

Kidney Function Indices

Renal function was evaluated using the following parameters: Urea, Creatinine and Electrolytes, (sodium [Na^+] and potassium [K^+]). These indices were used to assess glomerular filtration efficiency and electrolyte homeostasis. Serum urea and creatinine are established biomarkers of renal function, while electrolyte levels provide insight into tubular function and systemic fluid balance (Gounden, Bhatt, & Jialal, 2024).

Determination of No-Observed-Adverse-Effect Level (NOAEL)

The No-Observed-Adverse-Effect Level (NOAEL) was defined as the highest dose at which no treatment-related adverse effects were observed across all evaluated parameters, including clinical signs, body weight, hematological indices, biochemical markers, organ weights, and histopathological findings, in accordance with OECD guideline recommendations (Agathokleous, Saitanis, & Markouizou, 2021) (OECD, 2025).

Histopathological Examination

Excised organs were fixed in 10% neutral buffered formalin for 24–48 h, processed using standard paraffin embedding techniques, and sectioned at 4–5 µm thickness. Sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope

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by a blinded histopathologist. Histological alterations were graded using a semi-quantitative scoring system to assess the severity of tissue damage.

Statistical Analysis

Data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way or two-way analysis of variance (ANOVA) using statistical software (SPSS Software version 21).

Results

Phytochemical composition of crude methanol extract of *Catunaregam nilotica* root bark

The present investigation extends our earlier findings (Idris *et al.,* 2024), which demonstrated that the crude extracts of *Catunaregam nilotica* are rich in diverse phytochemical constituents, including alkaloids, anthraquinones, flavonoids, phenolic compounds, saponins, steroids, tannins, and terpenoids (Table 1).

Table 1. Qualitative Phytochemical Composition of Crude Methanol Extract of *Catunaregam nilotica* Root Bark

Test	CMECNR
Alkaloids	+
Anthocyanins	-
Anthraquinones	+
Flavonoids	+
Phenols	+
Saponins	+
Steroids	+
Tannins	+
Terpenoids	+

Key CMECNR =*Catunaregam nilotica* Root bark methanol extract, + = Presence, - = Not detected

Clinical Observations and Mortality Following Repeated Oral Administration of Crude Methanol Extract of *Catunaregam nilotica* Root Bark

Consistent with our previous acute toxicity study, which demonstrated the absence of observable toxicity or mortality at a single oral dose of 5000 mg/kg (Sani *et al.,* 2026), repeated oral administration of crude methanol extract of *Catunaregam nilotica* root bark at doses of 250, 500, and 1000 mg/kg for 28 days produced no mortality or treatment-related clinical signs of toxicity. All animals remained physiologically stable, exhibiting normal posture, locomotor activity, grooming behavior, and feeding patterns. No

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abnormalities were observed in skin, fur, eyes, mucous membranes, respiratory function, or autonomic responses.

Body Weight Changes Following Repeated Oral Administration of Crude Methanol Extract of *Catunaregam nilotica* Root Bark

Baseline body weights were comparable across all experimental groups, ranging from 128.00 ± 0.58 g to 129.17 ± 0.60 g. Repeated oral administration of crude methanol extract of *Catunaregam nilotica* root bark for 28 days resulted in a progressive increase in body weight in both control and treated animals. Mean body weights increased from 128.50 ± 0.76 g to 146.00 ± 0.86 g in the control group, from 129.17 ± 0.60 g to 145.50 ± 0.76 g in the 250 mg/kg group, from 128.50 ± 0.76 g to 144.50 ± 0.76 g in the 500 mg/kg group, and from 128.00 ± 0.58 g to 146.50 ± 0.76 g in the 1000 mg/kg group over the study period. Although minor variations in body weight gain were observed among treatment groups (Fig.1). Body weight data satisfied the assumptions of normality across all groups and time points (Shapiro–Wilk, $p > 0.05$), confirming the appropriateness of parametric statistical analyses, including repeated-measures ANOVA.

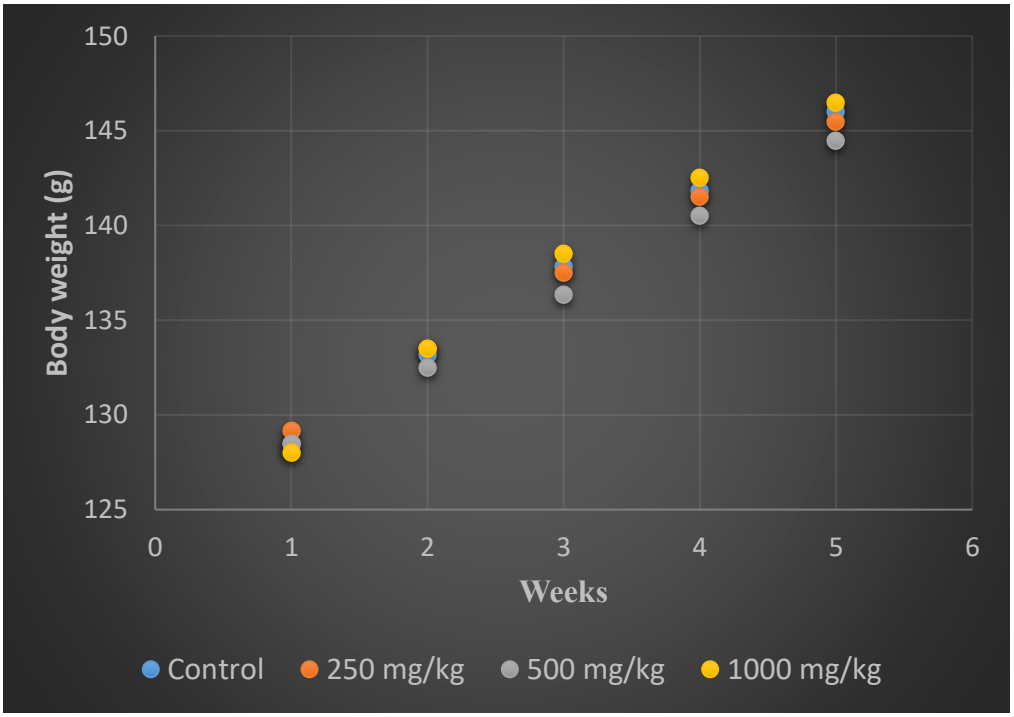


Figure 1: Changes in body weight of rats following repeated oral administration of crude methanol extract of *Catunaregam nilotica* root bark for 28 days. Values are expressed as Mean ± SEM (n = 6).

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Effect of Repeated Oral Administration of Crude Methanol Extract of *Catunaregam nilotica* Root Bark on Relative Organ Weights in Albino Rats

Figure 2 shows that the relative weights of the liver, kidneys, heart, and spleen in the treated groups (250–1000 mg/kg) were comparable to those of the control group. Although there were slight variations across the treatment groups, including a marginal increase in liver and kidney weights with increasing dose, these changes were not statistically significant (P > 0.05). In addition, the relative weights of the heart and spleen remained relatively stable across all groups, with no significant differences (p>0.05) observed when compared to the control.

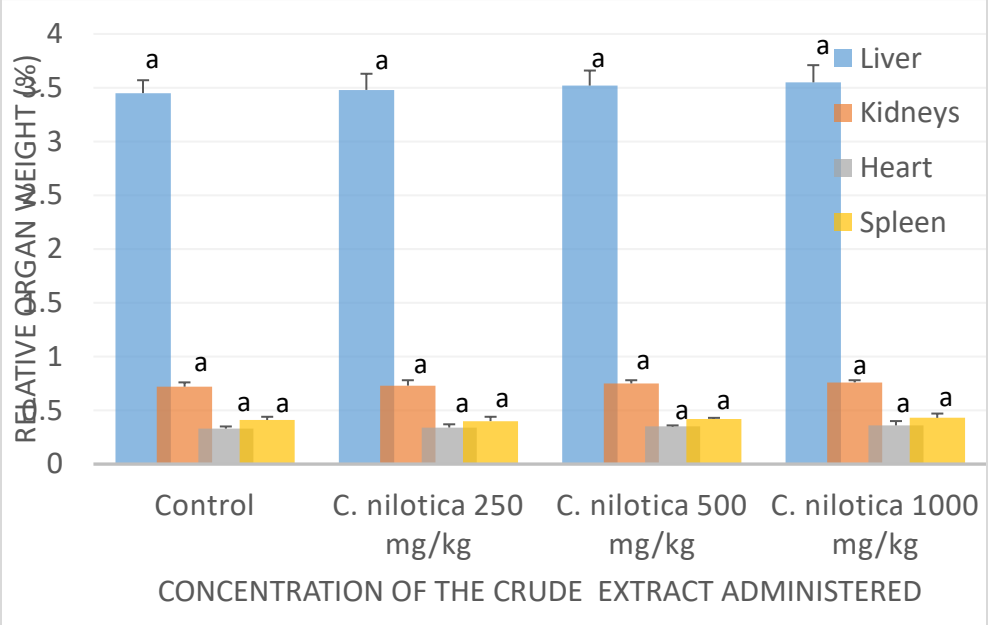


Figure 2: Relative organ weights (%) of rats following repeated oral administration of crude methanol extract of *Catunaregam nilotica* root bark.

Values are expressed as mean ± SE (n = 6). Mean having the same superscripts are not significantly (P>0.05) different, as determined by one-way ANOVA followed by Tukey's post hoc test, using SPSS version 20.

Effect of Repeated Oral Administration of *Catunaregam nilotica* Root Bark Extract on Hematological Parameters in Albino Rats

Table 2 shows that red blood cell (RBC) count, hemoglobin (Hb) concentration, and packed cell volume (PCV) exhibited slight decreases in the treated groups (250–1000 mg/kg) compared to the control; however, these changes were not statistically significant (P > 0.05). Similarly, white blood cell (WBC) count showed a marginal increase across the treated groups relative to the control, which was also not statistically significant (P >

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0.05). Platelet (PLT) counts demonstrated a slight decrease in the treated groups compared to the control, but this change was likewise not significant (P > 0.05).

Table 2. Effects of repeated oral administration of crude methanolic extract of Catunaregam nilotica root bark on hematological parameters in albino rats.

Group	RBC ($\times 10^{12}$/L)	Hb (g/dL)	PCV (%)	WBC ($\times 10^{9}$/L)	PLT ($\times 10^{9}$/L)
Control	7.1 ± 0.3[a]	14.8 ± 0.6[a]	44.2 ± 1.5[a]	6.2 ± 0.4[a]	295 ± 18[a]
250 mg/kg	7.0 ± 0.4[a]	14.6 ± 0.5[a]	43.9 ± 1.4[a]	6.3 ± 0.5[a]	292 ± 17[a]
500 mg/kg	6.9 ± 0.3[a]	14.5 ± 0.6[a]	43.7 ± 1.6[a]	6.4 ± 0.4[a]	291 ± 19[a]
1000 mg/kg	6.9 ± 0.4[a]	14.5 ± 0.7[a]	43.5 ± 1.6[a]	6.5 ± 0.5[a]	290 ± 20[a]

Values are expressed as mean ± SE (n = 6). Mean having the same superscripts are not significantly (P>0.05) different, as determined by one-way ANOVA followed by Tukey's post hoc test, using SPSS version 20.

Effect of Crude Methanol Extract of *Catunaregam nilotica* Root Bark on Serum Biochemical Parameters in Albino Rats

Liver Function Indices

Table 2 shows that serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) activities exhibited a slight dose-dependent increase in the treated groups (250–1000 mg/kg) compared to the control. However, these increases were not statistically significant (P > 0.05). Similarly, serum albumin and total protein levels showed a marginal decrease with increasing dose of the extract, but these changes were also not statistically significant (P > 0.05). In addition, total bilirubin levels showed a slight, non-significant increase across the treated groups compared to the control.

Table 3. Effects of repeated oral administration of crude methanol extract of *Catunaregam nilotica* root bark on liver function parameters in Albino Rats.

Group	ALT (U/L)	AST (U/L)	ALP (U/L)	Albumin (g/dL)	Total Protein (g/dL)	Bilirubin (mg/dL)
Control	29.4 ± 1.3[a]	13.6 ± 0.5[a]	84.2 ± 3.1[a]	7.75 ± 0.11[a]	6.82 ± 0.18[a]	0.42 ± 0.03[a]
250 mg/kg	31.2 ± 1.5[a]	14.5 ± 0.6[a]	85.1 ± 3.4[a]	7.62 ± 0.12[a]	6.75 ± 0.20[a]	0.43 ± 0.03[a]
500 mg/kg	32.6 ± 1.7[a]	15.4 ± 0.7[a]	86.4 ± 3.6[a]	7.55 ± 0.14[a]	6.72 ± 0.19[a]	0.44 ± 0.04[a]
1000 mg/kg	34.2 ± 1.9[a]	16.8 ± 0.8[a]	87.6 ± 3.8[a]	7.38 ± 0.13[a]	6.69 ± 0.21[a]	0.45 ± 0.04[a]

Values are expressed as mean ± SE (n = 6). Mean having the same superscripts are not significantly (P>0.05) different, as determined by one-way ANOVA followed by Tukey's post hoc test, using SPSS version 20.

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Kidney Function Indices

The results showed that serum urea and creatinine levels exhibited a slight dose-dependent increase in the treated groups (250–1000 mg/kg) compared to the control. However, these increases were not statistically significant ($P > 0.05$). Similarly, serum sodium (Na^+) levels showed a slight, non-significant decrease with increasing dose of the extract, while potassium (K^+) levels showed a marginal, non-significant increase across the treated groups compared to the control (Table 4).

Table 4. Effects of repeated oral administration of crude methanol extract of *Catunaregam nilotica* root bark on renal function parameters in Albino Rats.

Group	Urea (mg/dL)	Creatinine (mg/dL)	Na^+ (mmol/L)	K^+ (mmol/L)
Control	31.2 ± 1.4^a	0.78 ± 0.05^a	142.6 ± 2.1^a	4.2 ± 0.3^a
250 mg/kg	32.5 ± 1.5^a	0.81 ± 0.05^a	142.2 ± 2.0^a	4.3 ± 0.3^a
500 mg/kg	33.6 ± 1.6^a	0.85 ± 0.06^a	142.0 ± 2.2^a	4.3 ± 0.4^a
1000 mg/kg	34.9 ± 1.7^a	0.88 ± 0.06^a	141.8 ± 2.3^a	4.4 ± 0.4^a

Values are expressed as mean ± SE (n = 6). Mean having the same superscripts are not significantly ($P>0.05$) different, as determined by one-way ANOVA followed by Tukey's post hoc test, using SPSS version 20.

Histopathological Findings

The liver sections from the control group (Plate 1A) showed normal hepatic architecture, characterized by well-arranged hepatocytes radiating from the central vein, with clearly defined sinusoids and intact portal triads. Similarly, liver sections from the treated groups (Plates 1B–D; 250–1000 mg/kg) revealed preserved hepatic architecture comparable to the control. The hepatocytes appeared normal with intact nuclei and cytoplasmic integrity. In the kidney sections, the control group (Plate 2A) exhibited normal renal histology, with well-defined glomeruli and intact renal tubules. The treated groups (Plates 2B–D; 250–1000 mg/kg) also showed normal renal architecture, comparable to the control. The glomeruli appeared intact, and the tubular structures were well preserved without evidence of degeneration, necrosis, or inflammatory infiltration. There were no signs of tubular dilation or interstitial damage.

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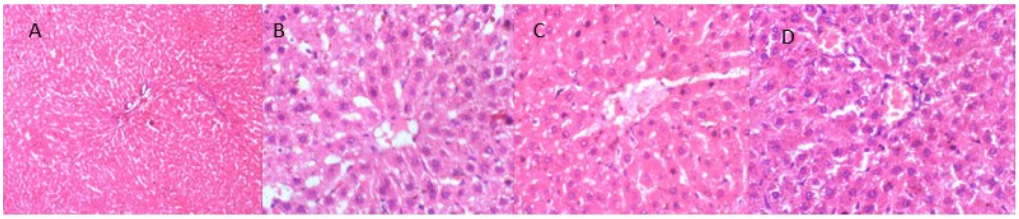


Plate 1. Liver histology (H&E, X100)
Representative liver sections from control (A) and treated groups (B-D, 250–1000 mg/kg) showing preserved hepatic architecture with intact central vein and portal triad. No degenerative or inflammatory changes were

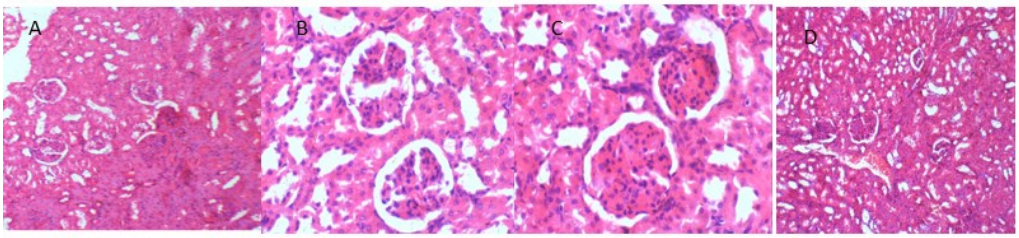


Plate 2. Kidney histology (H&E, X100)
Representative kidney sections from control (A) and treated groups, (B-D, 250–1000 mg/kg) showing normal glomeruli and renal tubules without evidence of structural damage.

NOAEL Determination

Based on the absence of treatment-related adverse effects across clinical, hematological, biochemical, organ weight, and histopathological endpoints, the No-Observed-Adverse-Effect Level (NOAEL) for *Catunaregam nilotica* methanol extract was determined to be 1000 mg/kg body weight/day following repeated oral administration for 28 days.

Discussion

The present study systematically evaluated the sub-acute oral toxicity profile of the methanol extract of *Catunaregam nilotica* root bark following repeated administration over 28 days in albino rats, in accordance with OECD Test Guideline 407. Overall, the findings demonstrate that the extract was well tolerated across all tested dose levels, with no evidence of treatment-related mortality or overt clinical signs of toxicity, thereby indicating a favorable systemic safety profile.

Body weight progression, a sensitive and integrative indicator of physiological and metabolic status in repeated-dose toxicity studies (Haschek *et al*., 2013), remained comparable between treated and control groups throughout the experimental period. The absence of significant differences in weight gain suggests that the extract did not adversely affect metabolic processes, appetite, or nutrient utilization. These observations are consistent with previous studies reporting that non-toxic phytochemicals do not disrupt growth patterns or energy homeostasis during sub-acute exposure (Nouioura *et al*., 2023; Yuet *et al*., 2013; Jegnie *et al*., 2023). The lack of systemic toxicity is further supported by the stability of relative organ weights. The liver and kidneys, as primary sites

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of xenobiotic metabolism and excretion, exhibited no signs of hypertrophy or atrophy. Since alterations in organ weight are widely regarded as early indicators of toxic insult or adaptive responses, their stability in this study suggests that *C. nilotica* does not induce structural or functional stress under the tested conditions. This finding aligns with reports on other medicinal plants where unchanged organ weights correlate with low toxicity potential (Ijioma *et al*., 2018; Aletan *et al*., 2024).

Hematological analysis provided additional evidence of systemic safety (Bamidele *et al.,* 2024). Erythrocytic indices, leukocyte counts, and platelet parameters remained within normal physiological ranges across all treatment groups, indicating that the extract did not adversely affect erythropoiesis, immune competence, or hemostatic balance. The preservation of hematological integrity is a critical indicator of non-toxicity and has been consistently reported in sub-acute toxicity studies of phytochemically rich plant extracts (Hafez *et al*., 2024). In agreement with these findings, serum biochemical parameters showed no significant alterations in hepatic or renal function markers. Liver enzymes (ALT, AST, and ALP), which serve as sensitive indicators of hepatocellular integrity, remained within normal limits, suggesting the absence of hepatocellular injury (Olumoh-Abdul *et al*., 2023). Although slight, non-significant increases in transaminase levels were observed at the highest dose, these changes were not accompanied by histopathological abnormalities, indicating that they likely reflect adaptive metabolic responses rather than toxic effects (Moriles *et al*., 2024) such transient enzyme fluctuations have been previously attributed to enzyme induction or enhanced metabolic activity associated with phytochemical-rich extracts ((Tilaye *et al*., 2018). Similarly, renal function parameters including urea, creatinine, and electrolyte levels remained within normal physiological ranges across all treatment groups, indicating preserved renal function. Given the susceptibility of the kidneys to xenobiotic-induced damage due to their role in excretion, the absence of biochemical perturbations further supports the renal safety of the extract ((Emosho and Bolaji, 2026).

Histopathological examination provided definitive morphological confirmation of these biochemical and hematological findings. The liver, kidneys, and heart tissues of treated animals exhibited normal histoarchitecture, with no evidence of necrosis, inflammatory infiltration, vascular congestion, or degenerative changes. The concordance between normal histology and stable biochemical indices strengthens the validity of the safety assessment and minimizes the likelihood of subclinical organ toxicity (Yuan *et al*., 2014). The favorable safety profile observed in this study may be attributed, in part, to the phytochemical composition of *C. nilotica*, which includes flavonoids, flavonol, and phenolic compounds known for their antioxidant and cytoprotective properties (Foyzun *et al*., 2022). These compounds are capable of mitigating oxidative stress and stabilizing cellular membranes, thereby reducing the risk of tissue injury and contributing to the

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overall tolerability of the extract (Elhassaneen *et al*., 2025). Collectively, the absence of treatment-related adverse effects across all evaluated endpoints supports the establishment of a No-Observed-Adverse-Effect Level (NOAEL) of 1000 mg/kg body weight/day for the methanol extract of *Catunaregam nilotica*. This finding indicates a relatively wide margin of safety and provides scientific support for its traditional use at moderate doses (Agathokleous *et al*., 2021).

Despite these encouraging results, certain limitations should be acknowledged. Sub-acute toxicity studies are inherently limited in their ability to predict long-term, reproductive, or genotoxic effects. Furthermore, variations in extraction methods, phytochemical composition, and dosing regimens may influence toxicity outcomes. Therefore, further studies involving chronic exposure, reproductive toxicity, and mechanistic investigations are warranted to comprehensively establish the safety profile of *C. nilotica* and facilitate its development as a standardized phytotherapeutic or adjunct antivenom agent.

Conclusion

In conclusion, the methanol extract of *Catunaregam nilotica* root bark exhibited no significant sub-acute toxicity following repeated oral administration for 28 days in albino rats. The absence of adverse effects across clinical observations, hematological indices, biochemical parameters, and histopathological evaluations indicates a favorable safety profile and establishes a NOAEL of 1000 mg/kg body weight/day. These findings provide important toxicological evidence supporting the safe use of *C. nilotica* in traditional medicine. Nevertheless, further long-term and mechanistic studies are required to fully elucidate its safety and support its development as a standardized phytopharmaceutical agent.

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Competing Interests

Authors have declared that no competing interests exist.

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