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SUB-ACUTE ORAL TOXICITY EVALUATION OF AQUEOUS LEAF EXTRACT OF *Annona senegalensis* IN WISTAR ALBINO RATS

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Abstract

Annona senegalensis Pers. is widely used in African traditional medicine; however, comprehensive toxicological data supporting its safety following repeated exposure remain limited. This study evaluated the sub-acute oral toxicity of the aqueous leaf extract of *A. senegalensis* using a standardized regulatory framework. Sub-acute toxicity was assessed in accordance with OECD Test Guideline 407. Wistar albino rats (n = 6/group) received the aqueous leaf extract orally at doses of 250, 500, or 1000 mg/kg body weight daily for 28 days, while a control group received distilled water. Clinical signs, body weight changes, and mortality were monitored. At termination, relative organ weights were determined, and blood samples were analyzed for hematological indices and serum biochemical markers of liver and kidney function. Histopathological examination of the liver, kidneys, and heart was also performed. Data were analyzed using one-way ANOVA at $p < 0.05$. No mortality or treatment-related clinical signs were observed throughout the study period. Body weight progression and relative organ weights did not differ significantly between treated and control groups. Hematological parameters remained within normal physiological ranges. Serum biochemical markers of hepatic (ALT, AST, ALP, total protein, albumin, bilirubin) and renal (urea, creatinine, electrolytes) function showed no significant alterations following extract administration. Histopathological evaluation revealed

Keywords: *Annona senegalensis,* Sub-acute oral toxicity, OECD Test Guideline 407, No-Observed-Adverse-Effect Level (NOAEL), Histopathological evaluation, Wistar albino rats.

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preserved tissue architecture with no evidence of treatment-related lesions. Based on the evaluated endpoints, the No-Observed-Adverse-Effect Level (NOAEL) was established at 1000 mg/kg body weight/day. Repeated oral administration of the aqueous leaf extract of *Annona senegalensis* for 28 days did not produce observable adverse effects in Wistar albino rats. These findings provide robust preclinical evidence supporting the sub-acute safety of the extract and substantiate its traditional use, while highlighting the need for further long-term toxicological evaluation.

Introduction

Medicinal plants continue to play a central role in primary healthcare systems worldwide, particularly in low and middle-income countries where access to conventional medicines may be limited. The World Health Organization has reported that a large proportion of the global population relies on traditional medicine for primary healthcare needs (World Health Organization [WHO], 2025). Despite their widespread use and cultural acceptance, many medicinal plants lack comprehensive toxicological evaluation, especially under conditions of repeated or prolonged exposure. The common perception that "natural" products are inherently safe has contributed to gaps in safety data, underscoring the need for systematic toxicological assessment to support their rational and evidence-based use. *Annona senegalensis* Pers. (Annonaceae), commonly known as African custard apple, is a shrub or small tree widely distributed across tropical and sub-Saharan Africa. Various parts of the plant, including the leaves, stem bark, roots, and fruits, are traditionally used for the management of malaria, diarrhea, epilepsy, inflammatory disorders, pain, and snakebite envenomation (Ekor, 2014; Pandey *et al*., 2025). Phytochemical investigations of *A. senegalensis* have revealed the presence of flavonoids, alkaloids, acetogenins, terpenoids, and phenolic compounds, which are believed to contribute to its diverse biological activities (Almamy *et al*., 2021; Odebunmi *et al*., 2022). Experimental studies have reported several pharmacological properties of *A. senegalensis*, including antimicrobial, antioxidant, anti-inflammatory, antinociceptive, and antivenom activities (Diallo *et al*., 2024; Okoye *et al*., 2012). While these findings support its therapeutic potential, pharmacological efficacy does not necessarily equate to safety. Plant-derived bioactive compounds may exert dose-dependent or cumulative toxic effects, particularly when administered repeatedly or over extended periods. Consequently, toxicological evaluation remains a critical component of medicinal plant research. Acute toxicity studies of *A. senegalensis* leaf extracts have generally reported high median lethal dose (LD_{50}) values, suggesting low toxicity following single-dose exposure (Almamy *et al*., 2021; Victoria *et al*., 2019). However, acute

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toxicity data alone are insufficient to predict adverse effects associated with repeated administration. Sub-acute toxicity studies, typically conducted over a 28-day period, provide more relevant information regarding cumulative toxicity, physiological adaptation, and early signs of organ dysfunction. Studies on *A. senegalensis* and related *Annona* species have indicated that repeated exposure may be associated with subtle biochemical or hematological alterations at higher doses, highlighting the importance of dose selection and comprehensive endpoint evaluation (Odebunmi *et al*., 2022; Merlin *et al*., 2019). The liver and kidneys are among the most vulnerable organs to xenobiotic-induced toxicity due to their central roles in metabolism and excretion. Alterations in serum biochemical markers such as alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, urea, and creatinine are widely used indicators of hepatic and renal injury (Pandey *et al*., 2025; Thakur *et al*., 2024; Victoria *et al*., 2019). In addition, hematological parameters provide valuable insight into the effects of test substances on the hematopoietic and immune systems, while histopathological examination offers direct morphological evidence of tissue integrity or damage (Merlin *et al*., 2019; Almamy *et al*., 2021).

To ensure regulatory relevance and methodological consistency, repeated-dose toxicity studies are commonly conducted in accordance with the Organisation for Economic Co-operation and Development (OECD) Test Guideline 407. This guideline recommends the evaluation of clinical signs, body weight changes, hematological and biochemical parameters, relative organ weights, and histopathological features to support the identification of adverse effects and the determination of the No-Observed-Adverse-Effect Level (NOAEL) (Agathokleous *et al*., 2021; OECD, 2025). Establishing a NOAEL is particularly important for medicinal plants, as it provides a scientifically defensible reference point for future toxicological and translational studies. Despite the extensive ethnomedicinal use of *A. senegalensis*, available sub-acute toxicity data remain limited and fragmented, with considerable variation in plant part used, extraction method, dose range, and evaluated endpoints (Merlin *et al*., 2019; Victoria *et al*., 2019). Comprehensive studies integrating hematological, biochemical, relative organ weight, and histopathological assessments under standardized OECD-407 conditions are still scarce.

Therefore, the present study was designed to evaluate the sub-acute oral toxicity of the aqueous leaf extract of *Annona senegalensis* in Wistar albino rats following repeated administration for 28 days. The study systematically assessed clinical observations, body weight progression, relative organ weights, hematological indices, serum biochemical markers of liver and kidney function, and histopathological alterations, with the aim of generating robust preclinical safety data to support the rational use of *A. senegalensis* in traditional and complementary medicine.

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Materials and Methods

Plant Material Collection and Authentication

Fresh leaves of *Annona senegalensis* Pers. (Annonaceae) were collected from Yankaba village Kaura Namoda, Zamfara State, Nigeria. The plant material was authenticated by a qualified taxonomist at the Department of SLT, Federal Polytechnic Kaura Namoda, and a voucher specimen number was deposited in the institutional herbarium for future reference, following standard ethnobotanical documentation practices.

Preparation of Aqueous Leaf Extract

The collected leaves were washed thoroughly with clean water and air-dried under shade at room temperature. The dried leaves were pulverized into a fine powder using a mechanical grinder. A measured quantity of the powdered material was macerated in distilled water for 72hrs with intermittent stirring. The mixture was filtered through muslin cloth followed by Whatman No. 1 filter paper. The filtrate was concentrated on a water bath at 40–45 °C to obtain the aqueous extract, which was stored at 4 °C until use. This extraction approach is consistent with methods commonly employed in toxicological evaluation of medicinal plants (Diallo *et al*., 2024).

Experimental Animals and Ethical Approval

Healthy adult Wistar male albino rats weighing 120–180g, were obtained from the animal facility of Biological Department, Usmanu Danfodiyo University Sokoto. Animal were housed under standard laboratory conditions and provided with standard pellet diet and water ad libitum. The animals were acclimatized for one week prior to experimentation. All experimental procedures were conducted in accordance with institutional guidelines for the care and use of laboratory animals and complied with internationally accepted ethical standards for animal experimentation (OECD, 2008).

Sub-acute Oral Toxicity Study (OECD 407)

The sub-acute oral toxicity study was conducted in accordance with the Organisation for Economic Co-operation and Development (OECD) Test Guideline 407 for repeated-dose 28days oral toxicity in rodents (OECD, 2008). Rats were randomly assigned into four groups (n = 6 per group): a control group receiving distilled water and three extract-treated groups receiving 250, 500, or 1000 mg/kg body weight of the aqueous extract. The extract was administered orally once daily for 28 consecutive days using an oral gavage. Animals were observed daily for clinical signs of toxicity, behavioral changes, and mortality, as recommended under OECD 407.

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Body Weight Monitoring and Relative Organ Weights

Body weights were recorded at baseline and weekly throughout the study period. At the end of the experiment, animals were fasted overnight, anesthetized, and sacrificed. The liver, kidneys, heart, and spleen were excised, rinsed in normal saline, blotted dry, and weighed. Relative organ weights were calculated as:

Relative organ weight (%) = (organ weight / final body weight) × 100

Relative organ weight assessment was performed to detect potential organ hypertrophy or atrophy following repeated exposure (OECD, 2008).

Hematological Analysis

Blood samples were collected via cardiac puncture into EDTA-containing tubes for hematological evaluation. Hematological parameters, including red blood cell count (RBC), hemoglobin concentration (Hb), packed cell volume (PCV), total white blood cell count (WBC), and platelet count (PLT), were analyzed using an automated hematology analyzer. These indices were selected as sensitive indicators of hematopoietic, immunological, and hemostatic effects of xenobiotics (Arthur *et al*., 2011).

Serum Biochemical Analysis

Blood samples for biochemical analysis were collected into plain tubes, allowed to clot, and centrifuged to obtain serum. Serum biochemical parameters were analyzed using Randox diagnostic reagent kits in accordance with manufacturers' instructions.

Liver Function Parameters

Serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) activities were measured as indices of hepatocellular integrity and biliary function, while total protein, albumin, and total bilirubin were determined to assess hepatic synthetic and excretory capacity.

Kidney Function Parameters

Renal function was evaluated by measuring serum urea and creatinine concentrations, along with electrolytes (sodium and potassium), to assess glomerular filtration efficiency and electrolyte homeostasis.

Histopathological Examination

Representative samples of the liver, kidneys, and heart were fixed in 10% buffered formalin for at least 24hrs. The tissues were processed routinely, embedded in paraffin wax, sectioned at 4–5µm thickness, and stained with hematoxylin and eosin (H&E).

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Stained sections were examined under a light microscope for evidence of cellular degeneration, necrosis, inflammation, or other pathological alterations, as recommended for repeated-dose toxicity studies (OECD, 2008).

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

The No-Observed-Adverse-Effect Level (NOAEL) was determined based on the absence of treatment-related adverse effects across all evaluated endpoints, including clinical observations, body weight changes, hematological indices, serum biochemical markers, relative organ weights, and histopathological findings, in accordance with OECD Test Guideline 407 (OECD, 2008).

Human Equivalent Dose (HED) Estimation

For comparative toxicological interpretation, the NOAEL determined in rats was extrapolated to a Human Equivalent Dose (HED) using body surface area normalization, as recommended by regulatory authorities for interspecies dose conversion (Nair and Jacob, 2016; FDA, 2005). This extrapolation was performed strictly for scientific discussion and risk-assessment context and does not imply clinical safety or therapeutic dosing.

Statistical Analysis

Data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Differences were considered statistically significant at $p < 0.05$.

Results and Discussion

Clinical Observations and Mortality

No mortality or treatment-related adverse clinical signs were observed in any experimental group throughout the 28-day oral administration period. Animals in all treated groups appeared active and exhibited normal feeding and grooming behavior, comparable to the control group.

Body Weight Changes

All experimental groups showed a progressive increase in body weight over the 28-day study period. No statistically significant differences ($p > 0.05$) were observed between extract-treated groups and the control group, indicating that repeated administration of the aqueous leaf extract did not adversely affect growth or general metabolic status (Table 1).

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Table 1. Body weight changes in rats treated with aqueous leaf extract of *Annona senegalensis*

Group	Initial Weight (g)	Final Weight (g)	Weight Gain (g)
Control	128.4 ± 2.1[a]	146.2 ± 2.8 [a]	17.8 ± 1.2 [a]
250 mg/kg	129.0 ± 2.0 [a]	145.6 ± 2.5 [a]	16.6 ± 1.0 [a]
500 mg/kg	127.8 ± 1.9 [a]	144.2 ± 2.3 [a]	16.4 ± 1.1 [a]
1000 mg/kg	128.2 ± 2.2 [a]	143.0 ± 2.6 [a]	14.8 ± 1.3 [a]

Values are mean ± SD (n = 6). Values with the same superscript letter within a column are not significantly different ($p > 0.05$).

Relative Organ Weights

Relative weights of the liver, kidneys, heart, and spleen did not differ significantly ($p > 0.05$) between extract-treated groups and the control group, indicating the absence of organ hypertrophy or atrophy following repeated oral administration of the aqueous leaf extract of *Annona senegalensis* (Table 2).

Table 2. Relative organ weights of rats treated with aqueous leaf extract of *Annona senegalensis*

Group	Liver (%)	Kidneys (%)	Heart (%)	Spleen (%)
Control	3.45 ± 0.12[a]	0.72 ± 0.04 [a]	0.33 ± 0.02 [a]	0.41 ± 0.03 [a]
250 mg/kg	3.48 ± 0.15[a]	0.74 ± 0.05 [a]	0.34 ± 0.03 [a]	0.40 ± 0.04 [a]
500 mg/kg	3.52 ± 0.14[a]	0.75 ± 0.04 [a]	0.35 ± 0.02 [a]	0.42 ± 0.03 [a]
1000 mg/kg	3.56 ± 0.16[a]	0.76 ± 0.05 [a]	0.36 ± 0.03 [a]	0.43 ± 0.04 [a]

Values are expressed as mean ± SD (n = 6). Values with the same superscript letter within a column are not significantly different ($p > 0.05$).

Hematological Parameters

Hematological indices remained within normal physiological ranges across all treatment groups. No significant differences ($p > 0.05$) were observed in RBC, hemoglobin concentration, packed cell volume, total WBC count, differential leukocyte counts, or platelet count when compared with the control group, indicating absence of hematotoxic or immunotoxic effects (Table 3).

Table 3. Effects of *A. senegalensis* extract on hematological parameters

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 ± 0.7 [a]	43.8 ± 1.7 [a]	6.4 ± 0.5 [a]	290 ± 20 [a]
500 mg/kg	6.9 ± 0.3 [a]	14.4 ± 0.6 [a]	43.5 ± 1.6 [a]	6.5 ± 0.4 [a]	289 ± 19 [a]
1000 mg/kg	6.8 ± 0.4 [a]	14.3 ± 0.7 [a]	42.9 ± 1.8 [a]	6.6 ± 0.5 [a]	288 ± 20 [a]

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Values are expressed as mean ± SD (n = 6). Values with the same superscript letter within a column are not significantly different (p > 0.05).

Serum Biochemical Parameters

Liver Function Indices

Serum levels of ALT, AST, and ALP showed no statistically significant alterations (p > 0.05) in extract-treated groups compared with the control. Similarly, total protein, albumin, and bilirubin concentrations remained within normal physiological limits (Table 4).

Table 4. Effects of *A. senegalensis* extract on liver function parameters

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	31.1 ± 1.5 [a]	14.0 ± 0.6 [a]	85.0 ± 3.4 [a]	7.48 ± 0.13 [a]	6.75 ± 0.20 [a]	0.44 ± 0.03 [a]
500 mg/kg	33.6 ± 1.7 [a]	15.2 ± 0.7 [a]	86.3 ± 3.6 [a]	7.32 ± 0.15 [a]	6.68 ± 0.22 [a]	0.45 ± 0.04 [a]
1000 mg/kg	39.8 ± 2.1 [a]	18.4 ± 0.9 [a]	88.9 ± 4.1 [a]	7.21 ± 0.14 [a]	6.61 ± 0.21 [a]	0.46 ± 0.04 [a]

Values are expressed as mean ± SD (n = 6). Values with the same superscript letter within a column are not significantly different (p > 0.05).

Kidney Function Indices

Repeated administration of the extract did not produce significant changes (p > 0.05) in serum urea, creatinine, sodium, or potassium levels when compared with the control group (Table 5).

Table 5. Effects of *A. senegalensis* extract on kidney function parameters

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	33.0 ± 1.6 [a]	0.83 ± 0.06 [a]	142.0 ± 2.2 [a]	4.3 ± 0.3 [a]
500 mg/kg	34.5 ± 1.7 [a]	0.87 ± 0.06 [a]	141.6 ± 2.4 [a]	4.4 ± 0.4 [a]
1000 mg/kg	36.4 ± 1.8 [a]	0.91 ± 0.07 [a]	141.2 ± 2.3 [a]	4.5 ± 0.4 [a]

Values are expressed as mean ± SD (n = 6). Values with the same superscript letter within a column are not significantly different (p > 0.05).

Histopathological of Liver and Kidney

Histological examination of liver sections revealed normal hepatocellular architecture, with preserved hepatic cords, central veins, and sinusoids across all treatment groups. No evidence of necrosis, inflammatory infiltration, or cellular degeneration was observed, corroborating the normal serum transaminase and protein levels (Fig. 1). Kidney sections showed intact glomeruli and renal tubules, with no signs of tubular necrosis or interstitial

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congestion (Fig. 2).

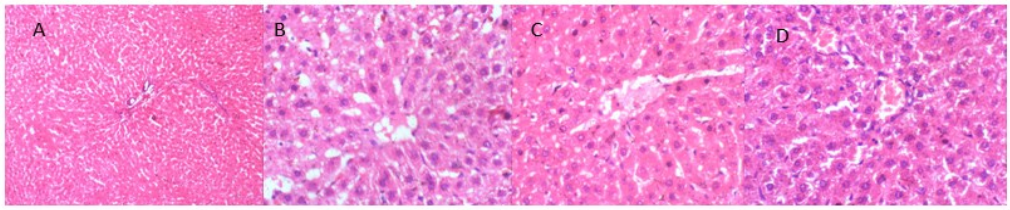


Figure 1. Liver histology (H&E, X100)
Representative liver sections from control (A) and treated groups (B-D, 250–1000 mg/kg) showing preserved hepatic architecture with intact central vein and portal triad. No degenerative or inflammatory changes were

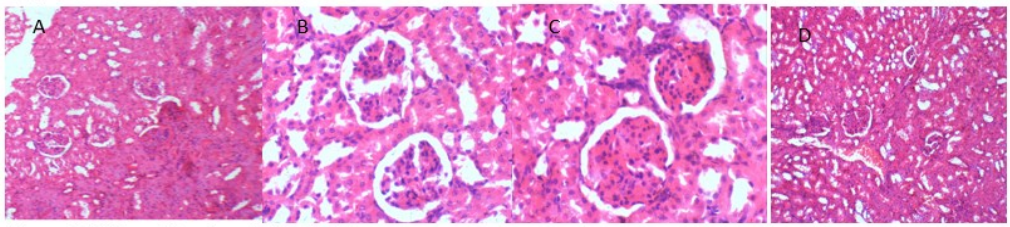


Figure 2. Kidney histology (H&E, X100)
Representative kidney sections from control (A) and treated groups, (B-D, 250–1000 mg/kg) showing normal glomeruli and renal tubules without evidence of structural damage.

Discussion

The present study evaluated the sub-acute oral toxicity of the aqueous leaf extract of *Annona senegalensis* following repeated administration for 28 days in Wistar male albino rats, in accordance with the repeated-dose toxicity framework recommended by the Organisation for Economic Co-operation and Development (OECD) Test Guideline 407. Sub-acute toxicity studies are essential for identifying cumulative physiological responses and early target-organ effects that may not be apparent in acute toxicity assessments. The integration of clinical observations, body weight monitoring, hematological analysis, serum biochemical profiling, organ weight evaluation, and histopathological examination provides a comprehensive framework for assessing the safety profile of medicinal plant extracts during repeated exposure. At the systemic level, the absence of mortality and treatment-related clinical signs indicates that the extract was well tolerated throughout the experimental period. Behavioral parameters such as feeding activity, locomotion, and grooming remained normal, suggesting that the extract did not induce neurobehavioral disturbances or systemic physiological stress (Okoye *et al*., 2012; Diallo *et al*., 2024). Body weight progression across all treatment groups also remained comparable to the control group, indicating that repeated administration of the extract did not interfere with nutrient assimilation, metabolic efficiency, or endocrine regulation of growth. In toxicological studies, sustained body weight gain is generally considered a sensitive indicator of systemic tolerance and metabolic stability. Relative organ weight analysis further supports the absence of systemic toxicity (OECD, 2008). Toxicants frequently

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induce organ hypertrophy or atrophy due to metabolic overload, inflammation, or cellular degeneration. In the present study, the relative weights of the liver, kidneys, and heart did not differ significantly from those of the control group, suggesting that repeated administration of the extract did not trigger compensatory structural changes in these metabolically active organs. Similar findings have been reported in toxicological studies involving *Annona senegalensis*, where sub-acute administration of plant extracts produced minimal changes in organ weights and physiological indices in experimental animals (Ugwah-Oguejiofor *et al*., 2024).

Hematological parameters provide valuable insight into the effects of xenobiotics on the hematopoietic and immune systems. In the present study, red blood cell count, hemoglobin concentration, packed cell volume, white blood cell count, and platelet levels remained within normal physiological ranges. These findings suggest that repeated administration of the extract did not impair erythropoiesis, bone marrow function, or immune competence. Stability of hematological indices during repeated exposure is particularly important because hematopoietic tissues are highly sensitive to toxic agents that interfere with cellular proliferation and oxidative balance (Okoye *et al*., 2012; Arthur *et al*., 2011). The biochemical evaluation of hepatic and renal function further supports the safety profile of the extract. The liver is the primary site for xenobiotic metabolism and is therefore highly susceptible to toxic injury. In this study, serum levels of alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase did not differ significantly between treated and control animals, indicating that hepatocyte membrane integrity and biliary function were preserved. Elevation of these enzymes typically reflects hepatocellular damage caused by oxidative stress, mitochondrial dysfunction, or inflammatory processes. The stability of these enzymes therefore suggests that the extract did not induce hepatotoxic effects during repeated exposure. These findings are consistent with recent experimental evidence demonstrating hepatoprotective effects of *Annona senegalensis* leaf extracts against aflatoxin-induced liver injury, where administration of the extract significantly reduced serum liver enzyme levels and promoted hepatocyte regeneration (Diallo *et al*., 2024; Okoye *et al*., 2012; Makhuvele *et al*., 2024). Similarly, renal function markers remained stable throughout the study. Serum urea and creatinine levels, which reflect glomerular filtration capacity and renal clearance efficiency, were not significantly altered in treated animals. The maintenance of electrolyte balance further indicates that renal tubular transport mechanisms remained intact. Because the kidneys play a central role in the elimination of xenobiotics and their metabolites, the absence of biochemical disturbances strongly supports the nephro-safety of the extract at the administered doses. The biochemical findings were corroborated by histopathological examination of vital organs. Liver sections from treated animals exhibited normal hepatocellular architecture with intact hepatic cords,

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well-defined central veins, and normal sinusoidal spaces. Kidney tissues showed preserved glomerular and tubular structures without evidence of necrosis, inflammation, or degeneration. Cardiac tissues also retained normal myocardial fiber organization. The strong concordance between biochemical indices and histological observations strengthens that repeated administration of the extract did not induce sub-clinical tissue damage; (Nweke *et al*., 2019; Arthur *et al*., 2011). Multi-endpoint agreement of this nature is considered an important criterion in regulatory toxicology because it enhances the reliability of safety assessment. The favorable safety profile observed in this study may be partly explained by the phytochemical composition of *Annona senegalensis*. The plant contains a diverse range of bioactive compounds including flavonoids, phenolic compounds, alkaloids, terpenoids, and acetogenins. These phytochemicals are known to exhibit antioxidant and cytoprotective properties that can mitigate oxidative stress generated during xenobiotic metabolism. For instance, flavonoids and phenolic compounds can scavenge reactive oxygen species, stabilize cellular membranes, and enhance endogenous antioxidant defense systems. Experimental studies have also demonstrated that *A. senegalensis* extracts possess significant antioxidant activity and can improve hematological and biochemical abnormalities in disease models, suggesting that these phytochemicals may contribute to the maintenance of physiological homeostasis during repeated exposure (Enechi, 2023).

Comparative studies further support the relatively favorable toxicological profile of *A. senegalensis*. Sub-acute and sub-chronic toxicity investigations in rodents have generally reported minimal alterations in biochemical and hematological parameters following repeated administration of the plant extract. For example, recent studies evaluating the safety of aqueous methanol extracts of *A. senegalensis* stem bark in Wistar rats reported no significant adverse effects on hematological or biochemical parameters after repeated dosing, suggesting a relatively wide safety margin for the plant when administered at moderate doses (Ugwah-Oguejiofor *et al*., 2024). Despite these encouraging findings, certain limitations should be acknowledged. The present study evaluated sub-acute toxicity over a 28-day exposure period using only male animals. Consequently, potential long-term effects such as chronic toxicity, reproductive toxicity, or sex-specific physiological responses cannot be fully excluded. Future investigations involving longer exposure durations, both sexes, and molecular biomarkers of oxidative stress, inflammation, and mitochondrial function would provide deeper insight into the mechanistic toxicology of *Annona senegalensis*.

The results of the present study provide important preclinical evidence supporting the short-term safety of the aqueous leaf extract of *Annona senegalensis*. These findings contribute to the growing body of toxicological data required for the rational

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development of plant-derived therapeutics and support the continued scientific validation of traditional medicinal plants within evidence-based pharmacology.

Conclusion

The present study demonstrates that repeated oral administration of the aqueous leaf extract of *Annona senegalensis* for 28days was well tolerated in Wistar albino rats, with no evidence of systemic or organ-specific toxicity. The absence of mortality, clinical toxicity signs, and significant changes in body weight, hematological parameters, serum biochemical markers, relative organ weights, and histopathological architecture indicates that the extract did not adversely affect hepatic, renal, cardiovascular, or hematopoietic functions under the conditions of the study. In accordance with the criteria of the Organisation for Economic Co-operation and Development Test Guideline 407 for repeated-dose toxicity studies, the No-Observed-Adverse-Effect Level (NOAEL) was determined to be 1000mg/kg body weight/day following 28days of oral administration.

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Competing Interests

Authors have declared that no competing interests exist.

References

Agathokleous, E., Moore, M., & Calabrese, E. (2021). Estimating the no-observed-adverse-effect-level (NOAEL) of hormetic dose-response relationships in meta-data evaluations. *MethodsX, 8*(101568), 25-30. doi: 10.1016/j.mex.2021.101568.

Almamy K., Franck, D., Richard, W. S., Lotfi, F., Norah, B., Valérie, M., . . . Olivier, C. (2021). Therapeutic potential of Annona senegalensis Pers (Annonaceae), a plant used in traditional medicine for the treatment of epilepsy in Burkina Faso. *African Journal of Pharmacy and Pharmacology, 15*(1), 1-9. doi: DOI: 10.5897/AJPP2020.5203

Arthur, F. K. N., Woode, E., Terlabi, E. O., & Larbie, C. (2011). Evaluation of acute and sub-chronic toxicity of *Annona muricata* (Linn.) aqueous extract in animals. *European Journal of Experimental Biology, 1*(4), 115–124.

Diallo D., Drame, A., Niang, L., Badock, E., Diallo I., Sow, S., & Ayessou, N. (2024). Phytochemical Screening and Antioxidant Activity of Annona Senegalensis Extracts (Leaves and Stem Bark) Collected from Three Regions of Senegal. *Advance Biochemistry, 12*(3), 105-111. doi: https://doi.org/10.11648/j.ab.20241203.13

Ekor, M. (2014). The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Frontier Pharmacology, 4* (177), 12-22. doi: doi: 10.3389/fphar.2013.00177

Enechi, O. C. (2023). Attenuation of haematological and biochemical alterations by *Annona senegalensis* leaf extract in diabetic rats. *Proceedings*, 56(1), 263.

Makhuvele, R., Foubert, K., Hermans, N., Pieters, L., Verschaeve, L., & Elgorashi, E. (2024). Hepatoprotective effects of leaf extract of *Annona senegalensis* against aflatoxin B1 toxicity in rats. *Onderstepoort Journal of Veterinary Research*, 91(1), e1-e6. doi: 10.4102/ojvr. v91i1.2133

EDITOR-IN-CHIEF
Prof. E. Mclenna [Australia]

Merlin, L., Mensah, G. A., Forkuo, C. A., & D., R. A. (2019). Toxicity and Safety Implications of Herbal Medicines Used in Africa. *Herbal Medicine*, 20-35. doi:10.5772/intechopen.72437

Nair, A., & Jacob, S. (2016). A simple practice guide for dose conversion between animals and human. *Journal of Basic Clinical Pharmacy. , 7*(2), 27-31. doi:10.4103/0976-0105.177703.

Nweke, E. O., Ndukwe, G. U., & Opara, J. K. (2019). Ethanolic extract of Annona muricata leaf and its effect on the liver. *Journal of Drug Delivery and Therapeutics, 9*((3-s)), 463-466. doi: http://dx.doi.org/10.22270/jddt.v9i3-s.2880

Odebunmi C., Adetunji, T., Adetunji, A., Olatunde, A., Oluwole, O., Adewale, I., . . . Aremu, T. (2022). Ethnobotanical Survey of Medicinal Plants Used in the Treatment of COVID-19 and Related Respiratory Infections in Ogbomosho South and North Local Government Areas, Oyo State, Nigeria. *Plants (Basel)., 11*(19), 2667. doi: doi: 10.3390/plants11192667

Okoye T., Akah, P., Ezike, A., Okoye M., Onyeto, C., Ndukwu, F., . . . Ikele, L. (2012). Evaluation of the acute and sub acute toxicity of Annona senegalensis root bark extracts. *Asian Pac J Trop Med., 5*(4), 277-82. doi: doi:10.1016/S1995-7645(12)60039-X

Organisation for Economic Co-operation and Development (OECD) (2008). *OECD guideline for the testing of chemicals: Repeated dose 28-day oral toxicity study in rodents (Test Guideline 407).* Organisation for Economic Co-operation and Development, Paris.

Organisation for Economic Co-operation and Development (OECD). (2025). *Test No. 407: Repeated dose 28-day oral toxicity study in rodents*. OECD Publishing.

Pandey, A., singh, A., kumar, J. S., Singh, A., & Deeba, F. (2025). Ethnopharmacology and Pharmacognosy: Intersections in The Search for Novel Therapeutic Agents. *International Journal of Pharmacognosy and Herbal Drug Technology, 2*(8), 79-94. doi: https://doi.org/10.64063/3049-1630.vol.2.issue8.7

Thakur, S., Kumar, V., Das, R., Sharma, V., & Mehta, D. (2024). Biomarkers of Hepatic Toxicity: An Overview. *Curr Ther Res Clin Exp., 100*(100737), 55-60. doi:0.1016/j.curtheres.2024.100737.

U.S. Food and Drug Administration. (2005). *Guidance for industry: Estimating the maximum safe starting dose in initial clinical trials for therapeutics in adult healthy volunteers.* Center for Drug Evaluation and Research.

Ugwah-Oguejiofor, C., Nefai, M., Emmanuel, M., Alkali, I., Biambo, A., Lawal, M., . . . Muhammed, A. (2024). Safety assessment of crude aqueous methanol extract of Annona senegalensis stem bark: acute and sub-chronic toxicity studies. *Drug Chem Toxicol., 47*(6), 1155-1164. doi:10.1080/01480545.2024.2344640.

Victoria A., Ansari, A., Rizwanb, E., Karimah, M. R., Otanwa, O. O., & Amah, A. (2019). Toxicological Evaluation of Selected Medicinal Plants on Hepatic and Renal Functions. *International Journal of Natural Sciences: Current and Future Research Trends, 4*(1), 1-8.

World Health Organization. (2025). *WHO global report on traditional and complementary medicine 2023*. Geneva: WHO.

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