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# Twenty-eight-day repeated-dose oral toxicity study of fresh *Costus lucanusianus* stem sap in Wistar rats: biochemical, oxidative stress, and histopathological evaluation

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**Background:** *Costus lucanusianus* Lam. is widely utilized in traditional medicine across parts of West Africa for the treatment of inflammatory disorders, fever, gastrointestinal ailments, and wound healing. Despite its extensive ethnomedicinal use, scientific evidence regarding the safety of repeated oral consumption of the fresh stem sap remains limited.

**Methods:** This study investigated the biochemical, oxidative stress, and histopathological effects of 28-day repeated-dose oral administration of fresh *Costus lucanusianus* stem sap in male Wistar rats. Twenty healthy adults male Wistar rats (90 to 156 g) were randomly allocated into four groups (n = 5). The control group received distilled water, while treatment groups received freshly expressed *C. lucanusianus* stem sap at doses of 200, 400, and 600 mg/kg body weight by oral gavage once daily for 28 consecutive days. Following treatment, serum biochemical indices of hepatic and renal function, lipid profile, hepatic oxidative stress biomarkers, and histopathological changes in the spleen and pancreas were evaluated using standard laboratory procedures.

**Results:** Repeated administration of the stem sap produced selective biochemical alterations. Serum alkaline phosphatase activity increased significantly in a dose-dependent manner ($p < 0.05$), whereas creatinine and potassium concentrations exhibited treatment-dependent variations, with the greatest changes occurring at 400 mg/kg. Animals receiving the intermediate dose also demonstrated elevated serum total cholesterol, triglycerides, low-density lipoprotein cholesterol, and very low-density lipoprotein cholesterol, accompanied by a reduction in high-density lipoprotein cholesterol. Oxidative stress assessment revealed significantly increased hepatic malondialdehyde concentration together with reduced superoxide dismutase, catalase, and reduced glutathione activities at 400 mg/kg, indicating enhanced lipid peroxidation and compromised antioxidant defence. Histopathological examination showed mild lymphoid depletion and distortion of splenic architecture, as well as vacuolar degeneration and increased cellularity of pancreatic islet cells. These alterations were most evident in animals treated with 400 mg/kg, whereas the severity of histological alterations was reduced in animals receiving 600 mg/kg compared with the 400 mg/kg group.

**Conclusion:** Repeated oral administration of fresh *C. lucanusianus* stem sap produced mild to moderate biochemical, oxidative, and histopathological alterations. The observed non-linear dose-response suggests that the biological effects were not directly proportional to dose, although the mechanisms underlying this pattern remain unknown. These findings indicate relatively low 28-day repeated-dose oral toxicity under the experimental conditions employed but support the need for chronic toxicity studies, phytochemical standardization, and mechanistic investigations before the long-term safety of repeated consumption can be established.

***Keywords:*** *Costus lucanusianus, stem sap, 28-day repeated-dose oral toxicity, oxidative stress, histopathology, Wistar rats*

## Introduction

Medicinal plants continue to serve as an important source of healthcare for millions of people worldwide, particularly in developing countries where accessibility, affordability, and cultural acceptance favour the use of traditional herbal remedies. The World Health Organization estimates that nearly 80% of the population in many developing regions depends on herbal medicines for primary healthcare (WHO, 2013). In Nigeria, medicinal plants remain integral to indigenous healthcare systems, where fresh plant preparations are frequently consumed without standardised dosage or comprehensive safety evaluation. Although numerous medicinal plants have demonstrated significant pharmacological potential, prolonged or repeated exposure may produce adverse biological effects that are not apparent during acute toxicity studies (Sofowora et al., 2008). The increasing global interest in medicinal plants has stimulated extensive investigations into their phytochemical composition and pharmacological activities. Bioactive compounds such as flavonoids, phenolic acids, alkaloids, saponins, tannins, terpenoids, and glycosides have been associated with diverse biological activities, including antioxidant, anti-inflammatory, antimicrobial, and metabolic effects (Sharma et al., 2025; Devi et al., 2026; Wink, 2015) These phytochemicals contribute to the therapeutic efficacy of medicinal plants by modulating oxidative stress, inflammatory signalling pathways, and cellular metabolism. However, several naturally occurring phytochemicals also exhibit dose-dependent toxicity and may exert pro-oxidant effects when administered repeatedly or at relatively high concentrations, resulting in oxidative injury and organ dysfunction (Adinortey et al., 2012; Rocha et al., 2021). *Costus lucanusianus* Lam. (Family: *Costaceae*) is a perennial rhizomatous herb indigenous to the tropical rainforests of West and Central Africa. The plant is commonly found in southern Nigeria, particularly within the Niger Delta, where it is locally known as "Ukpete." Fresh stem sap extracted from the succulent stem is traditionally consumed for the management of fever, inflammatory disorders, gastrointestinal disturbances, body pains, wounds, and other ailments (Boison et al., 2019; Rocha et al., 2021). Unlike solvent extracts that undergo drying and chemical processing, the fresh stem sap represents the form most frequently consumed by local communities and therefore constitutes the preparation with the greatest public health relevance. Previous investigations involving *Costus* species have primarily focused on ethnobotanical documentation, phytochemical characterization, antimicrobial activity, antioxidant potential, hypoglycaemic effects, and acute toxicity evaluation (Ekpe et al., 2016). Acute toxicity studies conducted on the fresh stem sap of *C. lucanusianus* demonstrated no mortality following oral administration of doses up to 5000 mg/kg body weight, suggesting a relatively low level of acute toxicity. Nevertheless, acute toxicity studies provide only preliminary information regarding safety because they evaluate biological responses following a single exposure and may fail to detect cumulative or delayed toxic effects that develop after repeated administration. Consequently, 28-day repeated-dose oral toxicity studies remain essential for establishing the safety profile of medicinal plants intended for prolonged traditional use. Repeated exposure to phytochemicals may alter physiological homeostasis through several mechanisms, among which oxidative stress has received considerable attention. Oxidative stress develops when the production of reactive oxygen species exceeds the capacity of endogenous antioxidant systems, leading to lipid peroxidation, protein oxidation, DNA damage, mitochondrial dysfunction, and eventual cellular injury. Endogenous antioxidant enzymes, including superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH), constitute the primary enzymatic defence system against reactive oxygen species, whereas malondialdehyde (MDA), a product of membrane lipid peroxidation, serves as a reliable biomarker of oxidative damage (Aebi, 1984; McCord & Fridovich, 1969). Simultaneous assessment of oxidative stress biomarkers, serum biochemical indices, and tissue histopathology therefore provides a comprehensive evaluation of organ toxicity and offers insight into the underlying mechanisms of tissue injury. Serum biochemical parameters remain valuable indicators of the functional integrity of vital organs during toxicological investigations. Alterations in hepatic enzymes, including alanine aminotransferase, aspartate aminotransferase and alkaline phosphatase, together with changes in renal biomarkers such as urea and creatinine, frequently precede overt histopathological lesions and provide early evidence of organ dysfunction. Similarly, disturbances in serum lipid profile may reflect altered lipid metabolism arising from hepatic injury or oxidative stress, thereby contributing additional information regarding systemic toxicity. Although several studies have examined the pharmacological properties of members of the genus *Costus*, comprehensive repeated-dose toxicity studies involving the fresh stem sap of *C. lucanusianus* remain scarce. Existing reports have rarely integrated biochemical assessment with oxidative stress biomarkers and histopathological evaluation using the preparation commonly consumed in traditional medicine. This lack of evidence limits the scientific validation of the plant's safety and hinders the development of evidence-based recommendations regarding its prolonged use. Therefore, the present study evaluated the 28-day repeated-dose oral toxicological profile of freshly expressed *Costus lucanusianus* stem sap through integrated biochemical, oxidative stress and histopathological assessments in male Wistar rats. Serum biochemical indices of hepatic and renal function, lipid profile, hepatic antioxidant status, lipid peroxidation, and histological architecture of the spleen and pancreas were evaluated to provide an integrated assessment of the sub-acute toxicological profile of the plant. The

findings are expected to contribute to the growing body of evidence supporting the safe use, standardization, and future pharmacological development of *C. lucanusianus*.

## Materials and methods

**Study design:** This study employed a completely randomized experimental design to evaluate the 28-day repeated-dose toxicity of freshly expressed *Costus lucanusianus* stem sap following repeated oral administration in Wistar rats. Toxicological assessment was based on serum biochemical indices, hepatic oxidative stress biomarkers, and histopathological examination of selected organs after 28 days of treatment.

**Collection and authentication of plant material:** Fresh stems of *Costus lucanusianus* Lam. were collected from naturally growing plants in Mgbere Community, Ibaa, Emohua Local Government Area, Rivers State, Nigeria, during a single harvesting period to minimize seasonal variation in phytochemical composition. The plant was authenticated by a taxonomist in the Department of Plant Science and Biotechnology, University of Port Harcourt, Nigeria, where a voucher specimen (UPH/P/612) was deposited in the departmental herbarium for future reference.

**Preparation of fresh stem sap:** Freshly harvested stems were thoroughly washed with clean water to remove adhering soil and debris before being cut into small sections. The stems were mechanically macerated using a sterile mortar and pestle, and the expressed sap was filtered through sterile muslin cloth to remove fibrous materials and particulate matter. Fresh sap was prepared daily throughout the experimental period to minimize degradation of labile phytochemical constituents and to simulate the traditional mode of preparation. The prepared sap was stored in sterile containers at approximately 4°C and administered within a short period after preparation. The mass of stem material used per preparation, sap yield, dry-matter concentration and administration volume were not prospectively recorded. Consequently, the administered doses cannot be retrospectively expressed as a standardised sap concentration or volume-based dose. The nominal doses of 200, 400 and 600 mg/kg body weight were those used in the experimental protocol. Because the test material consisted of freshly expressed crude stem sap rather than a standardised extract, some variation in phytochemical composition between daily preparations was unavoidable. To minimize this variability, stems were collected from the same geographical location during a single harvesting period, processed using the same extraction procedure, and administered immediately after preparation under standardised laboratory conditions. This lack of quantitative physicochemical characterization should be considered a limitation of the present study. Future investigations should incorporate measurement of extraction yield, dry-matter content, administration volume, and phytochemical composition to improve dose standardization and reproducibility.

**Experimental animals:** Twenty healthy adult male Wistar rats (*Rattus norvegicus*) weighing between 90 and 156 g were obtained from the Animal House, Faculty of Pharmaceutical Sciences, University of Port Harcourt, Nigeria. Male animals were selected to eliminate physiological variations associated with the oestrous cycle in females. Animals were acclimatized for 14 days before commencement of the experiment under standard laboratory conditions of temperature (25 ± 2°C), relative humidity (60 to 80%), and a 12-hour light-dark cycle. Standard laboratory pellet diet and clean drinking water were provided *ad libitum* throughout the study.

The animals were observed daily during acclimatization to ensure good health before inclusion in the experiment.

**Experimental design and treatment:** Following acclimatization, the animals were randomly assigned to four experimental groups comprising five rats per group (n = 5).

**Group I (Control):** Distilled water
**Group II:** Fresh *Costus lucanusianus* stem sap (200 mg/kg body weight)
**Group III:** Fresh *Costus lucanusianus* stem sap (400 mg/kg body weight)
**Group IV:** Fresh *Costus lucanusianus* stem sap (600 mg/kg body weight)

The selected doses were based on the preliminary acute oral toxicity study, in which no mortality was observed after oral administration of freshly expressed *Costus lucanusianus* stem sap up to 5000 mg/kg body weight. Consequently, the experimental doses of 200, 400, and 600 mg/kg represented 4%, 8%, and 12%, respectively, of the highest acute dose administered (5000 mg/kg), at which no mortality was observed. Dose selection was further guided by published repeated-dose toxicity studies involving related *Costus* species and by the traditional consumption of the fresh stem sap, for which standardised therapeutic doses have not been established. The selected dose range therefore aimed to evaluate the toxicological effects of repeated oral exposure rather than replicate a defined human therapeutic dose. The test substance was administered once daily by oral gavage for 28 consecutive days between 08:00 and 10:00 a.m. The nominal treatment doses were 200, 400, and 600 mg/kg body weight. The actual administration volumes in mL/kg were not prospectively

recorded. Animals had unrestricted access to standard laboratory feed and clean drinking water throughout the study. Animals were observed daily for clinical signs of toxicity and behavioural changes, including alterations in feeding behaviour, locomotor activity, grooming, posture, respiration, and mortality. Each experimental group comprised five animals (n = 5), a sample size consistent with many exploratory 28-day repeated-dose toxicity studies involving laboratory rodents and compatible with the principle of Reduction within the 3Rs framework (Replacement, Reduction, and Refinement) for the ethical use of experimental animals. Although this sample size permitted assessment of multiple toxicological endpoints while minimizing animal use, no formal *a priori* statistical power calculation was performed during study design. This should be considered when interpreting the findings, particularly for endpoints exhibiting substantial biological variability.

**Acute oral toxicity study:** An acute oral toxicity study was conducted before the 28-day repeated-dose oral toxicity experiment using the method described by Lorke (1983). The study consisted of two sequential phases designed to estimate the median lethal dose ($LD_{50}$) following single oral administration. In Phase I, nine rats were randomly assigned to three groups and administered single oral doses of 10, 100, and 1000 mg/kg body weight, respectively. In Phase II, three additional rats each received single oral doses of 1600, 3000, and 5000 mg/kg body weight. Animals were continuously observed during the first 24 hours and thereafter for 72 hours for mortality and behavioural abnormalities, including salivation, tremors, changes in locomotion, feeding behaviour, respiratory pattern, and responsiveness to external stimuli. No mortality or severe treatment-related clinical signs were observed at any of the administered doses during the observation period. Based on the experimental design described by Lorke, the median lethal dose ($LD_{50}$) was therefore estimated to exceed 5000 mg/kg body weight under the conditions of this study. Since only one animal was evaluated at each dose during Phase II, this value should be interpreted as an estimate derived from the study design rather than a precisely determined $LD_{50}$. The findings nevertheless indicate that freshly expressed *Costus lucanusianus* stem sap possesses relatively low acute oral toxicity.

**Sample collection:** At the end of the 28-day treatment period, the animals were fasted overnight while water remained freely available. Each rat was anaesthetised, and blood samples were collected by cardiac puncture. Blood intended for biochemical analysis was transferred into plain sample tubes, allowed to clot, and centrifuged to obtain serum. Following blood collection, the animals were sacrificed, and the spleen and pancreas were carefully excised, rinsed in ice-cold physiological saline to remove residual blood, and processed for histopathological examination. Portions of liver tissue were immediately homogenized for oxidative stress analysis. Organ weights were unfortunately not recorded during tissue collection because the study protocol had initially focused on biochemical and histological endpoints.

**Serum biochemical analysis:** Serum biochemical analyses were performed using a Spectrum Map Lab Plus automated chemistry analyzer with commercially available diagnostic reagent kits according to the manufacturer's instructions. Markers of hepatic function included alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and total bilirubin (TB). Renal function was evaluated using serum creatinine, urea, blood urea nitrogen (BUN), and electrolyte concentrations, including potassium. Lipid profile analysis included determination of total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and very low-density lipoprotein cholesterol (VLDL-C). These parameters were selected because alterations in hepatic enzymes, renal biomarkers, and lipid metabolism provide sensitive indicators of systemic toxicity following repeated exposure to medicinal plant preparations.

**Determination of hepatic oxidative stress biomarkers:** Fresh liver tissues were excised immediately after sacrifice, rinsed in ice-cold physiological saline to remove residual blood, blotted dry, weighed, and homogenized (10% w/v) in ice-cold 0.1 M phosphate buffer (pH 7.4). The homogenates were centrifuged at 4,000 rpm for 15 minutes at 4°C, and the resulting supernatants were used for oxidative stress assays. Lipid peroxidation was evaluated by measuring malondialdehyde (MDA) concentration using the thiobarbituric acid reactive substances (TBARS) method described by Ohkawa et al. (1979). The intensity of the pink chromogen formed was measured spectrophotometrically at 532 nm, and MDA concentration was expressed as nmol/mg protein. Superoxide dismutase (SOD) activity was determined according to the method of McCord & Fridovich (1969), based on the enzyme's ability to inhibit the autoxidation of pyrogallol. Catalase (CAT) activity was measured using the spectrophotometric method of Aebi (1984), which quantifies the decomposition of hydrogen peroxide at 240 nm. Reduced glutathione (GSH) concentration was determined using Ellman's reagent (5,5′-dithiobis-(2-nitrobenzoic acid)) according to the method of Ellman (1959), with absorbance measured at 412 nm. Collectively, these biomarkers were used to evaluate the balance between oxidant generation and antioxidant defence following repeated oral administration of fresh *Costus lucanusianus* stem sap.

**Histopathological examination:** Immediately after sacrifice, spleen and pancreas tissues were fixed in 10% neutral buffered formalin for 48 hours before routine histological processing. Fixed tissues were dehydrated through ascending grades of ethanol, cleared in xylene, infiltrated with molten paraffin wax, and embedded in paraffin blocks. Sections

approximately 5 µm thick were prepared using a rotary microtome and mounted on clean glass slides. Sections were stained with haematoxylin and eosin (H&E) following standard histological procedures and examined using a light microscope. Representative photomicrographs were captured at ×100 and ×400 magnifications. Histological assessment focused on tissue architecture, cellular degeneration, inflammatory cell infiltration, vascular congestion, vacuolation, necrosis and other observable pathological alterations. The histopathological findings were interpreted qualitatively and, where applicable, using the semi-quantitative lesion scoring system described in the Results section. The original experimental protocol did not prospectively document the number of microscopic fields examined per section, predefined criteria for field selection, or blinded assessment of the histological sections. These methodological limitations were therefore not retrospectively reconstructed and should be considered when interpreting the histopathological findings.

**Statistical analysis:** Data are presented as mean ± standard deviation (SD). Statistical analyses were performed using IBM SPSS Statistics version 25.0 (IBM Corp., Armonk, NY, USA). Data normality was assessed using the Shapiro-Wilk test, while homogeneity of variance was evaluated using Levene's test. Differences among treatment groups were analysed using one-way analysis of variance (ANOVA), followed by Tukey's Honestly Significant Difference (HSD) post hoc test for pairwise comparisons where significant differences were observed. Statistical significance was accepted at $p < .05$.

**Statistical analysis**

Data analysis using SPSS version 28, p-value 0.05 for significant differences, mean± SE and least significant differences (LSD) post hoc test if ANOVA p-value is less than 0.05 (Ab Rahman, 2015).

## Results

### Acute oral toxicity

Acute oral administration of freshly expressed *Costus lucanusianus* stem sap produced no mortality or severe clinical signs of toxicity throughout the observation period. Mild transient reductions in spontaneous activity were observed at the higher dose levels but resolved without intervention. Based on the Lorke acute toxicity protocol employed, the median lethal dose ($LD_{50}$) was estimated to be greater than 5000 mg/kg body weight. This estimate reflects the absence of mortality within the experimental design and should not be interpreted as a precisely determined $LD_{50}$ value. The findings indicate relatively low acute oral toxicity under the conditions of this study, as shown in Table 1.0.

**Table 1. Acute oral toxicity evaluation of fresh *Costus lucanusianus* stem sap in Wistar rats.**

| Phase | Dose (mg/kg) | Number of animals | Mortality | Clinical observation |
|---|---|---|---|---|
| I | 10 | 3 | 0/3 | No observable toxicity |
| I | 100 | 3 | 0/3 | No observable toxicity |
| I | 1000 | 3 | 0/3 | No observable toxicity |
| II | 1600 | 1 | 0/1 | Mild transient behavioural changes |
| II | 3000 | 1 | 0/1 | Mild transient behavioural changes |
| II | 5000 | 1 | 0/1 | Mild transient behavioural changes |

$LD_{50}$ > 5000 mg/kg body weight.

### Effect of fresh *Costus lucanusianus* stem sap on body weight

Weekly body-weight measurements showed an overall increase in body weight in all experimental groups throughout the 28-day treatment period (Table 2). Baseline body weights differed significantly among the treatment groups (one-way ANOVA, $F(3,16) = 8.67$, $p = .0012$).

**Table 2. Weekly body weight (g) of Wistar rats following repeated oral administration of fresh *Costus lucanusianus* stem sap (Mean ± SD, n = 5).**

| Group | Day 0 (Baseline) | Week 1 | Week 2 | Week 3 | Week 4 |
|---|---|---|---|---|---|
| Control | 98.8 ± 4.15 | 97.0 ± 4.36 | 101.0 ± 12.39 | 125.0 ± 1.87 | 126.0 ± 3.16 |
| 200 mg/kg | 107.2 ± 10.06 | 104.6 ± 10.48 | 107.0 ± 12.32 | 122.8 ± 11.53 | 126.6 ± 10.21 |
| 400 mg/kg | 123.2 ± 9.20 | 120.2 ± 8.82 | 117.6 ± 7.67 | 132.6 ± 12.21 | 135.4 ± 11.82 |
| 600 mg/kg | 123.2 ± 11.73 | 120.0 ± 11.58 | 124.2 ± 10.30 | 142.8 ± 8.51 | 146.6 ± 8.32 |

**Note:** Values are presented as mean ± standard deviation (SD) (n = 5 animals per group). Body weights were recorded before commencement of treatment (Day 0) and weekly thereafter during the 28-day repeated-dose oral toxicity study. Baseline body weights differed significantly among treatment groups (one-way ANOVA, $F(3,16) = 8.67$, $p = .0012$).

Weekly body-weight values and percentage changes from baseline to Week 4 are presented descriptively because no repeated-measures or other longitudinal statistical analysis was performed.

Because no repeated-measures or other longitudinal statistical analysis was performed, changes in body weight over time are presented descriptively and are not interpreted as statistically significant treatment effects. Percentage weight change from baseline to Week 4 was 29.9%, 21.0%, 12.6%, and 22.2% in the control, 200, 400, and 600 mg/kg groups, respectively. Interpretation of these changes should be made cautiously because of the significant baseline imbalance among groups and the absence of quantitative food and water intake data.

**Effect of fresh *Costus lucanusianus* stem sap on serum biochemical parameters**

Repeated oral administration of fresh *C. lucanusianus* stem sap produced selective alterations in serum biochemical indices (Table 3). Serum alkaline phosphatase (ALP) activity increased progressively with dose and was significantly elevated in the treatment groups compared with the control ($p < 0.05$). The highest ALP activity was observed in animals treated with 600 mg/kg. Serum creatinine concentrations increased markedly to 400 mg/kg before declining below control values in the highest-dose group. Potassium concentration also differed significantly among treatment groups, with elevated values observed at 200 mg/kg, followed by lower concentrations at 400 and 600 mg/kg. In contrast, serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, urea, and blood urea nitrogen showed no statistically significant differences compared with the control group (Table 3.0).

**Table 3. Effect of fresh *Costus lucanusianus* stem sap on serum biochemical parameters following 28 days of oral administration (Mean ± SD; n = 5).**

| Parameter | Control | 200 mg/kg | 400 mg/kg | 600 mg/kg | F-value | P-value |
|---|---|---|---|---|---|---|
| Total bilirubin | 11.00 ±0.29 | 12.22 ±4.86 | 10.96 ±6.09 | 11.04 ±1.08 | 0.121 | 0.947 |
| ALT (IU/L) | 9.88 ±0.48 | 7.95 ±0.71 | 8.70 ±1.57 | 8.57 ±1.11 | 2.934 | 0.065 |
| AST (IU/L) | 17.86 ±10.05 | 10.26 ±1.11 | 16.91 ±13.37 | 9.58 ±1.99 | 1.320 | 0.303 |
| ALP (IU/L) | 119.80 ±3.42 | 123.80 ±3.77 | 140.80 ±5.72 | 151.00 ±5.66 | 47.155 | <0.001[a] |
| Potassium (mmol/L) | 8.88 ±0.30 | 10.08 ±1.10 | 5.94 ±2.35 | 6.72 ±0.63 | 10.130 | <0.001[b] |
| Urea (mmol/L) | 7.23 ±0.24 | 7.66 ±2.09 | 8.94 ±1.54 | 8.90 ±2.98 | 0.962 | 0.435 |
| BUN (mg/dL) | 11.86 ±0.42 | 12.54 ±3.42 | 14.64 ±2.53 | 14.54 ±4.87 | 0.944 | 0.443 |
| Creatinine (µmol/L) | 59.20 ±2.77 | 58.20 ±1.79 | 77.40 ±19.28 | 52.60 ±5.27 | 5.647 | 0.008[c] |

Significant at $p < 0.05$.

**Effect of fresh *Costus lucanusianus* stem sap on lipid profile**

Fresh stem sap administration altered serum lipid metabolism (Table 4). The most pronounced changes occurred in animals receiving 400 mg/kg body weight, which exhibited increased concentrations of total cholesterol, triglycerides, LDL cholesterol and VLDL cholesterol, accompanied by reduced HDL cholesterol. Lipid parameters returned towards control values in the 600 mg/kg group, suggesting a non-linear dose-response pattern.

**Table 4. Effect of fresh *Costus lucanusianus* stem sap on serum lipid profile following 28 days of treatment (Mean ± SD; n = 5).**

| Parameter | Control | 200 mg/kg | 400 mg/kg | 600 mg/kg | F-value | P-value |
|---|---|---|---|---|---|---|
| Total cholesterol (mg/dL) | 84.20 ± 3.03[a] | 89.80 ± 3.11[a] | 105.40 ± 5.77[b] | 86.20 ± 2.59[a] | 31.427 | <0.001 |
| Triglycerides (mg/dL) | 77.20 ± 2.59[a] | 84.60 ± 4.04[a] | 101.20 ± 6.61[b] | 81.20 ± 3.49[a] | 28.176 | <0.001 |
| HDL-C (mg/dL) | 45.40 ± 1.14[a] | 41.60 ± 1.14[b] | 36.80 ± 1.92[c] | 43.00 ± 1.58[ab] | 29.910 | <0.001 |
| LDL-C (mg/dL) | 25.00 ± 2.24[a] | 31.60 ± 2.70[a] | 48.00 ± 7.07[b] | 28.20 ± 2.59[a] | 30.322 | <0.001 |
| VLDL-C (mg/dL) | 15.60 ± 0.55[a] | 16.80 ± 0.84[a] | 20.20 ± 1.30[b] | 16.00 ± 0.71[a] | 27.377 | <0.001 |

**Note.** Values are presented as mean ± SD (n = 5). Means within the same row with different superscript letters differ significantly according to Tukey's HSD post-hoc test ($p < 0.05$). Means sharing at least one superscript letter are not significantly different ($p > 0.05$).

**Effect of Fresh *Costus lucanusianus* stem sap on hepatic oxidative stress biomarkers**

Repeated administration of fresh *C. lucanusianus* stem sap produced marked alterations in hepatic antioxidant status (Table 5). Hepatic malondialdehyde concentration increased progressively from the control to the intermediate dose group, indicating enhanced lipid peroxidation. The highest MDA concentration was recorded at 400 mg/kg. Conversely, antioxidant defence markers exhibited an opposite pattern. Activities of SOD and CAT, together with GSH concentration,

declined markedly at the intermediate dose, whereas values at 600 mg/kg were higher than those observed at 400 mg/kg. These findings indicate increased oxidative stress following repeated administration of the stem sap, particularly at the intermediate dose, as shown in Table 5.0.

**Table 5. Effect of fresh *Costus lucanusianus* stem sap on hepatic oxidative stress biomarkers (Mean ± SD; n = 5).**

| Parameter | Control | 200 mg/kg | 400 mg/kg | 600 mg/kg | F-value | P-value |
|---|---|---|---|---|---|---|
| MDA (nmol/mg) | $2.11 \pm 0.10^{a}$ | $2.76 \pm 0.20^{b}$ | $3.65 \pm 0.37^{c}$ | $2.37 \pm 0.15^{ab}$ | 43.333 | <0.001 |
| SOD (U/mg) | $14.84 \pm 0.27^{a}$ | $12.54 \pm 0.79^{b}$ | $9.92 \pm 0.63^{c}$ | $13.24 \pm 0.46^{b}$ | 64.386 | <0.001 |
| CAT (U/mg) | $58.00 \pm 1.58^{a}$ | $51.60 \pm 2.30^{b}$ | $43.00 \pm 3.16^{c}$ | $52.80 \pm 1.92^{b}$ | 36.073 | <0.001 |
| GSH (µmol/g) | $38.40 \pm 1.14^{a}$ | $33.80 \pm 1.92^{b}$ | $27.80 \pm 1.92^{c}$ | $35.20 \pm 1.30^{ab}$ | 38.035 | <0.001 |

**Note.** Values are presented as mean ± SD (n = 5). Means within the same row with different superscript letters differ significantly according to Tukey's HSD post-hoc test ($p < 0.05$), whereas means sharing at least one superscript letter are not significantly different ($p > 0.05$).

**Histopathological findings**

**Spleen**

Microscopic examination of spleen sections from the control group demonstrated preserved splenic architecture, characterized by well-defined white pulp, intact lymphoid follicles, and normal red pulp organization (Plate 1A).
Animals treated with 200 mg/kg exhibited mild congestion without appreciable disruption of tissue architecture. More pronounced histopathological changes were observed at 400 mg/kg, including mild lymphoid depletion, distortion of white pulp architecture and reduced lymphocyte density. Although mild architectural alterations persisted in the 600 mg/kg group, the severity of architectural alterations was reduced in the 600 mg/kg group, with reduced severity of morphological alterations relative to the 400 mg/kg group, as shown in Table 6.0.

**Table 6. Summary of histopathological findings in the spleen.**

| Group | Histopathological observations |
|---|---|
| Control | Normal splenic architecture |
| 200 mg/kg | Mild vascular congestion |
| 400 mg/kg | Lymphoid depletion, architectural distortion |
| 600 mg/kg | Mild architectural distortion with reduced lesion severity. |

**Pancreas**

Histological examination of pancreatic tissues from control animals showed normal acinar organisation with intact islets of Langerhans. Administration of fresh stem sap was associated with morphological alterations that varied across treatment groups, with the greatest lesion severity observed at 400 mg/kg.

**Table 7. Summary of histopathological findings in the pancreas.**

| Group | Histopathological observations |
|---|---|
| Control | Normal pancreatic architecture |
| 200 mg/kg | Mild vacuolation |
| 400 mg/kg | Marked vacuolation, increased islet cellularity |
| 600 mg/kg | Reduced severity of histological alterations. |

Sections from the 200 mg/kg group demonstrated mild cytoplasmic vacuolation. The 400 mg/kg group exhibited the most pronounced lesions, characterized by vacuolar degeneration, increased cellularity of pancreatic islets and mild architectural distortion. Pancreatic sections from animals receiving 600 mg/kg showed reduced severity of the histological alterations observed at 400 mg/kg, with no widespread necrosis or severe inflammatory cell infiltration.

**Semi-quantitative scoring table for pancreatic and splenic lesions**

| Lesion | Control | 200 mg/kg | 400 mg/kg | 600 mg/kg |
|---|---|---|---|---|
| Congestion | 0 | 1 | 2 | 1 |
| Vacuolation | 0 | 1 | 3 | 1 |
| Lymphoid depletion | 0 | 0 | 2 | 1 |

Histopathological lesions were scored semi-quantitatively as follows:
0 = absent, 1 = mild, 2 = moderate, and 3 = severe.

**Figure 1.** Representative H&E-stained spleen sections from rats following 28 days of repeated oral administration of fresh *Costus lucanusianus* stem sap. (A) Control showing normal splenic architecture. (B) 200 mg/kg showing mild vascular congestion. (C) 400 mg/kg showing lymphoid depletion and distortion of the white pulp (arrows). (D) 600 mg/kg showing mild residual architectural distortion with reduced lesion severity compared with the 400 mg/kg group. Scale bar = 100 µm. Original magnification ×400.

**Spleen**

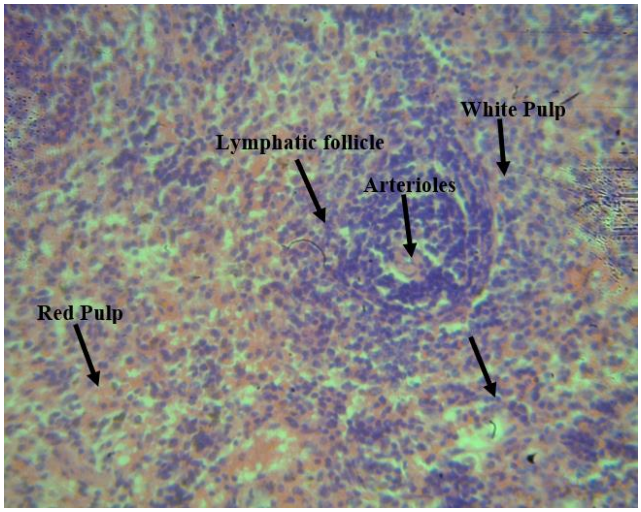



**Plate 1a:** Group 1. Photomicrograph (H&E X400) of the spleen showing the white pulp with lymphatic follicles and granulations with arterioles located at the marginal zone, and visible red pulp with reduced macrophages within the spleen tissue. **Diagnosis:** Normal spleen tissue.

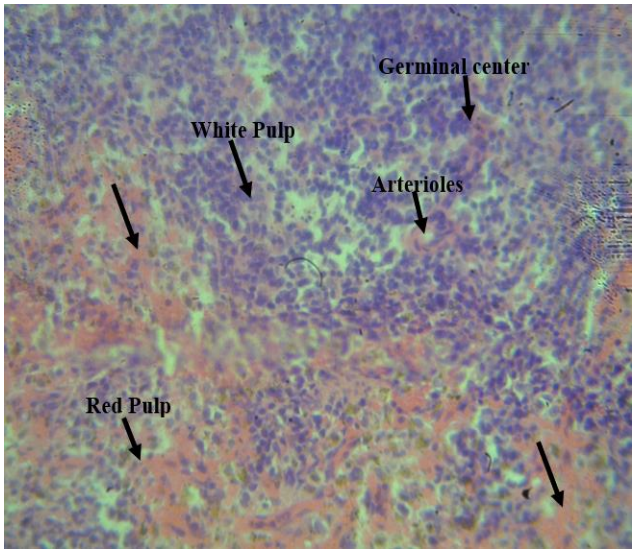


**Plate 1b:** Group 2. Photomicrograph (H&E X400) of the spleen showing normocellularity of lymphoid cells within the white pulp with associated hematologic proliferation of the red pulp. (arrows). **Diagnosis:** Normal spleen tissue with mild distortion.

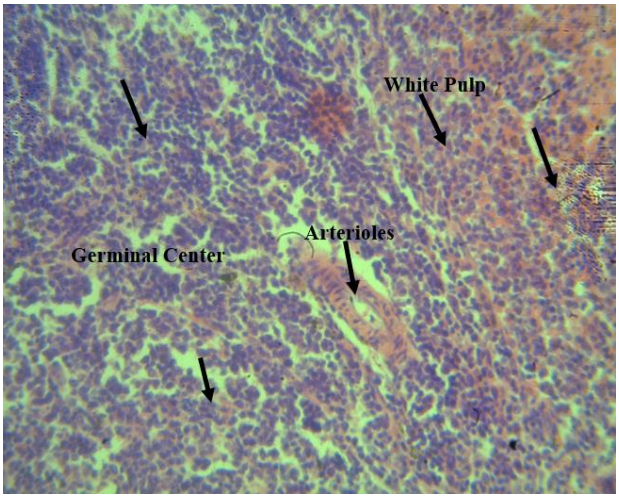



**Plate 1c:** Group 3. Photomicrograph (H&E X400) of the spleen showing formative white pulp with decreased lymphocytic activities, visible arterioles (arrows). **Diagnosis:** Mild distortion of the spleen tissue**.**

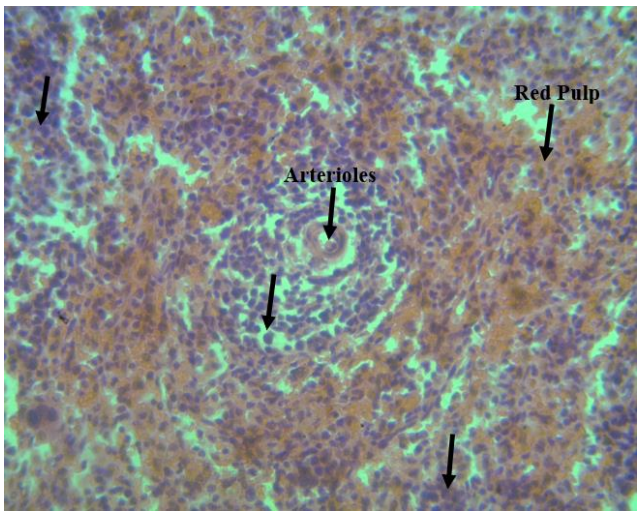



**Plate 1d:** Group 4. Photomicrograph (H&E X400) of the spleen showing the white and red pulp with dispersed lymphocytic proliferation surrounding the mantle and marginal zone of the spleen tissue (arrows).
**Diagnosis:** Mild distortion of the spleen tissue.

**Figure 2.** Representative photomicrographs of pancreatic tissue from rats following 28-day repeated oral administration of fresh Costus lucanusianus stem sap (H&E, ×400). (A) Control group showing normal pancreatic architecture with intact acinar cells and islets of Langerhans. (B) 200 mg/kg showing mild cytoplasmic vacuolation. (C) 400 mg/kg showing vacuolar degeneration, increased islet cellularity, and mild architectural distortion (arrows). (D) 600 mg/kg showing reduced lesion severity compared with the 400 mg/kg group, with mild residual architectural distortion (arrows).

**Pancreas**

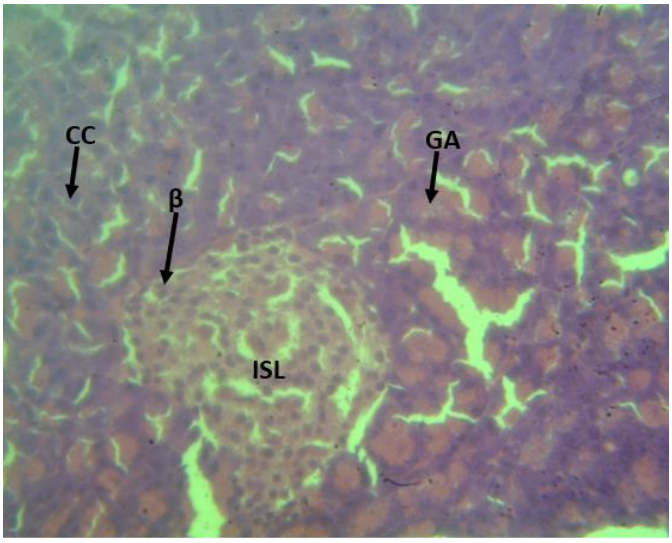



**Plate 2a:** Group 1. Photomicrograph (H&E X400) of the Pancreas showing the normal exocrine pancreas with patterned glandular acini (GA), centroacinar cells (CC), interlobular ducts (S) and Endocrine pancreas with normocellularity of beta cells dispersed evenly in islet of Langerhans (ISL).
**Diagnosis:** Normal appearance of the pancreatic tissue.

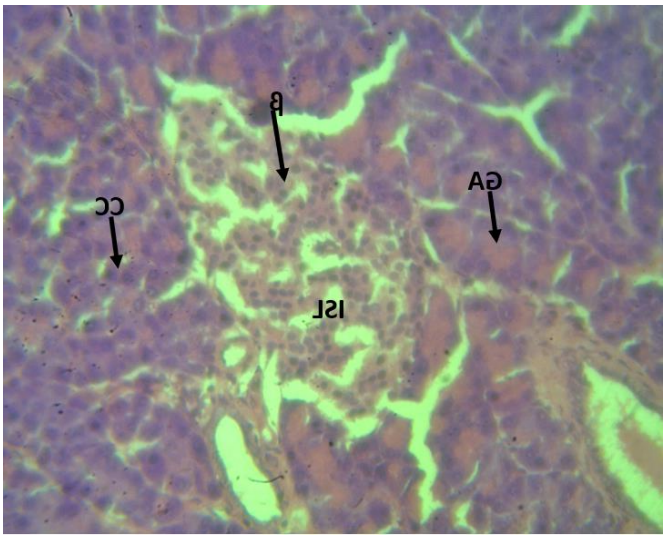



**Plate 2b:** Group 2. Photomicrograph (H&E X400) of the Pancreas showing proliferative vacuolation of the islet of Langerhans associated with hypercellularity of the beta cells.
**Diagnosis:** Vacuolar degeneration of islet cells of Langerhans.

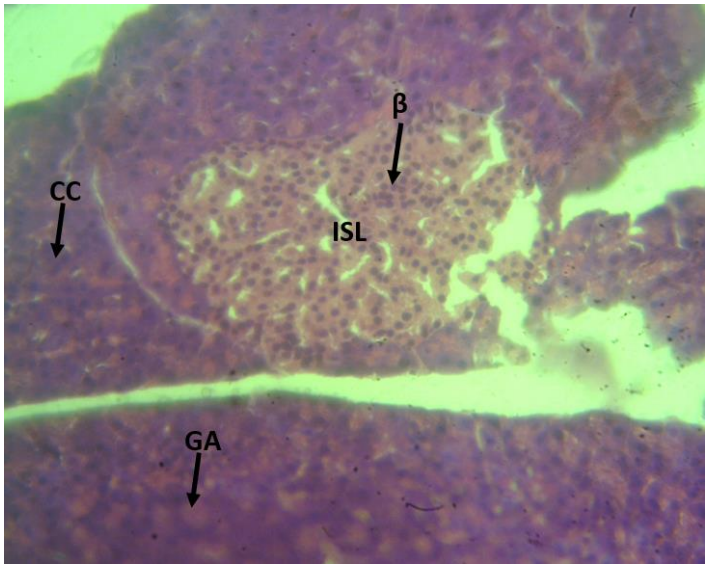



**Plate 2c** Group 3. Photomicrograph (H&E X400) of the Pancreas showing moderate disruption with hypercellularity of the islet (arrows).
**Diagnosis:** Moderate pancreatic islet hypercellularity with mild architectural distortion.

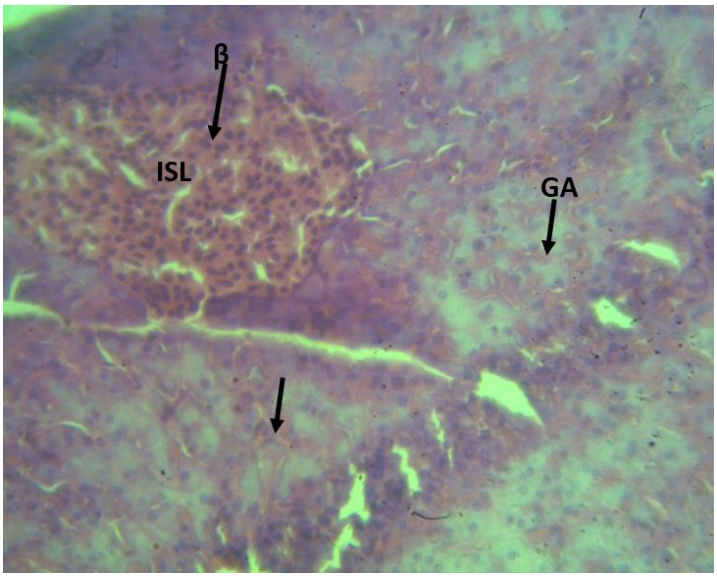



**Plate 2d:** Group 4. Photomicrograph (H&E X400) of the Pancreas showing moderate disruption with hypercellularity of the islet (arrows).
**Diagnosis:** Mild pancreatic islet vacuolar degeneration with reduced lesion severity relative to the 400 mg/kg group.

## Discussion

The present study evaluated the biochemical, oxidative stress, and histopathological effects of repeated oral administration of fresh *Costus lucanusianus* stem sap in male Wistar rats over 28 days. The findings demonstrate that repeated exposure to the fresh stem sap produced selective biochemical and tissue alterations that were predominantly mild to moderate in severity. Notably, the observed responses did not increase proportionally with dose, as the most pronounced biochemical disturbances, oxidative stress, and histopathological lesions occurred in animals administered 400 mg/kg body weight, whereas reduced severity was evident in the 600 mg/kg treatment group. This non-linear pattern indicates that the observed biological effects were not directly proportional to dose. The absence of mortality or severe clinical signs during the treatment period, together with the acute oral toxicity study indicated an $LD_{50}$ greater than 5000 mg/kg body weight. This observation agrees with the general toxicological profile reported for several members of the genus *Costus*, which are widely regarded as possessing broad margins of safety following acute administration (Boison et al., 2019; Kumari et al., 2024). However, acute toxicity studies alone provide limited information regarding cumulative tissue injury because repeated exposure may result in progressive biochemical and cellular alterations that are not evident after a single dose (OECD, 2008). Consequently, the present investigation provides additional evidence regarding the safety profile of *C.*

*lucanusianus* under conditions involving repeated experimental exposure, which may provide information relevant to the repeated traditional use of the plant, although the experimental dosing regimen cannot be directly equated with human exposure. One of the principal findings of this study was the significant elevation of serum alkaline phosphatase (ALP) activity following repeated administration of the stem sap. In contrast, serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and total bilirubin remained largely unchanged. ALP is predominantly associated with hepatobiliary tissues, although it is also present in bone, intestinal mucosa, and other organs. An isolated increase in ALP without corresponding elevations in hepatocellular enzymes generally suggests mild cholestatic changes or membrane perturbations rather than extensive hepatocellular necrosis (Olatunji et al., 2016). The preservation of normal ALT and AST activities in the present study therefore indicates that repeated administration of fresh *C. lucanusianus* stem sap did not produce widespread hepatocellular injury despite evidence of mild hepatobiliary stress. This interpretation should be considered alongside the biochemical findings because histopathological examination of liver tissue was not performed in the present study. Consequently, the observed biochemical alterations cannot be directly correlated with hepatic microscopic changes. Serum creatinine concentrations showed an isolated increase in the 400 mg/kg group before returning to values comparable to the control group at 600 mg/kg. Although serum creatinine is widely used as an indicator of renal function, interpretation of this finding should be cautious because no corresponding changes were observed in serum urea or blood urea nitrogen, and renal histopathological examination was not performed. Therefore, the observed increase should be regarded as an isolated biochemical alteration rather than definitive evidence of renal dysfunction.

Similarly, serum potassium concentrations differed among treatment groups, with relatively large variability observed in the 400 mg/kg group. Because serum potassium is susceptible to pre-analytical influences, including sample haemolysis and handling procedures, and these factors were not specifically evaluated in the present study, the biological significance of the observed differences cannot be established with certainty. Further studies incorporating confirmatory electrolyte measurements and renal histopathological assessment are required to clarify whether these findings represent treatment-related effects. The lipid profile findings provide further evidence that repeated administration of fresh *C. lucanusianus* stem sap influences intermediary lipid metabolism. Animals treated with 400 mg/kg exhibited significantly higher concentrations of total cholesterol, triglycerides, LDL-cholesterol, and VLDL-cholesterol, together with significantly reduced HDL-cholesterol, compared with the control group and, for most parameters, the other treatment groups. These alterations are consistent with treatment-related disturbances in lipid metabolism. The liver is central to cholesterol synthesis, lipoprotein metabolism, and triglyceride homeostasis; consequently, disturbances in hepatic function may be accompanied by alterations in circulating lipid concentrations (Nelson & Cox, 2021). Increased oxidative stress may also promote lipid peroxidation and influence lipoprotein metabolism, potentially contributing to the observed changes in serum lipid concentrations. Interestingly, lipid parameters in the 600 mg/kg group generally approached those observed in the control group. This pattern suggests that the biochemical response to fresh stem sap was not strictly dose-dependent and may reflect a non-linear toxicological response. However, the mechanisms underlying this apparent attenuation at the higher dose cannot be established from the present findings alone.

A particularly important finding was the significant, non-monotonic alteration in hepatic oxidative stress biomarkers. The 400 mg/kg dose produced the greatest oxidative disturbance, characterised by a significant increase in malondialdehyde (MDA) and significant reductions in superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH). MDA concentrations increased from 2.11 ± 0.10 nmol/mg in the control group to 3.65 ± 0.37 nmol/mg at 400 mg/kg, with a significant overall treatment effect ($F = 43.333$, $p < 0.001$). Tukey's HSD analysis further demonstrated that the 400 mg/kg group differed significantly from the control, 200 mg/kg, and 600 mg/kg groups. MDA is a relatively stable end-product of lipid peroxidation and is widely used as an indicator of oxidative damage to cellular membranes (Ayala et al., 2014). The marked increase in MDA at 400 mg/kg therefore indicates enhanced lipid peroxidation and is consistent with increased oxidative stress. SOD activity showed a significant treatment effect, with the lowest activity recorded at 400 mg/kg (9.92 ± 0.63 U/mg) compared with 14.84 ± 0.27 U/mg in the control group ($F = 64.386$, $p < 0.001$). CAT activity showed a similar pattern, declining from 58.00 ± 1.58 U/mg in the control group to 43.00 ± 3.16 U/mg at 400 mg/kg ($F = 36.073$, $p < 0.001$). GSH concentration also decreased significantly, from 38.40 ± 1.14 µmol/g in the control group to 27.80 ± 1.92 µmol/g at 400 mg/kg ($F = 38.035$, $p < 0.001$). SOD, CAT, and GSH constitute important components of the endogenous antioxidant defence system involved in the neutralisation of superoxide radicals, hydrogen peroxide, and other reactive oxygen species (Aebi, 1984; McCord & Fridovich, 1969). The simultaneous elevation of MDA and reductions in SOD, CAT, and GSH at 400 mg/kg are therefore consistent with a shift towards oxidative imbalance and reduced antioxidant capacity.

Notably, the oxidative stress biomarkers did not demonstrate a progressive dose-dependent response. At 600 mg/kg, MDA decreased to 2.37 ± 0.15 nmol/mg, while SOD, CAT, and GSH increased to 13.24 ± 0.46 U/mg, 52.80 ± 1.92 U/mg, and 35.20 ± 1.30 µmol/g, respectively. Tukey's HSD analysis showed that the 600 mg/kg group was not significantly different from the 200 mg/kg group for SOD and CAT, while MDA and GSH values shared statistical groupings with both the control and 200 mg/kg groups. Collectively, these findings support a non-monotonic biochemical response, with the

greatest oxidative perturbation occurring at the intermediate dose rather than at the highest dose. Although adaptive antioxidant responses, differences in absorption or metabolism, and other pharmacokinetic factors could potentially contribute to such a pattern, these mechanisms were not directly investigated in the present study and therefore require further experimental confirmation. The relationship between oxidative stress and phytochemical exposure is complex because many plant-derived polyphenols exhibit dual biological activities depending on concentration, exposure duration, and cellular redox state. At lower concentrations, flavonoids and phenolic compounds may exert antioxidant effects by scavenging free radicals and enhancing endogenous antioxidant enzyme activity. However, under certain conditions, these same compounds may undergo auto-oxidation or redox cycling, thereby generating reactive oxygen species and exerting pro-oxidant effects (Wink, 2015; Rocha et al., 2021). Such concentration-dependent behaviour has been reported for medicinal plants and may provide a possible context for the marked oxidative stress observed at the intermediate dose in the present study. However, this interpretation remains tentative because the phytochemical constituents responsible for the observed effects were not characterized in the present investigation. An important observation in the present study was that several biochemical, oxidative stress, and histopathological changes were less pronounced in animals receiving 600 mg/kg than in those receiving 400 mg/kg. This finding indicates that the biological response to repeated administration of fresh *Costus lucanusianus* stem sap was non-linear and not strictly dose-dependent. The present study did not measure molecular signalling, gene expression, pharmacokinetics, or other mechanistic endpoints that could explain this pattern. Therefore, mechanisms such as cellular adaptation, hormesis, or Nrf2 activation cannot be established from the present findings. These mechanisms may represent possible explanations, but they remain speculative. Future mechanistic studies incorporating phytochemical profiling, pharmacokinetic assessment, and molecular endpoints are required to determine the biological basis of the observed non-linear response.

Studies involving other members of the genus *Costus* provide useful context for interpreting the present findings. Investigations on *Costus* afer have consistently demonstrated antioxidant, anti-inflammatory, nephroprotective, and hepatoprotective activities, which have largely been attributed to its abundance of flavonoids, phenolic compounds, steroidal saponins and terpenoids. Experimental studies have further shown that extracts of *C. afer* can reduce lipid peroxidation and enhance endogenous antioxidant enzyme activities in chemically induced models of oxidative stress (Rocha et al., 2021). Although the present study also demonstrates biological activity of *C. lucanusianus*, the transient oxidative stress observed at the intermediate dose suggests that differences in phytochemical composition, extraction method, or dosage may influence the toxicological profile of individual *Costus* species. Similarly, *Costus* speciosus has been widely reported to possess hepatoprotective, antidiabetic, and antioxidant properties. Experimental studies have shown that extracts of *C. speciosus* improve hepatic antioxidant status by increasing the activities of superoxide dismutase, catalase, and glutathione while simultaneously reducing malondialdehyde concentrations in animal models of chemically induced liver injury (Eliza et al., 2009; Kumari et al., 2024). These findings contrast with the temporary reduction in antioxidant defence observed in the present investigation following administration of fresh *C. lucanusianus* stem sap. The discrepancy may be attributable to differences in plant species, phytochemical composition, extraction solvent, dosage regimen or duration of exposure. Unlike solvent extracts, the freshly expressed stem sap used in the present study likely contains a distinct profile and concentration of water-soluble bioactive constituents that may influence its biological effects.

Comparable findings have also been reported for *Costus pictus*, a medicinal plant recognized for its antidiabetic and antioxidant activities. Extracts of *C. pictus* have been shown to attenuate oxidative stress and preserve tissue architecture in experimental models of diabetes, thereby supporting the protective effects of several *Costus* species against oxidative injury (Daisy et al., 2008). Collectively, these reports indicate that although members of the genus share several pharmacologically active phytochemicals, important interspecies differences exist in their toxicological and therapeutic profiles. The present study therefore provides novel information specific to freshly expressed *C. lucanusianus* stem sap and highlights the importance of evaluating each medicinal species independently rather than extrapolating safety data from related taxa. The histopathological findings in the spleen and pancreas complemented the biochemical and oxidative stress findings by demonstrating that repeated administration of the stem sap was associated with mild tissue alterations. However, because liver and kidney tissues were not examined histologically, direct correlations between biochemical markers of hepatic or renal function and microscopic tissue changes cannot be made. Histological examination of the spleen revealed mild lymphoid depletion, reduced cellular density within the white pulp, and distortion of splenic architecture, particularly in animals receiving 400 mg/kg. The spleen plays a critical role in immune surveillance, antigen presentation, lymphocyte maturation, and the removal of damaged erythrocytes. Consequently, structural alterations within splenic tissue may indicate transient immunomodulatory effects or oxidative injury to lymphoid cells (Abbas et al., 2021). Nevertheless, the lesions observed in the present study were relatively mild and were not accompanied by extensive necrosis, fibrosis, or severe inflammatory infiltration, suggesting that immune tissue integrity remained largely preserved.

Similarly, pancreatic sections demonstrated vacuolar degeneration and increased cellularity of the islets of Langerhans, with the greatest severity occurring at the intermediate dose. Vacuolar degeneration can occur in response to cellular or

metabolic stress (Kumar et al., 2021). Pancreatic sections from animals receiving 600 mg/kg showed less severe morphological alterations than those observed at 400 mg/kg. This finding is appropriately described as reduced lesion severity rather than recovery or restoration because the study did not include a recovery period or longitudinal histopathological assessment. The concordance between the biochemical, oxidative stress, and histopathological findings suggests that oxidative stress may have been associated with the observed tissue alterations. However, because mechanistic pathways were not directly investigated, this interpretation should be regarded as tentative. Elevated MDA concentrations coincided with reduced antioxidant enzyme activities and GSH concentrations, together with histological evidence of cellular alterations in the spleen and pancreas. This integrated pattern is consistent with an association between oxidative imbalance and tissue alterations; however, a causal sequence cannot be established from the present study. Similar relationships between oxidative stress biomarkers and histopathological lesions have been described in repeated-dose toxicity studies involving other medicinal plants rich in phenolic compounds (Kumari et al., 2024; Rocha et al., 2021).

The study demonstrates measurable biological effects following repeated administration of fresh *C. lucanusianus* stem sap. Although no mortality or severe clinical signs were observed, ALP, lipid parameters, oxidative stress biomarkers, creatinine, and potassium showed treatment-related differences, while mild-to-moderate histological alterations were observed in the spleen and pancreas. The 400 mg/kg group showed the clearest adverse biochemical and oxidative changes. These findings indicate that the absence of overt clinical toxicity did not preclude measurable biochemical, oxidative, and histopathological responses to repeated exposure. Accordingly, the observed effects should be interpreted as evidence of treatment-related biological responses rather than definitive evidence of clinically significant toxicity. Although several parameters differed statistically among treatment groups, statistical significance does not necessarily imply biological or toxicological importance. The magnitude of change, inter-animal variability, consistency across related endpoints, and absence of overt clinical toxicity should therefore be considered when interpreting the biological relevance of the findings. The non-linear pattern observed across several endpoints, particularly the greater severity of biochemical, oxidative, and histopathological changes at 400 mg/kg compared with 600 mg/kg, further indicates that the biological responses were not directly proportional to dose. The present study provides an integrated toxicological evaluation of freshly expressed *Costus lucanusianus* stem sap using biochemical, oxidative stress, and histopathological endpoints. Unlike previous investigations that primarily focused on ethnomedicinal use, phytochemical composition, or acute toxicity, the present work demonstrates that repeated exposure was associated with organ-specific biochemical and histopathological alterations accompanied by changes in hepatic oxidative-stress biomarkers. These findings contribute to the current understanding of the toxicological profile of *C. lucanusianus*.

## Conclusion

The present study demonstrated that repeated oral administration of freshly expressed *Costus lucanusianus* stem sap for 28 days produced selective biochemical, oxidative stress, and histopathological alterations in male Wistar rats. The most pronounced changes occurred at 400 mg/kg body weight and included increased alkaline phosphatase activity, altered lipid profile, increased hepatic lipid peroxidation, reduced antioxidant defence, and mild histological alterations in the spleen and pancreas. Although no mortality or severe clinical signs of toxicity were observed during the study period, these findings indicate that repeated exposure to the fresh stem sap is associated with measurable biological effects under the experimental conditions employed. The observed non-linear dose-response warrants further investigation, and longer-term studies incorporating comprehensive histopathological and mechanistic evaluations are required before conclusions regarding long-term safety can be made.

### Study Limitations

The present study has several limitations that should be considered when interpreting the findings. First, only male Wistar rats were included, and potential sex-related differences in toxicological responses were therefore not evaluated. Second, although the study followed a 28-day repeated-dose design, it does not provide information on the effects of longer-term exposure. Chronic toxicity studies are therefore required to establish the safety of prolonged consumption. Third, freshly expressed stem sap was administered as a crude preparation without quantitative determination of extraction yield, dry matter content, or phytochemical standardization. Consequently, batch-to-batch variation in phytochemical composition cannot be excluded, which may influence reproducibility across studies. Fourth, histopathological examination was limited to the spleen and pancreas. Liver and kidney tissues were not examined microscopically, preventing direct correlation between biochemical findings and structural changes in these organs. Fifth, absolute and relative organ weights were not recorded. Organ-weight assessment is an important endpoint in repeated-dose toxicity studies and would have strengthened interpretation of potential target-organ effects. Sixth, food and water consumption were not quantitatively monitored during the treatment period. Consequently, changes in nutritional intake cannot be excluded as possible

contributors to variations in body weight or biochemical parameters. Seventh, histopathological assessment was qualitative and semi-quantitative, and the original study protocol did not prospectively document the number of microscopic fields examined per section, predefined field-selection criteria, or blinded assessment. These limitations may introduce observer or sampling variability and should be considered when interpreting the histopathological findings. Finally, no prospective sample size or statistical power calculation was performed before the study. Although the sample size was consistent with many exploratory repeated-dose toxicity studies, the relatively small number of animals per group may have limited the ability to detect subtle biological differences. Future investigations should incorporate larger sample sizes, standardised phytochemical characterization of the fresh sap, comprehensive organ-weight measurements, histopathological evaluation of additional organs including the liver and kidneys, monitoring of food and water intake, and mechanistic analyses to provide a more complete assessment of the toxicological profile of *Costus lucanusianus* stem sap.

**Future Perspectives**

Future investigations should extend the present findings through comprehensive repeated-dose toxicity studies conducted over 90 days or longer in accordance with OECD guidelines for chronic toxicity assessment. Such studies should incorporate both male and female animals to determine possible sex-related differences in biological response. Comprehensive phytochemical profiling using high-performance liquid chromatography (HPLC), gas chromatography-mass spectrometry (GC-MS), and liquid chromatography-tandem mass spectrometry (LC-MS/MS) should be undertaken to identify and quantify the major bioactive constituents present in the fresh stem sap. Isolation of individual phytochemicals would facilitate correlation between specific compounds and observed toxicological effects. Mechanistic studies should evaluate oxidative stress signalling pathways, inflammatory cytokines, apoptotic proteins, and hepatic phase I and phase II drug-metabolizing enzymes to clarify the adaptive responses observed following repeated administration. Investigation of transcriptional regulators such as nuclear factor erythroid 2-related factor 2 (Nrf2), nuclear factor-kappa B (NF-κB), and mitogen-activated protein kinase (MAPK) signalling pathways would provide valuable mechanistic information. Histopathological assessment was qualitative and semi-quantitative. Because the study protocol did not prospectively define the number of microscopic fields examined per section, criteria for field selection, or observer blinding, the histological findings should be interpreted with appropriate caution. Future studies should incorporate standardised lesion-scoring protocols, blinded assessment, and predefined sampling strategies to improve reproducibility. Future studies should also assess reproductive toxicity, developmental toxicity, genotoxicity, and pharmacokinetic behaviour before recommendations regarding long-term therapeutic use can be made. Standardization of the fresh stem sap based on its phytochemical composition would further facilitate its development as a safe and evidence-based herbal medicinal product.

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## Author contribution

Conceptualization: HOA, VTI, MES; Methodology: HOA, VTI, MES; Formal analysis: MES; Investigation: MES; Literature search: MES; Supervision: HOA, VTI; Writing – original draft: MES; Writing – review & editing: HOA, VTI, MES; Project administration: MES; Final approval: HOA, VTI, MES.

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## Conflict of interest

The authors declare that there are no competing financial or personal interests that could have influenced the work reported in this manuscript.

## Ethical approval

All experimental procedures involving animals were reviewed and approved by the University of Port Harcourt Research Ethics Committee, University of Port Harcourt, Nigeria (Approval No. UPH/R&D/REC/EXEC/004/034; 29 April 2026). Animal handling and experimental procedures complied with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and the Organization for Economic Co-operation and Development (OECD) guidelines for laboratory animal experimentation.

## AI tool declaration

The authors have not used AI and it's related tools to write this manuscript. The authors declare that no AI and related tools are used to write the scientific content of this manuscript.

## Data availability

The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

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