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# Acute and Sub-chronic (90 days) Toxicity Assessments of Hydroethanol Leaf Extract of *Costus afer* Ker Gawl (Costaceae) in Rodents

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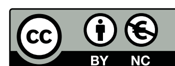

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## ABSTRACT

**Background.** *Costus afer* is widely used in traditional medicine for diverse therapeutic purposes, yet its long-term safety profile remains poorly defined. This study evaluated the acute and 90-day sub-chronic oral toxicity of the hydroethanolic leaf extract of *Costus afer* and assessed the reversibility of observed effects after treatment withdrawal.

**Material and methods.** Acute toxicity was evaluated in mice following oral and intraperitoneal administration. Sub-chronic toxicity was assessed in male and female Sprague-Dawley rats administered the extract orally at 10, 50, or 250 mg/kg daily for 90 days, in accordance with OECD guideline 408, followed by a 30-day recovery period. Body weight, food and water intake, organ weights, haematological, biochemical, hormonal, urine, sperm parameters, and histopathology of major organs were evaluated.

**Results.** The oral $LD_{50}$ was greater than 2 g/kg, while the intraperitoneal $LD_{50}$ was 298 mg/kg. Sub-chronic administration produced dose- and sex-dependent effects. At 10–50 mg/kg, changes in body weight, haematological, biochemical, hormonal, and urine parameters were largely mild and reversible, with no histopathological lesions. However, at 250 mg/kg, the extract induced adverse effects, including reductions in body weight, alterations in lipid profile, elevations in hepatic enzymes, suppression of reproductive hormones, irreversible reduction in sperm count, and histopathological changes in the liver, kidney, and brain, which were mostly reversible except for selected reproductive indices.

**Conclusions.** *Costus afer* hydroethanolic leaf extract appears relatively safe at sub-therapeutic and therapeutic doses following repeated administration. However, prolonged exposure to high doses produces systemic and reproductive toxicity, underscoring the need for dose regulation and caution in its medicinal use. The NOAEL for the hydroethanol leaf extract of *Costus afer* was determined to be 50 mg/kg**,** while the LOAEL was 250 mg/kg.

## Introduction

*Costus afer* is a rapidly growing flowering plant belonging to the family Costaceae. It is a perennial, herbaceous, rhizomatous species with a partially woody stem that grows year-round, particularly in regions with high rainfall, and can also be cultivated in gardens [1]. The plant is widely distributed in the wild and forest regions of tropical Africa. It is commonly referred to as bush cane, ginger lily, or spiral ginger [2,3]. It is known locally as "Ireke-omede" and "Tete-egun" in Yoruba, "Dodonkodi" and "Kakizawa" in Hausa, "Okpete" and "Ejula" in Igbo, and "Ogbodou" in Ijaw [4,5].

Ethnomedicinally, the stem of *C. afer* is used in the treatment of inflammation, gout, cough, sore throat, and gonorrhea [6–8]. The leaves have been traditionally used to manage diabetes mellitus, oligospermia, colic, jaundice, fever, stomach ache, and measles [3,8]. At the same time, rhizomes are used to treat malaria, diabetes mellitus, and central nervous system (CNS) disorders [6,9]. Ethnopharmacological studies have reported the phytochemical composition, proximate constituents, mineral content, and antioxidant activity of *C. afer* extracts [4,10]. In addition, several pharmacological properties of the plant, including wound-healing, antihyperglycemic, CNS depressant, analgesic, anti-arthritic, and antimicrobial activities, have been investigated in separate studies [1,2,11–13].

Despite the widespread traditional use and reported pharmacological activities of *C. afer*, limited scientific studies have evaluated its safety profile. Globally, ethnomedicines are often presumed safe due to their natural origin [14]. However, factors such as sex, age, ethnicity, level of education, economic status, and social background have been reported to influence the prevalence of herbal medicine use [15]. The consumption of herbal products without rigorous toxicological evaluation to establish their safety and tolerability may result in organ damage and, in severe cases, death [16]. Several factors, including individual body chemistry, the presence of secondary metabolites in plants, dosage, duration of exposure, genetic makeup, and physiological status, have been implicated in herbal-induced toxicity [17]. To date, only a few acute and sub-acute toxicity studies have been reported on *C. afer* extracts [6,18].

However, an extensive review of the literature revealed that no subchronic toxicity study involving 90-day administration has been reported for any C. afer extract. In view of this knowledge gap, the present study was undertaken to investigate the toxicological effects of the hydroethanolic leaf extract of *Costus afer* following 90 days of administration, as well as the potential reversibility of any observed effects after a 30-day treatment-free recovery period.

## Methods

### Chemicals and materials

Standard diagnostic test kits for blood chemistry were obtained from Randox Laboratories (Crumlin, UK). A Sysmex XS-1000i automated haematology analyser was used for full blood count analysis. Forceps and Petri dishes were employed for sperm analysis. Sex hormone assay kits were used with a DSX Automated Four-Plate ELISA system (Dynex Technologies, Virginia, USA). Other materials included formalin, hematoxylin and eosin for slide preparation, metabolic cages for urine collection, an automatic urine analyser and commercial urine analysis strips for urine chemistry (Cybow™, DFI Co. Ltd., Gimhae, Korea), *Costus afer* leaves, ethanol, and an oven (Gallenkamp®, Leicestershire, UK).

### Plant material

The leaves of *Costus afer* were identified and authenticated by Mr TK Odewo of the Department of Botany, Faculty of Science, University of Lagos, Lagos, Nigeria. The plant material was collected from Ikenne Town, Ogun State, Nigeria. A voucher specimen (LUH8018) was deposited in the herbarium of the University of Lagos.

### Extraction

The *C. afer* leaves were air-dried until a constant weight was obtained. The dried leaves were then ground into a powder, weighed (510 g), and macerated in 2 L of hydroethanol (1:1) for 72 hours. The extract was subsequently decanted and filtered first through muslin cloth and then through Whatman filter paper. The three preparatory stages of extraction, decanting, and filtration were repeated twice more using the residues obtained. The combined filtrates were evaporated to dry-

ness in an oven maintained at 40°Cyielding, yielding a dark-brown solid extract with a 10.8% yield. Distilled water was used to dissolve the extract before oral administration to experimental animals. Dose selection was based on findings from a previous study on the anticonvulsant and muscle-relaxant activities of the hydroethanolic leaf extract of *C. afer* in mice [19].

### Experimental animals

Swiss mice (15–25 g) and Sprague-Dawley rats of either sex (150–200 g; 6–12 weeks old) were obtained from the Laboratory Animal Centre, Faculty of Pharmacy, Olabisi Onabanjo University, Sagamu Campus, Ogun State, Nigeria. The animals were housed in clean, well-ventilated cages under standard laboratory conditions and properly labelled. Standard rodent feed (Jafel Agro Service, Sagamu, Ogun State, Nigeria) and water were provided *ad libitum*. The animals were allowed a 14-day acclimatisation period before the experiment commenced and were weighed before treatment initiation. Ethical approval was obtained from the Health Research Ethics Committee (HREC) of the College of Medicine, University of Lagos, Lagos, Nigeria (CMUL/HREC/08/18/415).

### Acute toxicity test

Mice were fasted for 12 hours before the acute toxicity study. A group of five (5) mice received *C. afer* extract at a dose of 2 g/kg orally (*p.o.*) using the limit test approach. Other groups of five (5) mice each were administered the extract intraperitoneally (*i.p.*) at doses of 25, 50, 100, 200, and 400 mg/kg using the Behrens-Karber method. Control groups received distilled water (10 mL/kg) via oral or intraperitoneal routes. Animals were closely observed for behavioural changes and signs of toxicity for two hours post-treatment. Mortality within the first 24 hours was recorded, and surviving animals were monitored for 14 days for signs of delayed toxicity. The median lethal dose ($LD_{50}$) for the intraperitoneal route was estimated using the Behrens-Karber method [20,21].

### Sub-chronic toxicity test

This was a repeated-dose oral toxicity study conducted over 90 days, including a reversibility assessment. Sixty-four (64) albino rats were randomly assigned into four (4) groups of sixteen (16) animals each (eight males and eight females). Male and female rats were housed separately within each group. Animals received *C. afer* extract orally at doses of 10 mg/kg (low dose, 1/5×), 50 mg/kg (medium dose, 1×), and 250 mg/kg (high dose, 5×) daily for 90 consecutive days. The control group received distilled water (10 mL/kg). The study followed OECD guideline 408 for sub-chronic oral toxicity. Body weights were recorded weekly from weeks 0 to 13, and weight changes were calculated relative to baseline values. Feed and water consumption were measured daily throughout the study period. On day 89, urine samples were collected overnight using metabolic cages for urinalysis [22–24]. At the end of the treatment period (day 90), animals were fasted overnight (12–16 hours) and anaesthetised with an intraperitoneal injection of 1% chloralose in 25% urethane (w/v) at 5 mL/kg. Blood samples were collected through the retro-orbital plexus into EDTA, lithium heparin, and plain sample bottles for haematological, hormonal, and biochemical analyses. Five males and five females per group were humanely sacrificed. Blood samples in plain tubes were allowed to clot for 1 hour, then centrifuged at 3,500 rpm for 10 minutes. Semen samples were collected from male rats and evaluated for sperm motility, count, and morphology using established methods [25–27]. For the reversibility study, three males and three females per group were withdrawn from treatment after 90 days and observed for an additional 30 days without extract administration, after which the same procedures and analyses were conducted. Sample size determination was based on previously reported experimental designs [23]. Reversibility, in this study, is defined in operational terms as the extent to which alterations induced by treatment in biochemical, physiological, and/or histological parameters revert to baseline (control) values following a 30-day recovery period following treatment termination. A parameter was deemed reversible if its value post-recovery did not exhibit a statistically significant difference from that of the control group or demonstrated a pronounced trend towards normalisation in comparison with the treatment group.

### Effect on vital organs

Vital organs were carefully excised, rinsed in normal saline, blotted dry, and examined macroscop-

ically for visible lesions. Organs, including the liver, heart, lungs, spleen, kidneys, testes, brain, and pancreas, were weighed and expressed relative to 100 g body weight. Tissue samples were fixed in 10% formol-saline for histopathological evaluation. These procedures were also applied in the reversibility study.

### Hematological analysis

Haematological parameters were assessed using an automated haematology analyser (Sysmex XS-1000i). Parameters measured included packed cell volume (PCV), red blood cell (RBC) count, haemoglobin concentration (Hb), platelet count, total and differential white blood cell (WBC) count, mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC).

### Biochemical analysis

Serum biochemical parameters were analysed using standard diagnostic kits (Randox Laboratories, Crumlin, UK) on an automated clinical chemistry analyser (Synchron Clinical Systems, model CX5PRO; Beckman Coulter Inc., Galway, Ireland). Parameters measured included albumin, high-density lipoprotein cholesterol (HDL-c), low-density lipoprotein cholesterol (LDL-c), total cholesterol, triglycerides, glucose, uric acid, alkaline phosphatase (ALP), aspartate transaminase (AST), alanine transaminase (ALT), and total bilirubin.

### Sperm analysis

Male rats were euthanised, and the vas deferens and cauda epididymides were carefully dissected. Spermatozoa were expressed from the vas deferens, while the cauda epididymides were incised to allow semen to flow into a beaker maintained at 36°C in a water bath. Semen samples were evaluated for sperm motility, count, and percentage abnormalities [25–27].

### Urine analysis

Fresh urine samples were collected over 12 hours (8:00 pm–8:00 am) using metabolic cages on days 89/90 and 119/120. Urinalysis was performed using an automatic urine analyser and commercial urine test strips (Cybow™, DFI Co. Ltd., Gimhae, Korea) to assess glucose, bilirubin, ketones, specific gravity, pH, protein, urobilinogen, nitrite, and blood [28].

### Hormonal analysis

Serum samples obtained after centrifugation were used to determine follicle-stimulating hormone (FSH), luteinizing hormone (LH), testosterone, estrogen, and progesterone levels following validated procedures [29,30]. Hormonal assays were conducted using enzyme-linked immunosorbent assay (ELISA) with a DSX Automated Four-Plate ELISA system (Dynex Technologies, Virginia, USA).

### Histopathological assessment

Major internal organs, including the brain, testes, liver, and kidneys, were excised for histopathological examination. Tissues were fixed in 10% formol-saline, dehydrated in graded alcohol, embedded in paraffin, sectioned at 4–5 μm thickness, and stained with hematoxylin and eosin (H&E). Histological sections were examined and photomicrographed at ×100 magnification using a Model N-400ME photomicroscope (CEL-TECH Diagnostics, Hamburg, Germany) [24,31,32].

### Statistical analysis

Data were expressed as mean ± standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's post-hoc test with GraphPad Prism version 6 (GraphPad Software Inc., CA, USA). Differences were considered statistically significant at $P < 0.05$. Sample sizes were selected based on prior studies and standard preclinical guidelines to ensure adequate sensitivity for detecting biologically relevant effects.

## Results

### Acute toxicity

Oral administration of *C. afer* at 2 g/kg in mice did not result in mortality within 24 hours. However, mild signs of toxicity, such as inactivity, dullness, and reduced locomotor activity, were observed within the first 2 hours post-treatment. No palpable signs of immediate or delayed toxicity were observed during the 2-week post-treatment monitoring period. For the intraperitoneal (*i.p.*) route, the median lethal dose ($LD_{50}$) was estimated to be 298 mg/kg in mice.

### Effect on body weight, food, and water intake

In male rats, *C. afer* (10–250 mg/kg) produced a dose-dependent reduction ($p < 0.05–0.0001$) in body weight compared with the control (distilled water) group. Conversely, food and water intake significantly increased ($p < 0.05–0.001$) following extract administration. These effects were reversed following the 30-day recovery period. In female rats, *C. afer* at 10 mg/kg significantly and reversibly increased body weight ($p < 0.001$). However, administration at 250 mg/kg resulted in a reversible decrease ($p < 0.01–0.001$) in body weight, food intake, and water consumption (**Table 1**).

### Effect on vital organ weights (per 100 g body weight)

In male rats, *C. afer* (10–250 mg/kg) produced a reversible increase ($p < 0.05–0.001$) in the weights of most vital organs compared with the control group (**Table 2**). In female rats, the extract did not significantly affect ($p > 0.05$) most organ weights, except for the lungs, which showed a significant but reversible reduction in weight ($p < 0.01$) at doses of 10–50 mg/kg compared with the control (**Table 3**).

### Effect on haematological parameters

In male rats, *C. afer* (10–250 mg/kg) significantly reduced neutrophils, monocytes, and mean corpuscular volume (MCV) ($p < 0.05–0.01$) relative to the control. Conversely, white blood cell (WBC) count, differential counts, lymphocytes, and platelets were significantly increased ($p < 0.05–0.001$) at all doses. Red blood cell (RBC) count and haemoglobin (Hb) levels decreased significantly ($p < 0.05–0.001$) after 90 days of extract administration compared with distilled water (**Table 4**). In female rats, *C. afer* (10–250 mg/kg) produced a reversible reduction ($p < 0.05–0.001$) in WBC, neutrophils, and lymphocytes compared with the control. At 10 mg/kg, the extract caused an irreversible increase ($p < 0.001$) in RBC and

**Table 1.** Effect of hydroethanol leaf extract of *Costus afer* on changes in body weight, food and water intakes in male and female rats (Main and Reversibility).

MALE

| Treatment | Dose (mg/kg) | Body weight change (g) | Food intake(g) | Water intake (L) |
|---|---|---|---|---|
| **MAIN** | | | | |
| Distilled water | 10 mL/kg | 52.50±5.15 | 38.79±3.52 | 18.80±2.25 |
| C. afer | 10 | 36.08±4.30$^{a}$ | 57.39±3.14$^{c}$ | 60.82±7.01$^{c}$ |
| C. afer | 50 | 32.33±3.51$^{b}$ | 53.21±3.29$^{b}$ | 55.64±5.02$^{c}$ |
| C. afer | 250 | 13.56±4.34$^{d}$ | 64.87±4.41$^{c}$ | 36.65±3.94$^{a}$ |
| **REV** | | | | |
| Distilled water | 10 mL/kg | 38.75±0.85 | 24.93±2.55 | 52.14±2.60 |
| C. afer | 10 | 25.50±6.44 | 22.86±2.16 | 55.90±5.83 |
| C. afer | 50 | 28.50±2.75 | 24.48±1.84 | 56.72±5.40 |
| C. afer | 250 | 40.75±2.65 | 22.55±2.20 | 53.90±4.19 |

FEMALE

| Treatment | Dose (mg/kg) | Body weight change (g) | Food intake(g) | Water intake (L) |
|---|---|---|---|---|
| **MAIN** | | | | |
| Distilled water | 10 mL/kg | 33.50±4.08 | 106.1±4.61 | 66.70±4.83 |
| C. afer | 10 | 66.83±6.05$^{c}$ | 93.90±4.57 | 75.70±6.00 |
| C. afer | 50 | 37.25±4.53 | 100.1±4.51 | 69.34±8.07 |
| C. afer | 250 | 14.50±1.91$^{b}$ | 69.18±4.26$^{c}$ | 35.11±3.03$^{c}$ |
| **REV** | | | | |
| Distilled water | 10 mL/kg | 28.50±3.50 | 26.28±3.19 | 53.38±5.49 |
| C. afer | 10 | 42.25±4.23 | 24.90±2.24 | 50.93±3.89 |
| C. afer | 50 | 36.25±6.15 | 25.21±2.38 | 51.72±5.02 |
| C. afer | 250 | 38.75±2.95 | 27.00±2.40 | 54.45±5.67 |

Values are mean ± S.E.M. (n=8 per sex; 5 per sex for the main study and 3 per sex for the reversibility study). $^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$, $^{d}p<0.0001$ vs. distilled water (One way ANOVA followed by Dunnett's multiple comparison test). **REV:** Reversibility study.

**Table 2.** Effect of hydroethanol leaf extract of *Costus afer* on weight of vital organs per 100 g body weight of male rats.

| Treatment | Dose (mg/kg) | Liver (g) | Kidney (g) | Lungs (g) | Heart (g) | Testes (g) | Brain (g) | Pancreas (g) | Spleen (g) |
|---|---|---|---|---|---|---|---|---|---|
| **MAIN** | | | | | | | | | |
| Distilled water | 10 (mL/kg) | 1.94±0.02 | 0.59±0.01 | 0.46±0.02 | 0.28±0.05 | 0.59±0.00 | 0.62±0.02 | 0.96±0.02 | 0.19±0.01 |
| C. afer | 10 | 3.91±0.21[c] | 0.78±0.09 | 1.21±0.18[b] | 0.42±0.04 | 0.83±0.13 | 0.84±0.01[b] | 1.44±0.04[a] | 0.47±0.08[a] |
| C. afer | 50 | 2.65±0.02[a] | 0.69±0.01 | 0.77±0.01 | 0.33±0.02 | 0.73±0.01 | 1.10±0.05[c] | 1.96±0.08[c] | 0.28±0.01 |
| C. afer | 250 | 3.70±0.21[c] | 0.82±0.02[a] | 0.78±0.01 | 0.39±0.06 | 0.71±0.04 | 1.10±0.00[c] | 1.39±0.10[a] | 0.32±0.02 |
| **REV** | | | | | | | | | |
| Distilled water | 10 (mL/kg) | 3.62±0.01 | 0.63±0.03 | 0.81±0.05 | 0.31±0.04 | 0.62±0.01 | 0.77±0.01 | 0.34±0.03 | 0.21±0.00 |
| C. afer | 10 | 3.73±0.04 | 0.66±0.05 | 0.95±0.02 | 0.32±0.01 | 0.69±0.03 | 0.90±0.01 | 0.23±0.00 | 0.21±0.02 |
| C. afer | 50 | 3.66±0.26 | 0.66±0.02 | 0.84±0.01 | 0.34±0.03 | 0.68±0.03 | 0.71±0.08 | 0.25±0.01 | 0.20±0.00 |
| C. afer | 250 | 4.16±0.22 | 0.67±0.02 | 0.79±0.02 | 0.35±0.01 | 0.63±0.01 | 0.81±0.01 | 0.28±0.02 | 0.19±0.02 |

Values are mean ± S.E.M. (n=8 per sex; 5 per sex for the main study and 3 per sex for the reversibility study).[a]p<0.05, [b]p<0.01, [c]p<0.001 vs. distilled water (One way ANOVA followed by Dunnett's multiple comparison test). **REV:** Reversibility study.

**Table 3.** Effect of hydroethanol leaf extract of *Costus afer* on weight of vital organs per 100 g bodyweight of female rats.

| Treatment | Dose (mg/kg) | Liver (g) | Kidney (g) | Lungs (g) | Heart (g) | Brain (g) | Pancreas (g) | Spleen (g) |
|---|---|---|---|---|---|---|---|---|
| **MAIN** | | | | | | | | |
| Distilled water | 10 (mL/kg) | 3.00 ±0.11 | 0.70 ±0.00 | 0.92±0.01 | 0.36±0.01 | 0.75±0.01 | 1.10±0.05 | 0.30±0.01 |
| C. afer | 10 | 2.62 ±0.13 | 0.70±0.01 | 0.72±0.01[b] | 0.31±0.01 | 0.71±0.01 | 1.21±0.04 | 0.24±0.01 |
| C. afer | 50 | 2.83±0.01 | 0.74±0.03 | 0.70±0.04[b] | 0.40±0.01 | 0.77±0.05 | 1.58±0.16 | 0.27±0.05 |
| C. afer | 250 | 2.91±0.08 | 0.79±0.07 | 0.95±0.01 | 0.39±0.01 | 0.76±0.01 | 1.42±0.08 | 0.27±0.01 |
| **REV** | | | | | | | | |
| Distilled water | 10 (mL/kg) | 3.24 ±0.35 | 0.68±0.02 | 0.88±0.46 | 0.31±0.01 | 0.86±0.01 | 0.26±0.03 | 0.23±0.01 |
| C. afer | 10 | 2.95±0.32 | 0.64±0.01 | 0.87±0.71 | 0.26±0.00 | 0.80±0.03 | 0.21±0.00 | 0.23±0.02 |
| C. afer | 50 | 3.89±0.25 | 0.70±0.04 | 0.93±0.04 | 0.35±0.02 | 0.77±0.06 | 0.22±0.03 | 0.29±0.03 |
| C. afer | 250 | 3.66±0.19 | 0.69±0.02 | 0.70±0.01 | 0.33±0.00 | 0.75±0.03 | 0.23±0.01 | 0.16±0.02 |

Values are mean ± S.E.M. (n=8 per sex; 5 per sex for the main study and 3 per sex for the reversibility study).[b]p<0.01 vs. distilled water (One way ANOVA followed by Dunnett's multiple comparison test). **REV:** Reversibility study.

Hb, along with a reversible increase in packed cell volume (PCV). Following the 30-day recovery period, WBC, differential counts, and lymphocytes significantly increased ($p < 0.05$–$0.001$) compared with the control. Platelet counts showed a dose-dependent response, increasing at 10 mg/kg ($p < 0.001$) and decreasing at 250 mg/kg ($p < 0.001$). PCV significantly decreased ($p < 0.05$) after the 30-day recovery period (**Table 5**).

## Effect on serum biochemical parameters

In male rats, most serum biochemical parameters were not significantly altered ($p > 0.05$) compared with the control. However, *C. afer* at 250 mg/kg irreversibly elevated alkaline phosphatase (ALP), alanine aminotransferase (ALT), and triglyceride levels ($p < 0.05$–$0.0001$). High-density lipoprotein cholesterol (HDL-c) significantly decreased ($p < 0.05$–$0.01$) across all doses. Following the 30-day recovery period, there was a significant increase in HDL-c ($p < 0.05$, 250 mg/kg) and a decrease in low-density lipoprotein cholesterol (LDL-c) ($p < 0.01$–$0.001$, 50–250 mg/kg). Uric acid levels significantly increased ($p < 0.05$–$0.001$) at all doses following extract administration (**Table 6**). In female rats, *C. afer* increased HDL-c at 250 mg/kg ($p < 0.05$) and decreased it at 50 mg/kg ($p < 0.01$). Triglycerides and LDL-c were irreversibly reduced ($p < 0.05$–$0.01$) at 250 mg/kg. ALP ($p < 0.05$, 250 mg/kg) and ALT ($p < 0.01$, 10 mg/kg)

**Table 4.** Effect of hydroethanol leaf extract of *Costus afer* on hematological parameters in male rats.

| Treatment | Dose (mg/kg) | WBC (×10$^{3}$/µl) | NEU (%) | BAS (%) | MON (%) | EOS (%) | LYM (%) | PLT (×10$^{6}$/µl) | MCHC (g/dl) | MCH (g/dl) | PCV (%) | Hb (g/dl) | RBC (×10$^{6}$/µl) | MCV (g/dl) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **MAIN** | | | | | | | | | | | | | | |
| Distilled water | 10 (mL/kg) | 11.62 ±2.71 | 5.28 ±0.71 | 0.02 ±0.01 | 0.10 ±0.01 | 0.05 ±0.01 | 6.18 ±1.98 | 833.0 ±104.50 | 26.35 ±0.20 | 16.85 ±0.03 | 49.80 ±2.83 | 13.10 ±0.64 | 7.77 ±0.36 | 64.05 ±0.66 |
| C. afer | 10 | 6.33 ±0.21 | 2.2 ±0.06$^{b}$ | 0.03 ±0.01 | 0.02 ±0.00$^{a}$ | 0.0 ±0.01 | 3.98 ±0.27 | 667.5 ±30.30 | 30.15 ±2.28 | 17.25 ±0.61 | 45.30 ±1.91 | 13.50 ±0.46 | 7.82 ±5.00 | 57.90 ±2.43$^{a}$ |
| C. afer | 50 | 8.90 ±0.52 | 2.83 ±0.00$^{b}$ | 0.02 ±0.00 | 0.03 ±0.00$^{a}$ | 0.0 ±0.00 | 6.84 ±0.00 | 624.7 ±13.17 | 25.90 ±0.00 | 17.23 ±0.67 | 54.90 ±0.00 | 14.20 ±0.00 | 7.93 ±0.00 | 69.20 ±0.00$^{a}$ |
| C. afer | 250 | 7.90 ±0.35 | 2.06 ±0.08$^{b}$ | 0.05 ±0.00 | 0.10 ±0.01 | 0.0 ±0.01 | 5.69 ±0.44 | 766.00 ±46.77 | 28.30 ±0.81 | 17.05 ±0.03 | 44.35 ±2.45 | 12.50 ±0.35 | 7.35 ±0.19 | 60.25 ±1.82 |
| **REV** | | | | | | | | | | | | | | |
| C. afer | 10 | 6.86 ±0.11$^{c}$ | 2.76 ±0.01$^{c}$ | 0.03 ±0.0$^{b}$ | 0.04 ±0.00$^{a}$ | 0.0 ±0.00$^{c}$ | 3.97 ±0.11$^{c}$ | 944.0 ±2.30$^{c}$ | 27.7 ±0.69$^{b}$ | 17.50 ±0.11 | 45.30 ±1.91 | 13.30 ±0.11$^{c}$ | 7.6 ±0.17$^{b}$ | 63.2 ±1.15$^{b}$ |
| C. afer | 50 | 5.83 ±0.06$^{c}$ | 2.3 ±0.02$^{c}$ | 0.02 ±0.01$^{a}$ | 0.02 ±0.00 | 0.0 ±0.01$^{c}$ | 3.42 ±0.05$^{c}$ | 824.0 ±1.73 | 27.4 ±1.24$^{b}$ | 17.20 ±1.21 | 54.90 ±0.00 | 14.20 ±0.46$^{b}$ | 7.97 ±0.01$^{a}$ | 65.0 ±0.75$^{c}$ |
| C. afer | 250 | 4.72 ±0.01$^{c}$ | 1.71 ±0.01$^{c}$ | 0.00 ±0.003 | 0.003 ±0.00$^{a}$ | 0.02 ±0.01$^{b}$ | 2.98 ±0.05$^{c}$ | 997.0 ±6.928$^{c}$ | 27.7 ±0.69$^{b}$ | 16.10 ±0.57 | 44.3 ±2.45 | 14.50 ±0.11$^{a}$ | 9.00 ±0.20 | 58.1 ±1.67 |

Values are mean ± S.E.M.(n=8 per sex; 5 per sex for the main study and 3 per sex for the reversibility study).$^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$ vs. distilled water (One way ANOVA followed by Dunnett's multiple comparison test). **REV:** Reversibility study. **WBC:** White blood cell, **NEU:** Neutriphils, **BAS**: Basophils, **MON**: Monocytes, **EOS**: Eosinophils, **LYM**: Lymphocytes, **PLT**: Platelets, **MCHC**: Mean corpuscular hemoglobin concentration, **MCH**: Mean corpuscular hemoglobin, **MCV**: Mean corpuscular volume, **PCV**: Parked cell volume, **Hb**: hemoglobin, **RBC**: Red blood cell, **MCV**: Mean corpuscular volume.

**Table 5.** Effect of hydroethanol leaf extract of *Costus afer* on hematological parameters in female rats.

| Treatment | Dose (mg/kg) | WBC (×10$^{3}$/µl) | NEU (%) | BAS (%) | MON (%) | EOS (%) | LYM (%) | PLT (×10$^{6}$/µl) | MCHC (g/dl) | MCH (g/dl) | PCV (%) | Hb (g/dl) | RBC (×10$^{6}$/µl) | MCV (g/dl) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **MAIN** | | | | | | | | | | | | | | |
| Distilled water | 10 (mL/kg) | 6.41 ±0.63 | 1.74 ±0.39 | 0.02 ±0.00 | 0.04 ±0.01 | 0.04 ±0.01 | 4.38 ±0.30 | 635.5 ±58.02 | 26.7 ±0.20 | 17.1 ±0.06 | 44.70 ±0.87 | 11.95 ±0.32 | 6.99 ±0.21 | 64.0 ±0.66 |
| C. afer | 10 | 1.82 ±0.16$^{c}$ | 0.76 ±0.03$^{a}$ | 0.00 ±0.00$^{c}$ | 0.02 ±0.00 | 0.04 ±0.01 | 1.01 ±0.13$^{c}$ | 526.5 ±59.76 | 27.75 ±0.38 | 17.10 ±0.06 | 51.60 ±1.33$^{b}$ | 14.30 ±0.17$^{c}$ | 8.36 ±0.12$^{c}$ | 61.75 ±0.6 |
| C. afer | 50 | 4.99 ±0.84$^{a}$ | 1.72 ±0.27 | 0.02 ±0.00 | 0.02 ±0.01 | 0.05 ±0.01 | 3.19 ±0.56$^{b}$ | 599.5 ±52.25 | 27.75 ±0.66 | 17.85 ±0.03$^{c}$ | 48.70 ±0.52 | 13.50 ±0.17$^{b}$ | 7.57 ±0.09 | 64.40 ±1.44 |
| C. afer | 250 | 4.72 ±0.29$^{a}$ | 1.89 ±0.07 | 0.03 ±0.00 | 0.05 ±0.00 | 0.02 ±0.01 | 2.74 ±0.23$^{b}$ | 758.50 ±43.01 | 30.20 ±0.06$^{c}$ | 18.15 ±0.03$^{c}$ | 43.20 ±0.35 | 13.05 ±0.09$^{a}$ | 7.18 ±0.06 | 60.1 ±0.03$^{b}$ |
| **REV** | | | | | | | | | | | | | | |
| Distilled water | 10 (mL/kg) | 3.63 ±0.12 | 1.70 ±0.11 | 0.02 ±0.00 | 0.01 ±0.00 | 0.04 ±0.01 | 1.86 ±0.11 | 981.3 ±0.66 | 26.70 ±0.11 | 17.0 ±0.03 | 50.0 ±0.01 | 13.4 ±0.14 | 7.88 ±0.11 | 63.7 ±0.115 |
| C. afer | 10 | 11.89 ±0.05$^{c}$ | 5.78 ±0.12$^{c}$ | 0.05 ±0.00$^{c}$ | 0.03 ±0.001 | 0.11 ±0.05$^{c}$ | 5.82 ±0.10$^{c}$ | 1222.0 ±5.77$^{c}$ | 31.0 ±1.15$^{b}$ | 18.2 ±1.79 | 49.1 ±0.05 | 15.2 ±0.63$^{a}$ | 8.36 ±0.01$^{a}$ | 58.7 ±0.05$^{c}$ |
| C. afer | 50 | 8.07 ±0.06$^{b}$ | 3.12 ±0.01$^{a}$ | 0.02 ±0.001 | 0.06 ±0.02$^{b}$ | 0.15 ±0.01$^{c}$ | 4.66 ±0.01$^{c}$ | 772.0 ±12.12$^{c}$ | 27.9 ±1.15 | 13.6 ±0.05 | 53.10 ±1.21$^{a}$ | 14.4 ±0.49 | 7.97 ±0.24 | 66.6 ±0.57$^{c}$ |
| C. afer | 250 | 16.76 ±1.15$^{c}$ | 11.7 ±0.57$^{c}$ | 0.08 ±0.00$^{c}$ | 0.97 ±0.01$^{c}$ | 0.22 ±0.01$^{c}$ | 3.77 ±0.12$^{c}$ | 1087.0 ±11.5$^{c}$ | 26.6 ±0.57 | 17.3 ±0.57 | 47.30 ±0.63$^{a}$ | 12.6 ±0.05 | 7.30 ±0.11$^{a}$ | 64.8 ±0.40 |

Values are mean ± S.E.M. (n=8 per sex; 5 per sex for the main study and 3 per sex for the reversibility study).$^{a}p<0.05$, $^{b}p<0.01$, $^{c}p<0.001$ vs. distilled water (One way ANOVA followed by Dunnett's multiple comparison test). **REV:** Reversibility study. **WBC:** White blood cell, **NEU:** Neutriphils, **BAS**: Basophils, **MON**: Monocytes, **EOS**: Eosinophils, **LYM**: Lymphocytes, **PLT**: Platelets, **MCHC**: Mean corpuscular hemoglobin concentration, **MCH**: Mean corpuscular hemoglobin, **MCV**: Mean corpuscular volume, **PCV**: Parked cell volume, **Hb**: hemoglobin, **RBC**: Red blood cell, **MCV**: Mean corpuscular volume.

**Table 6.** Effect of hydroethanol leaf extract of *Costus afer* on biochemical parameters in male rats.

| Treatment | Dose (mg/kg) | ALB (umol/l) | CHO (mmol/l) | HDL (mmol/l) | LDL (mmol/l) | TRIG (mmol/l) | GLUCOSE (mmol/l) | URIC ACID (umol/l) | ALP (u/l) | AST (u/l) | ALT (u/l) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **MAIN** | | | | | | | | | | | |
| Distilled water | 10 (mL/kg) | 3.05 ±0.09 | 63.00 ±16.17 | 85.50 ±6.06 | 33.50 ±15.26 | 16.50 ±2.59 | 74.00 ±5.77 | 12.00 ±1.16 | 94.00 ±2.31 | 37.00 ±0.58 | 21.50 ±0.87 |
| C. afer | 10 | 3.05 ±0.14 | 50.00 ±2.89 | 25.50 ±8.37[b] | 16.50 ±7.79 | 38.50 ±9.52 | 80.00 ±27.14 | 19.00 ±1.16 | 120.00 ±9.24 | 14.50 ±4.91 | 20.00 ±0.58 |
| C. afer | 50 | 2.79 ±0.16 | 45.49 ±7.86 | 52.61 ±3.75[a] | 16.87 ±11.33 | 32.16 ±14.71 | 57.32 ±4.62 | 23.09 ±6.05 | 115.40 ±10.57 | 60.61 ±19.14 | 20.59 ±1.26 |
| C. afer | 250 | 2.60 ±0.12 | 74.50 ±6.06 | 52.50 ±6.64[a] | 11.17 ±1.16 | 59.33 ±2.60[a] | 54.00 ±7.51 | 27.00 ±4.62 | 140.00 ±17.90[a] | 43.50 ±4.91 | 50.00 ±2.89[d] |
| **REV** | | | | | | | | | | | |
| Distilled water | 10 (mL/kg) | 2.91 ±0.31 | 53.37 ±1.33 | 42.50 ±1.44 | 23.50 ±0.866 | 22.00 ±0.577 | 75.50 ±2.59 | 22.5 ±0.86 | 52.00 ±10.39 | 35.00 ±2.08 | 21.50 ±0.86 |
| C. afer | 10 | 3.40 ±0.05 | 52.00 ±0.57 | 47.00 ±1.16 | 21.00 ±0.577 | 22.00 ±0.57 | 79.00 ±0.57 | 34.00 ±1.15[c] | 136.0 ±1.15[c] | 14.50 ±4.90 | 19.00 ±1.15 |
| C. afer | 50 | 2.33 ±0.33 | 51.00 ±0.28 | 48.33 ±2.40 | 12.00 ±1.15[c] | 18.00 ±1.73 | 81.67 ±4.91 | 25.00 ±1.15[a] | 121.0 ±0.28[c] | 53.95 ±12.70 | 19.26 ±0.37 |
| C. afer | 250 | 2.60 ±0.05 | 59.00 ±0.57[a] | 54.67 ±3.38[a] | 17.00 ±0.577[b] | 22.00 ±1.15 | 77.00 ±1.15 | 32.00 ±1.15[c] | 121.0 ±0.57[c] | 40.17 ±2.61 | 33.67 ±4.66[a] |

Values are mean ±S.E.M.(n=8 per sex; 5 per sex for the main study and 3 per sex for the reversibility study). [a]p<0.05, [b]p<0.01, [c]p<0.001 vs. distilled water (One way ANOVA followed by Dunnett's multiple comparison test). **REV:** Reversibility study. **ALB**: Albumin, **CHO**: Cholesterol, **HDL**: High-density lipoprotein cholesterol (HDL-c), **LDL**: Low-density lipoprotein cholesterol (LDL-c), **TRIG**: Triglyceride, **ALP**: Alkaline Phosphatase, AST: Aspartate Aminotransferase, ALT: Alanine Aminotransferase

**Table 7.** Effect of hydroethanol leaf extract of *C. afer* on biochemical parameters in female rats.

| Treatment | Dose (mg/kg) | ALB (umol/l) | CHO (mmol/l) | HDL (mmol/l) | LDL (mmol/l) | TRIG (mmol/l) | GLUCOSE (mmol/l) | URIC ACID (umol/l) | ALP (u/l) | AST (u/l) | ALT (u/l) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **MAIN** | | | | | | | | | | | |
| Distilled water | 10 (mL/kg) | 3.30 ±0.06 | 51.50 ±11.26 | 57.50 ±1.44 | 12.00 ±8.66 | 30.50 ±6.64 | 56.50 ±0.87 | 22.50 ±0.29 | 87.50 ±1.44 | 43.00 ±13.88 | 19.50 ±2.02 |
| C. afer | 10 | 3.20 ±0.17 | 45.00 ±13.86 | 47.50 ±5.49[b] | 12.00 ±5.20 | 32.00 ±6.35 | 60.00 ±5.78 | 26.00 ±0.00 | 65.00 ±2.89[b] | 58.00 ±0.00 | 2.00 ±0.00[b] |
| C. afer | 50 | 2.70 ±0.23 | 44.00 ±6.93 | 49.00 ±5.77[a] | 10.00 ±0.58 | 26.50 ±8.37 | 44.50 ±11.84 | 42.00 ±4.62 | 76.00 ±3.46 | 25.50 ±3.75 | 21.50 ±0.87 |
| C. afer | 250 | 3.35 ±0.14[a] | 54.00 ±5.20 | 73.50 ±1.44[a] | 34.50 ±9.53[b] | 26.00 ±1.44[a] | 53.00 ±1.16 | 36.50 ±8.37 | 70.00 ±2.31[a] | 29.00 ±6.35 | 23.50 ±3.18 |
| **REV** | | | | | | | | | | | |
| Distilled water | 10 (mL/kg) | 4.05 ±0.02 | 69.50 ±0.28 | 24.33 ±1.85 | 22.5 ±0.28 | 21.5 ±0.86 | 77.5 ±1.44 | 22.5 ±0.86 | 93.37 ±1.20 | 39.00 ±10.58 | 19.50 ±2.02 |
| C. afer | 10 | 3.93 ±0.20 | 77.0 ±1.15[c] | 20.00 ±1.73 | 6.00 ±0.57[c] | 33.00 ±1.15[c] | 56.00 ±1.15[c] | 34.0 ±0.57[c] | 94.33 ±14.62 | 55.67 ±2.33 | 19.33 ±0.88 |
| C. afer | 50 | 3.80 ±0.11 | 75.67 ±2.84 | 26.0 ±0.57 | 10.00 ±1.15[c] | 15.00 ±0.57[c] | 63.00 ±0.57[c] | 33.0 ±1.73[b] | 90.67 ±5.45 | 24.00 ±4.04 | 20.83 ±0.44 |
| C. afer | 250 | 4.00 ±0.12 | 74.00 ±0.57 | 23.37 ±3.18 | 19.0 ±0.577[c] | 16.00 ±1.15[c] | 88.0 ±2.88[c] | 27.0 ±1.15 | 93.00 ±1.52 | 26.00 ±0.04 | 21.83 ±2.45 |

Values are mean ± S.E.M.(n=8 per sex; 5 per sex for the main study and 3 per sex for the reversibility study).[a]p0.05, [b]p0.01, [c]p0.001 vs. distilled water (One way ANOVA followed by Dunnett's multiple comparison test).**REV:** Reversibility study. **ALB**: Albumin, **CHO**: Cholesterol, **HDL**: High-density lipoprotein cholesterol (HDL-c), **LDL**: Low-density lipoprotein cholesterol (LDL-c), **TRIG**: Triglyceride, **ALP**: Alkaline Phosphatase, AST: Aspartate Aminotransferase, ALT: Alanine Aminotransferase

were significantly decreased compared with the control. There was a significant increase in uric acid levels ($p < 0.05$–$0.001$) following the 30-day recovery period (**Table 7**).

## Effect on sperm motility, count, and morphology

Administration of *C. afer* at 250 mg/kg significantly reduced sperm count ($p < 0.05$), and this effect was not reversed following the 30-day recovery period. However, sperm motility was significantly and reversibly reduced ($p < 0.01$) at the same dose compared with the control group (**Table 8**).

## Effect on urine parameters

In male rats, urinary parameters were not significantly affected ($p > 0.05$), although a 30-day recovery period decrease in urine pH was observed at 50 mg/kg ($p < 0.05$) (**Table 9**). In female rats, *C. afer* significantly increased urine pH and reduced specific gravity ($p < 0.05$) at all doses. These changes were reversed following the 30-day recovery period (**Table 10**).

**Table 8.** Effect of hydroethanol leaf extract of *Costus afer* on sperm analysis in male rats.

| Treatment | Dose (mg/kg) | Sperm motility (%) | Sperm count ($\times 10^6$) | Sperm morphology (%) | Dead/Live ratio (%) |
|---|---|---|---|---|---|
| **MAIN** | | | | | |
| Distilled water | 10 (mL/kg) | 93.50±2.02 | 29.60±0.69 | 93.55±0.72 | 92.70±0.69 |
| C. afer | 10 | 96.00±1.15 | 33.90±1.15 | 94.10±1.21 | 96.90±1.15 |
| C. afer | 50 | 95.00±0.57 | 29.40±0.57 | 89.50±1.44 | 90.30±0.57 |
| C. afer | 250 | 79.17±2.20[b] | 24.57±2.09[a] | 86.37±2.89[a] | 88.48±1.68 |
| **REV** | | | | | |
| Distilled water | 10 (mL/kg) | 97.00±1.15 | 30.20±0.05 | 95.40±0.57 | 94.77±0.84 |
| C. afer | 10 | 93.67±2.40 | 33.37±0.35[d] | 95.47±0.14 | 67.20±1.10[d] |
| C. afer | 50 | 88.00±3.78 | 31.60±0.11[b] | 90.30±0.11[c] | 38.00±0.75[d] |
| C. afer | 250 | 84.67±6.74 | 29.40±0.12[a] | 83.13±1.08[d] | 93.00±1.04[a] |

Values are mean ± S.E.M. (n=8 per sex; 5 per sex for the main study and 3 per sex for the reversibility study). [a]p0.05, [b]p0.01, [c]p0.001, p<0.0001 vs. distilled water (One way ANOVA followed by Dunnett's multiple comparison test). **REV:** Reversibility study.

**Table 9.** Effect of hydroethanol leaf extract of *Costus afer* on urine parameters in male rats.

| Treatment | Dose (mg/kg) | Urobilinogen (mg/dL) | Bilirubin (mg/dL) | Blood (mL) | Nitrite (mg/L) | pH (m/l) | S.G. (g/cm$^3$) | Protein (g/l) | Glucose (mmol/L) | Ketones (mmol/L) |
|---|---|---|---|---|---|---|---|---|---|---|
| **MAIN** | | | | | | | | | | |
| Distilled water | 10 (mL/kg) | 1.67 ±0.33 | 2.00 ±0.57 | 0.66 ±0.33 | 1.66 ±0.33 | 7.66 ±0.33 | 1.01 ±0.00 | 2.66 ±0.33 | 2.16 ±1.42 | 2.67 ±0.33 |
| C. afer | 10 | 2.67 ±0.67 | 2.33 ±0.33 | 1.00 ±0.00 | 1.67 ±0.33 | 8.16 ±0.16 | 1.00 ±0.00 | 3.33 ±0.33 | 0.83 ±0.16 | 3.00 ±0.00 |
| C. afer | 50 | 2.00 ±0.00 | 2.67 ±0.33 | 1.33 ±0.33 | 2.00 ±0.57 | 8.33 ±0.16 | 1.00 ±0.00 | 2.66 ±0.33 | 1.00 ±0.00 | 3.33 ±0.33 |
| C. afer | 250 | 2.00 ±0.00 | 3.67 ±0.67 | 1.67 ±0.33 | 2.00 ±0.57 | 7.83 ±0.16 | 1.01 ±0.00 | 3.67 ±0.33 | 1.33 ±0.33 | 2.66 ±0.33 |
| **REV** | | | | | | | | | | |
| Distilled water | 10 (mL/kg) | 0.67 ±0.33 | 2.33 ±0.33 | 1.66 ±0.33 | 2.00 ±0.00 | 7.83 ±0.16 | 1.00 ±0.00 | 1.66 ±0.33 | 0.50 ±0.00 | 3.00 ±0.00 |
| C. afer | 10 | 1.33 ±0.33 | 2.33 ±0.33 | 2.00 ±0.57 | 2.33 ±0.33 | 8.33 ±0.16 | 1.00 ±0.00 | 2.33 ±0.33 | 0.67 ±0.16 | 2.66 ±0.33 |
| C. afer | 50 | 1.33 ±0.33 | 2.67 ±0.33 | 2.33 ±0.33 | 2.33 ±0.66 | 6.83 ±0.33[a] | 1.01 ±0.00 | 2.00 ±0.57 | 0.66 ±0.16 | 3.00 ±0.33 |
| C. afer | 250 | 1.66 ±0.33 | 3.00 ±0.00 | 2.66 ±0.33 | 2.66 ±0.33 | 7.16 ±0.16 | 1.00 ±0.00 | 2.00 ±0.00 | 0.83 ±0.16 | 3.33 ±0.33 |

Values are mean ± S.E.M. (n=8 per sex; 5 per sex for the main study and 3 per sex for the reversibility study). [a]p0.05 vs. distilled water (One way ANOVA followed by Dunnett's multiple comparison test). **REV:** Reversibility study. **S.G:** Specific gravity

**Table 10.** Effect of hydroethanol leaf extract of *Costus afer* on urine parameters in female rats.

| Treatment | Dose (mg/kg) | Urobilinogen (mg/dL) | Bilirubin (mg/dL) | Blood (mL) | Nitrite (mg/L) | pH (m/l) | S.G. (g/cm$^3$) | Protein (g/l) | Glucose (mmol/L) | Ketones (mmol/L) |
|---|---|---|---|---|---|---|---|---|---|---|
| **MAIN** | | | | | | | | | | |
| Distilled water | 10 (mL/kg) | 1.66 ±0.33 | 1.33 ±0.33 | 1.33 ±0.33 | 2.00 ±0.00 | 6.33 ±0.33 | 1.02 ±0.00 | 2.33 ±0.33 | 0.66 ±0.16 | 3.00 ±0.00 |
| C. afer | 10 | 2.33 ±0.33 | 2.00 ±0.57 | 2.00 ±0.33 | 2.00 ±0.00 | 8.16 ±0.33$^b$ | 1.00 ±0.00 | 3.00 ±0.00 | 1.00 ±0.00 | 2.66 ±0.33 |
| C. afer | 50 | 2.00 ±0.00 | 2.33 ±0.66 | 1.66 ±0.33 | 2.33 ±0.33 | 8.00 ±0.28$^b$ | 0.34 ±0.33$^a$ | 2.33 ±0.33 | 0.83 ±0.16 | 3.33 ±0.33 |
| C. afer | 250 | 3.00 ±0.57 | 2.66 ±0.33 | 2.00 ±0.00 | 2.33 ±0.33 | 8.16 ±0.16$^b$ | 1.00 ±0.00 | 3.33 ±0.33 | 1.00 ±0.00 | 2.33 ±0.33 |
| **REV** | | | | | | | | | | |
| Distilled water | 10 (mL/kg) | 1.00 ±0.00 | 1.66 ±0.33 | 1.00 ±0.57 | 1.66 ±0.33 | 6.33 ±0.33 | 1.01 ±0.00 | 2.00 ±0.00 | 0.66 ±0.16 | 2.00 ±0.00 |
| C. afer | 10 | 1.33 ±0.33 | 2.33 ±0.33 | 1.00 ±0.00 | 2.00 ±0.00 | 8.33 ±0.16 | 1.02 ±0.00 | 2.67 ±0.33 | 0.83 ±0.17 | 2.66 ±0.33 |
| C. afer | 50 | 1.33 ±0.66 | 2.00 ±0.57 | 1.33 ±0.33 | 2.33 ±0.33 | 8.16 ±0.16 | 1.00 ±0.00 | 2.66 ±0.33 | 1.16 ±0.44 | 2.33 ±0.66 |
| C. afer | 250 | 1.66 ±0.33 | 2.00 ±0.57 | 1.66 ±0.67 | 2.66 ±0.33 | 7.83 ±0.16 | 1.00 ±0.00 | 2.33 ±0.33 | 1.20 ±0.44 | 2.66 ±0.33 |

Values are mean±S.E.M. (n=8 per sex; 5 per sex for the main study and 3 per sex for the reversibility study). $^a$p0.05, $^b$p0.01 vs. distilled water (One way ANOVA followed by Dunnett's multiple comparison test). **REV:** Reversibility study. **S.G:** Specific gravity

**Table 11.** Effect of hydroethanol leaf extract of *Costus afer* on hormones in male rats.

| Treatment | Dose (mg/kg) | FSH (u/mg) | LH (u/mg) | Testosterone (u/mg) |
|---|---|---|---|---|
| **MAIN** | | | | |
| Distilled water | 10 (mL/kg) | 2.05±0.02 | 1.74±0.09 | 0.13±0.09 |
| C. afer | 10 | 1.53±0.08$^b$ | 0.15±0.01$^d$ | 1.02±0.24$^a$ |
| C. afer | 50 | 1.21±0.06$^c$ | 0.50±0.11$^d$ | 0.66±0.16 |
| C. afer | 250 | 0.73±0.12$^d$ | 0.24±0.09$^d$ | 0.44±0.06 |
| **REV** | | | | |
| Distilled water | 10 (mL/kg) | 2.00±0.10 | 0.41±0.11 | 0.56±0.12 |
| C. afer | 10 | 1.95±0.09 | 2.07±0.20$^a$ | 6.40±0.43$^d$ |
| C. afer | 50 | 0.33±0.12$^d$ | 0.28±0.13 | 1.55±0.30 |
| C. afer | 250 | 0.60±0.05$^d$ | 0.69±0.24 | 2.75±0.24$^b$ |

Values are mean ± S.E.M.(n=8 per sex; 5 per sex for the main study and 3 per sex for the reversibility study). $^a$p0.05, $^b$p0.01, $^c$p<0.001, $^d$p<0.0001 vs. distilled water (One way ANOVA followed by Dunnett's multiple comparison test). **REV:** Reversibility study. **FSH**: Follicle stimulating hormone, **LH**: Luteinizing Hormone

## Effect on hormonal parameters

In male rats, *C. afer* (10–250 mg/kg) significantly reduced follicle-stimulating hormone (FSH) and luteinizing hormone (LH) levels ($p < 0.01$–$0.0001$) compared with the control. The reduction in FSH was irreversible, whereas LH levels returned to normal following the 30-day recovery period. Testosterone levels were significantly increased ($p < 0.05$) at 10 mg/kg and remained elevated post-treatment (**Table 11**). In female rats, *C. afer* (10–250 mg/kg) produced a significant reduction in FSH levels< ($p < 0.05$–$0.0001$) that was not reversed following the 30-day recovery period. Estrogen levels decreased reversibly ($p < 0.05$) at 250 mg/kg. Additionally, LH levels significantly increased ($p < 0.05$) after 90 days of treatment at 250 mg/kg compared with the control (**Table 12**).

## Histopathological findings

Histological examination revealed that *C. afer* at 10–50 mg/kg did not alter the brain's microscopic architecture in either sex after 90 days of continuous administration. Neuronal morphology remained intact, with no observable lesions. How-

**Table 12.** Effect of hydroethanol leaf extract of *Costus afer* on hormones in female rats.

| Treatment | Dose (mg/kg) | FSH (u/mg) | LH (u/mg) | Progesterone (u/mg) | Estrogen (u/mg) |
|---|---|---|---|---|---|
| MAIN | | | | | |
| Distilled water | 10 (mL/kg) | 2.26±0.06 | 2.03±0.03 | 17.96±2.86 | 2.34±0.24 |
| C. afer | 10 | $0.87±0.11^b$ | 1.21±0.05 | 21.34±2.06 | 1.77±0.41 |
| C. afer | 50 | $0.85±0.10^c$ | 1.44±0.63 | 16.75±3.46 | 1.59±0.63 |
| C. afer | 250 | $1.60±0.11^d$ | 0.79±0.05 | 25.24±7.24 | $0.51±0.10^a$ |
| REV | | | | | |
| Distilled water | 10 (mL/kg) | 2.03±0.03 | 48.49±1.10 | 30.04±0.46 | 2.98±0.10 |
| C. afer | 10 | 1.21±0.05 | 50.63±0.91 | 22.07±1.80 | $10.91±1.24^a$ |
| C. afer | 50 | $1.44±0.63^d$ | 55.45±3.14 | 33.23±1.29 | 3.62±0.94 |
| C. afer | 250 | $0.76±0.05^d$ | $59.73±2.80^a$ | 41.33±1.14 | $14.99±3.10^b$ |

Values are mean ± S.E.M.(n=8 per sex; 5 per sex for the main study and 3 per sex for the reversibility study). [a]p0.05, [b]p0.01, [c]p<0.001, [d]p<0.0001 vs. distilled water (One way ANOVA followed by Dunnett's multiple comparison test). **REV:** Reversibility study. **FSH**: Follicle stimulating hormone, **LH**: Luteinizing Hormone

ever, at 250 mg/kg, moderate congestion of cerebral blood vessels and focal neuronal degeneration and necrosis were observed. These changes were reversed following the 30-day recovery period, with restoration of normal neuronal architecture (**Figure 1**). Liver sections from both male and female rats treated with 10–50 mg/kg showed normal hepatic plates with mild thinning of hepatic cords and dilation of hepatic sinusoids, without vascular alterations. At 250 mg/kg, marked thinning of hepatic cords, closely packed hepatic plates, and focal single hepatocellular necrosis were observed. These alterations were fully reversed after the 30-day recovery period (**Figure 2**). Renal histology revealed normal glomerular and tubular architecture at 10–50 mg/kg in both sexes.

In contrast, kidneys from rats treated with 250 mg/kg exhibited cellular degeneration, apoptosis,

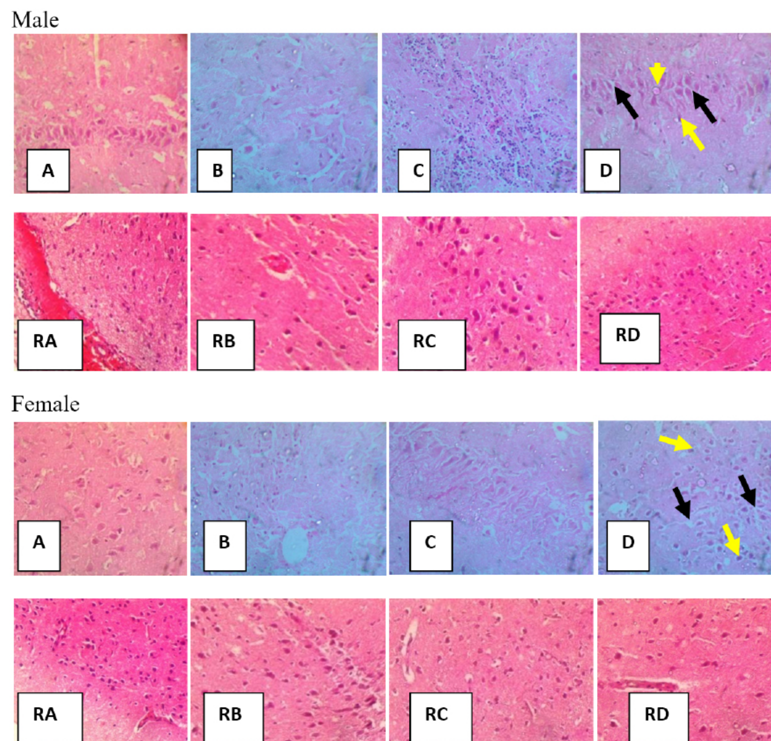


**Figure 1.** Photomicrographs of representative male and female brain tissues of rats treated with distilled water 10 mL/kg (A, RA), CA 10 mg/kg (B, RB), CA 50 mg/kg (C, RC), and CA 250 mg/kg (D, RD) (× 100). R: Reversibility study. In both male and female rat brain tissues, moderate congestion of cerebral blood vessels (yellow arrow) and few foci of neuronal degeneration and necrosis (black arrow) were seen at 250 mg/kg of the extract. RA, RB, RC, & RD: Reversibility

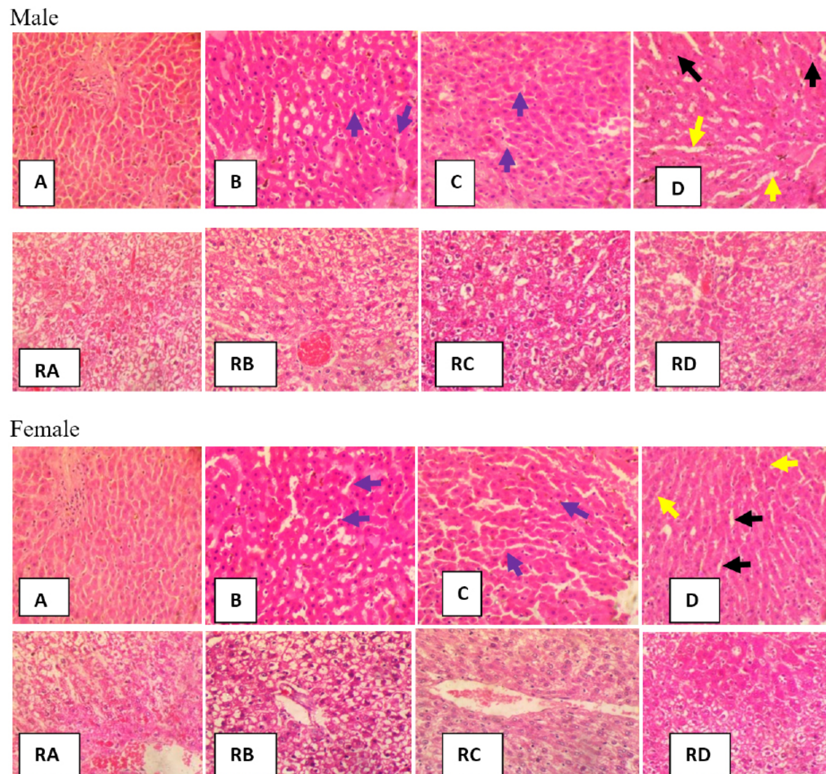


**Figure 2.** Photomicrographs of representative male and female liver tissues of rats treated with distilled water 10 mL/kg (A, RA), CA 10 mg/kg (B, RB), CA 50 mg/kg (C, RC), and 250 mg/kg (D, RD) (× 100). R: Reversibility study. In both male and female rat liver tissues, there was mild thinning of hepatic cord at 10 and 50 mg/kg (purple arrow); marked thinning of hepatic cord (black arrow) and foci of hepato-cellular necrosis at 250 mg/g (yellow arrow). RA, RB, RC, & RD: Reversibility

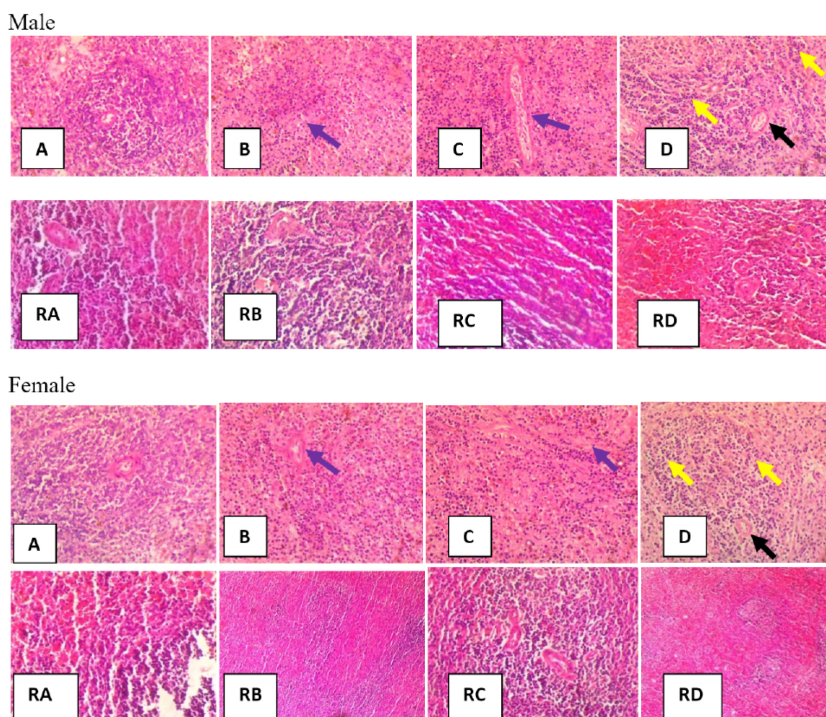


**Figure 3.** Photomicrographs of representative male and female kidney tissues of rats treated with distilled water 10 mL/kg (A, RA), CA 10 mg/kg (B, RB), CA 50 mg/kg (C, RC), and CA 250 mg/kg (D, RD) (× 100). R: Reversibility study. In both male and female rat kidney tissues, normal glomerular and tubular structures were observed at 10 and 50 mg/kg (purple arrow); atrophy and congestion of glomerular tubules (black arrow) and cellular degeneration and apoptosis (yellow arrow) were observed at 250 mg/kg of the extract. RA, RB, RC, & RD: Reversibility

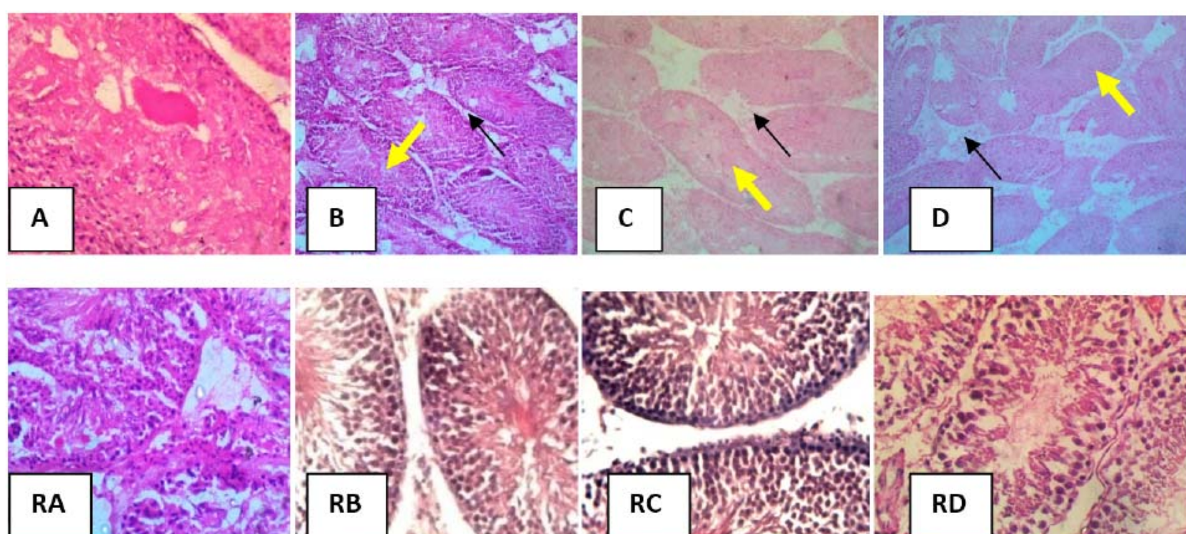


**Figure 4.** Photomicrographs of representative testes tissues of rats treated with distilled water 10 mL/kg (A, RA), CA 10 mg/kg (B, RB), CA 50 mg/kg (C, RC), and 250 mg/kg (D, RD) (× 100). R: Reversibility study. The rat testes tissues showing normal seminiferous tubules and spermatocytes (yellow arrow) and normal blood vessels (black arrow). RA, RB, RC, & RD: Reversibility

glomerular and tubular atrophy, and congestion. These pathological changes were reversed after the 30-day recovery period (**Figure 3**). Testicular histology showed no visible lesions at any dose. Interstitial cells appeared intact, spermatogenic cells were preserved, seminiferous tubules and spermatocytes were normal, and there was no evidence of vascular congestion (**Figure 4**).

## Discussion

Previous research on *Costus afer* Ker Gawl has largely focused on its medicinal properties and limited toxicity evaluations in rodent models. Several studies have reported biological activities of various *C. afer extracts*, including antioxidant, anti-inflammatory, and organ-protective effects, supporting its traditional use in managing metabolic and toxicant-induced conditions in experimental animals. Despite these findings, there is a paucity of systematic acute and extended (90-day) subchronic toxicity data specifically for the hydroethanol leaf extract, particularly with detailed histopathological evaluation of multiple internal organs, alongside haematological, biochemical, urine, and reproductive parameters. This gap limits a clear understanding of the safety profile, dose-dependent effects, and potential reversible versus irreversible tissue responses to prolonged exposure. The present study, therefore, addresses these limitations by providing a more thorough and extended assessment of hydroethanol leaf extract toxicity in rodents, thereby providing essential toxicological evidence to guide its safe therapeutic use.

Medinat et al. and Akindele et al. [23,33] reported that acute and sub-chronic toxicity studies are widely accepted experimental models for determining the safety profile of substances. Acute toxicity testing is a standard *in vivo* method used to evaluate toxicokinetics, toxicodynamics, dose–response relationships, and the median lethal dose ($LD_{50}$) [34,35]. The oral $LD_{50}$ of *Costus afer* was estimated to be greater than 2 g/kg in mice, while the intraperitoneal $LD_{50}$ was found to be 298 mg/kg. The extract did not cause mortality within 24 hours of administration, and no immediate or delayed toxicity was observed during the 2-week post-treatment monitoring period. Extracts with acute oral $LD_{50}$ values greater than 2 g/kg are generally considered practically non-toxic and safe for use [36,37]. Therefore, *C. afer* may be considered relatively safe based on acute toxicity testing.

Body weight changes, together with food and water intake, are fundamental physiological indices commonly used to assess the systemic and metabolic effects of bioactive plant extracts in experimental models. In this study, administration of *C. afer* (10–250 mg/kg) produced a reversible, dose-dependent reduction in body weight

alongside a reversible increase in feed and water intake in male rats. The reduction in body weight despite increased feed intake suggests that the extract may improve metabolic efficiency or nutrient utilisation rather than suppress appetite. Such effects may be mediated through enhanced energy expenditure, altered lipid or carbohydrate metabolism, or reduced intestinal nutrient absorption. Increased water intake may also represent a compensatory response to elevated metabolic activity or subtle fluid loss. Importantly, the reversibility of these effects following the 30-day recovery period indicates the absence of permanent metabolic or organ dysfunction at the tested doses.

In contrast, female rats exhibited a dose-specific response. At the highest dose (250 mg/kg), *C. afer* induced reversible reductions in body weight, food intake, and water consumption, suggesting possible sex-dependent sensitivity to higher extract concentrations. The observed reduction in feed and water intake at supra-therapeutic doses may reflect mild central or peripheral anorectic effects or gastrointestinal discomfort. Assessment of vital organ weights serves as an important indicator of systemic toxicity and organ-specific effects. In male rats, administration of *C. afer* (10–250 mg/kg) produced a reversible increase in the weights of most vital organs, suggesting a dose-related physiological adaptation rather than overt toxicity. Conversely, no significant changes in the weights of most vital organs were observed in female rats, indicating a sex-dependent response. An exception was observed in the lungs, which showed a significant but reversible reduction in weight at doses of 10 and 50 mg/kg. This effect may reflect transient alterations in pulmonary hydration status, vascular tone, or smooth muscle activity mediated by bioactive phytochemicals present in the extract. Reductions in body weight gain, organ weights, and food and water intake are indicative of the toxic effects of substances in experimental animals [33,37,38]. At the same time, organ hypertrophy has also been reported as a potential manifestation of toxicity [34]. Alam and Najam [39] reported that reduced food and water intake may reflect central nervous system effects capable of inducing anorexia. Based on these findings, *C. afer* cannot be regarded as entirely safe at supra-therapeutic doses due to its effects on body weight and consumption parameters. However, the extract appears safe at sub-therapeutic and therapeutic doses, where no adverse effects on these parameters were observed.

Haematological parameters are sensitive indicators of immune status, oxygen-carrying capacity, and systemic toxicity. In male rats, administration of *C. afer* (10–250 mg/kg) resulted in a 30-day increase in total white blood cell count, differential leukocyte counts, lymphocytes, and platelets, suggesting stimulation of immune and hematopoietic activity. Conversely, reductions in red blood cell count and haemoglobin concentration indicate a possible transient effect on erythropoiesis or red cell survival, potentially arising from mild bone marrow suppression, altered iron utilisation, or increased red cell turnover. In female rats, administration of the extract produced a reversible reduction in total white blood cell count, neutrophils, and lymphocytes, suggesting a transient immunomodulatory effect. The reversibility of these changes indicates that the extract did not induce sustained immunosuppression or bone marrow toxicity but rather a temporary adaptive response, possibly mediated by bioactive phytochemicals with immunomodulatory properties. Hanga-Farcaș et al. [40] reported that certain phytochemical components can depress bone marrow activity and reduce blood cell synthesis. Haematological analysis is a valuable predictor of the toxic potential of plant extracts [16,41]. Maxwell et al. [42] identified the hematopoietic system as a primary target of toxic compounds and a key indicator of systemic response to injury. According to Akindele et al. [23] and Kelly [43], non-significant effects on haemoglobin, red blood cells, and packed cell volume following long-term extract administration reduce the likelihood of extract-induced anaemia. Based on these findings, the post-treatment increase in WBC, its differentials and lymphocytes in male animals reflects immune activation or enhanced bone marrow activity, possibly mediated by bioactive phytochemicals known to possess immunomodulatory properties. At the same time, the reduction in red blood cell count and haemoglobin suggests mild bone marrow suppression, which is likely adaptive rather than indicative of severe hematotoxicity. The 30-day recovery period decreased WBC, neutrophils and lymphocytes. The significant increase in RBC,

Hb, and PCV at a sub-therapeutic dose of the extract in female rats suggests dose-dependent and parameter-specific haematological effects, with reversible changes in leukocyte profiles and a sustained enhancement of erythroid indices at low dose, highlighting both the immunomodulatory and possible hematine potential of the extract.

Biochemical parameters provide sensitive indices of metabolic and organ-specific effects. In this study, administration of *C. afer* extract at 250 mg/kg produced irreversible elevations in triglycerides, alkaline phosphatase (ALP), and alanine aminotransferase (ALT), suggesting potential disturbances in lipid metabolism and hepatic function at high doses. Across the dose range of 10–250 mg/kg, the extract reduced high-density lipoprotein cholesterol (HDL-c), suggesting altered lipid transport or impaired reverse cholesterol transport. Additionally, male rats exhibited a post-administration increase in uric acid, suggesting possible effects on purine metabolism or renal excretory function. Conversely, the dose-dependent reduction in low-density lipoprotein cholesterol (LDL-c) at 50–250 mg/kg may indicate a partial hypolipidemic effect, potentially mediated by phytochemicals known to modulate cholesterol synthesis and clearance. At 250 mg/kg, the extract irreversibly reduced serum triglycerides and LDL-cholesterol while increasing HDL-cholesterol, indicating a potentially beneficial effect on lipid metabolism and cardiovascular risk profile. These changes suggest that bioactive constituents of *C. afer* may enhance lipid utilisation or clearance and promote a favourable lipoprotein balance.

In contrast, the reduction in HDL-cholesterol observed at 50 mg/kg reflects a non-linear dose response, a phenomenon commonly reported with phytochemicals and indicative of differential pathway activation at varying doses. Regarding liver function indices, the extract decreased alkaline phosphatase (ALP) at 250 mg/kg and alanine aminotransferase (ALT) at 10 mg/kg. Reductions in these enzymes are generally not associated with hepatocellular injury and may reflect preserved hepatic integrity or mild hepatoprotective activity. Du and Qin [44] implicated high levels of lipids and lipoproteins in the blood (dyslipidemia) as possible risk factors for cardiovascular diseases and atherosclerosis. A low level of HDL-c increases the risk of cardiovascular disease, while a high level of HDL-c shows cardioprotective properties [45]. An elevated level of LDL-c, cholesterol, and triglycerides increases the risk of heart dysfunction, while a low level protects the heart [46]. ALT, AST, and ALP are major biomarkers for evaluating liver function [47]. An increase in serum ALT, AST, and ALP levels reflects major hepatic permeability, congestion, or rupture [48,49]. Medinat et al. [33] reported that increased levels of AST and ALT are more specific for liver diseases. AST is not only expressed in liver damage, as it is also found in abnormalities associated with the muscles, kidneys, heart, RBCs, and brain [50], unlike ALT, which is largely a specific indicator of liver [51]. In this study, the increase in ALP and ALT in male rats at 250 mg/kg of the extract suggests possible liver toxicity. The significant increase in triglycerides and decrease in HDL-c in male animals is a reflection of cardiovascular toxicity. The decrease in triglyceride and LDL-c, and the increase in HDL-c (250 mg/kg), suggest cardio-protective function of the extract in female animals. In contrast, the decrease in ALP and ALT indicates possible hepatoprotective activity of the extract in female rats.

Sperm parameters are sensitive indicators of male reproductive toxicity. In this study, administration of *C. afer* at 250 mg/kg resulted in a significant reduction in sperm count that was not reversed following the 30-day recovery period, suggesting a potential adverse effect on spermatogenesis at high doses. This irreversible decrease may indicate disruption of germ cell development or sustained impairment of testicular function. Conversely, the reversible reduction in sperm motility observed at the same dose suggests a transient functional effect, possibly related to alterations in epididymal maturation, energy metabolism, or spermatozoon membrane integrity. The recovery of motility after the 30-day recovery period implies that this effect is not associated with permanent structural damage to mature sperm cells. These findings align with reports by Anna-Lizia et al. [52], Akindele et al. [23], and Panter et al. [53], who observed the restoration of spermatogenesis following withdrawal of toxic agents. Thus, *C. afer* at high doses may induce male infertility.

Urinalysis revealed no significant alterations in male rats, except for a decrease in urine

pH during the 30-day recovery period, suggesting delayed metabolic acidosis possibly associated with reduced water intake. In female rats, increased urine pH and reduced specific gravity were observed, likely reflecting increased fluid intake and altered physicochemical properties of the extract. These changes were reversible following the 30-day recovery period.

Hormonal evaluation revealed that *C. afer* (10–250 mg/kg) produced a significant reduction in follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in male rats, suggesting an effect on the hypothalamic–pituitary–gonadal (HPG) axis. Despite reductions in FSH and LH, administration of *C. afer* at 10 mg/kg resulted in a significant increase in testosterone levels, suggesting a direct stimulatory effect on testicular steroidogenesis independent of gonadotropin signalling. This dissociation between gonadotropin suppression and elevated testosterone suggests that the extract may enhance Leydig cell activity or steroidogenic enzyme expression. Notably, the persistence of elevated testosterone levels following the 30-day recovery period indicates a sustained biological effect, which may have implications for reproductive function. In female rats, *C. afer* (10–250 mg/kg) produced a significant and non-reversible reduction in follicle-stimulating hormone (FSH) levels, suggesting a sustained modulatory effect on the hypothalamic–pituitary–gonadal axis.

In contrast, the observed reversible decrease in estrogen levels indicates a transient effect on ovarian steroidogenesis, which normalised following the 30-day recovery period. This reversibility suggests functional rather than permanent ovarian impairment. Additionally, the 30-day recovery period increase in luteinizing hormone (LH) at the highest dose (250 mg/kg) may represent a compensatory response to reduced estrogen levels, consistent with negative feedback regulation within the reproductive endocrine system. Serum testosterone maintains the physiological functions of the male reproductive organ [54]. The synthesis and release of testosterone depend on the release of Luteinizing hormone via a negative feedback system [55]. The release of testosterone from the anterior pituitary gland, with the aid of Luteinizing hormone, can be disrupted by exposure to toxic compounds/plants, which negatively impacts the endocrine function of the testes [55]. Ebenyi et al. [56] reported that medicinal plants can damage the male reproductive system through their antioxidative, anti-androgenic, and inhibitory activities on sex hormone-induced benign prostate hyperplasia, as demonstrated in *in vivo* and *in silico* studies. Oduwole et al. [57] reported that elevated levels of FSH and LH increase testosterone production during spermatogenesis. A low FSH level may alter folliculogenesis and delay follicular maturation [58]. Musa et al. [59] and Ogunlakin [60] demonstrated that estrogenic plants enhance the functions of FSH and LH, thereby promoting ovulation. Based on these assertions, the significant increase in testosterone suggests that the extract may promote spermatogenesis at lower doses.

In contrast, the decrease in FSH and LH, especially at supra-therapeutic doses, may negatively affect male fertility. The decrease in FSH and estrogen levels in female rats suggests sustained suppression of FSH and transient alterations in estrogen and LH. While the reversibility of estrogen and LH changes implies adaptive endocrine modulation, the non-reversible reduction in FSH is indicative of female reproductive toxicity.

Histological assessment remains a critical tool for detecting subtle structural alterations that may not be apparent through biochemical or physiological indices alone. The observed microscopic changes across the examined organs provide important insights into the organ-specific responses to the extract and contribute to understanding its safety profile and biological activity. Histopathological assessment of the brain, liver, kidney, and testes in both genders revealed no lesions or pathologies, even at sub-therapeutic and therapeutic doses. However, at the supra-therapeutic dose, evidence of neuronal degeneration, necrosis, and moderate congestion of cerebral blood vessels was observed in the brain; necrosis and marked thinning of the hepatic cords were observed in the liver; and glomerular and tubular degeneration, atrophy, and congestion were observed in the kidney. Based on these findings, the extract is toxic at high doses. However, the testes did not show any lesions at supra-therapeutic doses.

The hydroethanol leaf extract of *C. afer* had previously been reported to contain flavonoids, steroids, glycosides, phenols, alkaloids, terpenoids and tannins [19]. Plant parts of *C. afer*,

including the leaves, have been reported to contain significant amounts of micronutrients and macronutrients, with the presence of steroidal sapogenins, aferosides, dioscin, and paryphyllin C and flavonoid glycoside kaempferol-3-O-α-L-rhamnopyranoside, which have been associated with various beneficial pharmacological effects [12]. Although the mechanism of action of most phytotoxins is unclear, previous studies have shown that some phytoconstituents may be the toxicants responsible for plant toxicity [44,61].

In our previous study, FT-IR analysis, as reported by Murtala *et al.* [19], demonstrated the presence of key functional groups in *Costus afer*, including carbonyl groups (C=O, 1795.60 $cm^{-1}$), unsaturated carbon–carbon bonds (C=C, 1631.53 $cm^{-1}$), phenolic hydroxyl groups (O–H, 3355.71 $cm^{-1}$), and C–O stretching vibrations (1075.86 and 1030.08 $cm^{-1}$), indicative of alcohols, ethers, and glycosides. These functional moieties, particularly carbonyl, phenolic hydroxyl, unsaturated C=C, and C–O–linked glycosidic groups, are the most plausible contributors to the dose-dependent toxicological effects of *C. afer*. Such chemical functionalities are widely implicated in oxidative stress generation, membrane perturbation, endocrine modulation, and organ-specific toxicity, especially under conditions of phytochemical accumulation at supra-therapeutic doses.

Similarly, in a separate investigation by Murtala *et al.* [62], GC–MS analysis of *C. afer* identified hexadecanoic acid ethyl ester (peak #18), (E)-9-octadecenoic acid ethyl ester (peak #16), 13-octadecenal (Z) (peak #25), octadecanoic acid, 2-hydroxy-1,3-propanediyl (peaks #19 and #26), and octadecanoic acid (peak #20) as the predominant constituents of the extract. Among these compounds, 13-octadecenal (Z) appears to have the strongest association with toxicity, attributable to its reactive aldehyde functional group and its propensity to induce oxidative stress and cellular injury. In contrast, the identified fatty acid esters and saturated fatty acids, such as hexadecanoic acid ethyl ester, ethyl oleate, and stearic acid derivatives, are generally considered biologically inert at low concentrations. However, at very high doses, these lipophilic constituents may contribute to lipotoxicity, membrane disruption, and metabolic stress, thereby amplifying the extract's overall toxic potential at supra-therapeutic exposure levels.

## Limitations of the study

These include the lack of chemical and chromatographic standardisation of the extract and a smaller sample size in the recovery arm, which could have prevented the assessment of sex-specific manifestations, reduced the statistical power and limited confidence in the reported reversibility effects.

## Conclusion

This study demonstrates that sub-chronic oral administration of *Costus afer* hydroethanolic leaf extract produces dose- and sex-dependent toxicological effects in rats. At sub-therapeutic and therapeutic doses, the extract was generally well tolerated, with reversible changes in body weight, food and water intake, organ weights, haematological indices, biochemical parameters, hormonal profiles, urinalysis findings, and no histopathological lesions in vital organs. These findings suggest the relative safety of *C. afer* at lower doses following repeated administration. However, supra-therapeutic dosing (250 mg/kg) was associated with adverse outcomes, including reductions in body weight and intake parameters, alterations in hematological and biochemical indices, evidence of hepatic, renal, and neural histopathological damage, disruption of lipid metabolism, irreversible suppression of certain reproductive parameters (notably sperm count and FSH levels), and endocrine disturbances in both sexes. These effects indicate that *C. afer* possesses toxic potential at high doses, particularly with prolonged exposure. The No-Observed-Adverse-Effect Level (NOAEL) for the hydroethanol leaf extract of Costus afer in rats was determined to be 50 mg/kg body weight, as doses up to this level did not produce persistent or biologically significant adverse effects across the assessed parameters. The Lowest-Observed-Adverse-Effect Level (LOAEL) was established at 250 mg/kg body weight, based on clear evidence of adverse outcomes, including irreversible elevations in hepatic enzymes (ALT and ALP), persistent dyslipidemia, irreversible reduction in sperm count, sustained suppression of follicle-stimulating hormone in females, and histopathological lesions in the brain, liver, and kidney. Overall, the results

highlight that while *C. Afer* may be considered relatively safe within defined low and therapeutic dose ranges; its use at higher doses poses significant systemic and reproductive toxicity risks. The study underscores the importance of dose regulation in the medicinal use of *C. afer.*


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**Authors' contributions**
AAM – Conception, conduct, data analysis, result interpretation and manuscript writing; ODA – Methodology, result interpretation and manuscript review; AJA – Conception, design, result interpretation and manuscript review; IAO – Conception, result interpretation and manuscript review.

**Conflict of interest statement**
The authors declare no conflict of interest.

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