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Acute and Subacute Oral Toxicity Study of *Anthocleista djalonensis* Hydroethanolic Leaf Extract in male Swiss mice

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ABSTRACT

Background: *Anthocleista djalonensis* is a medicinal plant used traditionally in West and Central Africa to treat gastrointestinal, hepatic, and inflammatory conditions.

Objectives: This study assessed the acute and subacute oral toxicity of *Anthocleista djalonensis* hydroethanolic leaf extract (ADHE) in male Swiss mice.

Methodology: Acute toxicity was assessed in male Swiss mice using a single oral dose of 2000 mg/kg. For the subacute toxicity study, a separate set of mice (n=15) were divided into three groups (n=5/group). Group 1 served as the control and received distilled water (10 mL/kg, p.o.), while groups 2 and 3 received *Anthocleista djalonensis* hydroethanolic leaf extract orally at 400 mg/kg and 800 mg/kg, respectively, for 28 days, after which haematological, biochemical, metabolic, and histopathological parameters were evaluated.

Results: Acute toxicity assessment showed no mortality at 2000 mg/kg. Repeated administration of ADHE significantly increased serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, total protein, and blood urea nitrogen levels at both doses. At 800 mg/kg, ADHE caused hyperglycemia and significant elevations in total cholesterol, triglycerides, high-density lipoprotein, and low-density lipoprotein levels ($p < 0.05$). ADHE also produced significant reductions in packed cell volume [$F(2,6) = 18.52$, $p = 0.0027$], hemoglobin concentration [$F(2,6) = 17.51$, $p = 0.0031$], and red blood cell count [$F(2,6) = 14.96$, $p = 0.0047$]. Histopathological examination revealed hepatic cord atrophy at 400 mg/kg, progressing to centrilobular degeneration and necrosis at 800 mg/kg, while renal sections showed tubular epithelial coagulative necrosis at both doses. Cardiac tissue demonstrated myocyte nuclear hypertrophy at 800 mg/kg with preserved myocardial architecture.

Conclusion: These findings indicate that repeated exposure to ADHE induces hepatic, renal, hematological, and metabolic disturbances, suggesting potential safety concerns associated with its consumption despite its widespread traditional use.

Keywords: *Anthocleista djalonensis*; Subacute toxicity; Acute toxicity; Hepatotoxicity; Nephrotoxicity

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Étude de la toxicité orale aiguë et subaiguë d'un extrait hydroéthanolique de feuilles d'*Anthocleista djalonensis* chez des souris mâles suisses

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RÉSUMÉ

Contexte : *Anthocléista djalonensis* est une plante médicinale traditionnellement utilisée en Afrique de l'Ouest et centrale pour traiter les affections gastro-intestinales, hépatiques et inflammatoires.

Objectifs : Cette étude a évalué la toxicité orale aiguë et subaiguë de l'extrait hydroéthanolique de feuilles *d'Anthocleista djalonensis* (ADHE) chez des souris suisses mâles.

Méthodologie : La toxicité aiguë a été évaluée chez des souris mâles Swiss après administration d'une dose unique de 2 000 mg/kg par voie orale. Pour l'étude de toxicité subaiguë, un autre groupe de souris (n = 15) a été divisé en trois groupes (n = 5/groupe). Le groupe 1, servant de témoin, a reçu de l'eau distillée (10 mL/kg, p.o.), tandis que les groupes 2 et 3 ont reçu *l'extrait hydroéthanolique* de feuilles *d'Anthocleista djalonensis* par voie orale à des doses respectives de 400 mg/kg et 800 mg/kg pendant 28 jours, à l'issue desquels les paramètres hématologiques, biochimiques, métaboliques et histopathologiques ont été évalués.

Résultats : L'évaluation de la toxicité aiguë n'a révélé aucun cas de mortalité à la dose de 2 000 mg/kg. L'administration répétée d'ADHE a entraîné une augmentation significative des taux sériques d'aspartate aminotransférase (ASAT), d'alanine aminotransférase (ALAT), de phosphatase alcaline, de protéines totales et d'urée sanguine aux deux doses. À la dose de 800 mg/kg, l'ADHE a induit une hyperglycémie et une augmentation significative des taux de cholestérol total, de triglycérides, des lipoprotéines de haute densité et des lipoprotéines de basse densité ($p < 0,05$). L'ADHE a également entraîné une diminution significative de l'hématocrite [$F(2,6) = 18,52$; $p = 0,0027$], de la concentration d'hémoglobine [$F(2,6) = 17,51$; $p = 0,0031$] et du nombre de globules rouges [$F(2,6) = 14,96$, $p = 0,0047$]. L'examen histopathologique a révélé une atrophie des cordons hépatiques à 400 mg/kg, évoluant vers une dégénérescence centrolobulaire et une nécrose à 800 mg/kg, tandis que les coupes rénales ont montré une nécrose de coagulation de l'épithélium tubulaire aux deux doses. Le tissu cardiaque a présenté une hypertrophie nucléaire des myocytes à la dose de 800 mg/kg avec une architecture myocardique préservée.

Conclusion : Ces résultats indiquent qu'une exposition répétée à l'ADHE induit des troubles hépatiques, rénaux, hématologiques et métaboliques, ce qui suggère des préoccupations potentielles quant à l'innocuité de sa consommation malgré son usage traditionnel répandu.

Mots-clés : *Anthocleista djalonensis* ; toxicité subaiguë ; toxicité aiguë ; hépatotoxicité ; néphrotoxicité

INTRODUCTION

Herbal medicines are widely perceived as inherently safe because of their natural origin. Yet, organ toxicity from plant-derived bioactive compounds has been documented at supratherapeutic doses and with prolonged use.[1,2] This risk is particularly pronounced among children, pregnant women, and individuals with underlying hepatic or renal impairment, who frequently rely on plant-based preparations as primary healthcare in the absence of standardized dosing or formal toxicological guidance.[3,4]

Anthocleista djalonensis A. Chev. (Gentianaceae, formerly Loganiaceae), also known as cabbage tree, is a medicinal tree distributed across the forest zones of West and Central Africa, including Nigeria, Ghana, Cameroon, and Guinea, where the Yoruba call it Ewe Shapo and it is important in local ethnomedicinal practice.[5] Preparations derived from the leaves, roots, and stem bark are traditionally used to manage wound infections, diarrhea, dysentery, inflammatory disorders, hepatitis, jaundice, and sexually transmitted infections. The plant is also used as a purgative and diuretic.[6] Consequently, these preparations are consumed by individuals with pre-existing hepatic compromise, including those with hepatitis and jaundice, and by pregnant women seeking abortifacient effects.[6] Several pharmacological activities relevant to these traditional applications have been experimentally demonstrated, including hypoglycemic, antiplasmodial, antifungal, anthelmintic, analgesic, and antioxidant effects across different plant parts and extract types.[7-11]

Phytochemical investigations of *A. djalonensis* have identified xanthones, phthalides, triterpenes, iridoids, sesquiterpenes, alkaloids, flavonoids, tannins, and saponins in the leaves, roots, and stem bark.[12-16] Bioactive constituents isolated from *A. djalonensis*, including djalonenol, sweroside, and djalonensone, have demonstrated concentration-dependent cytotoxicity against ST-57 brain tumor-transformed fibroblasts *in vitro.*[17] Despite its promising profile and widespread use in physiologically vulnerable populations, the toxicological evaluation of *Anthocleista djalonensis* remains limited. Existing studies have primarily investigated aqueous and ethanolic stem-bark extracts, whereas the published literature lacks safety data on the *Anthocleista djalonensis* hydroethanolic leaf extract (ADHE). Accordingly, this study evaluated the acute toxicity and 28-day repeated-dose effects of *Anthocleista djalonensis* hydroethanolic leaf extract (ADHE) on histopathology, hematological parameters, lipid profile, body and relative organ weights, hepatic and renal biochemical indices in mice.

MATERIALS AND METHODS

Collection and Authentication of Plant Material

Leaves of *Anthocleista djalonensis* were harvested in March 2025 from Ikenne, Ogun State, Nigeria. Authentication of the plant material was carried out by a plant taxonomist at the Department of Pharmacognosy, Olabisi Onabanjo University, and a voucher specimen bearing the accession number PHS/OOU/496 was deposited in the departmental herbarium for reference.

Preparation of Extract

The authenticated leaves were dried in the shade at ambient temperature and ground to a fine powder. We macerated 1,500 g of the powdered material in 70 % hydroethanol for 72 hours with intermittent shaking. The resulting mixture was filtered, and the filtrate was concentrated under reduced pressure using a rotary evaporator to yield a crude hydroethanolic extract weighing 165.47 g (11.03 % w/w yield). The extract was stored at 4°C pending further use.

Experimental Animals

Twenty-one (21) male Swiss mice weighing 22 g - 30 g were sourced from the Central Animal House, Department of Pharmacology and Therapeutics, Olabisi Onabanjo University, Nigeria, and maintained throughout the study. Animals were housed under controlled laboratory conditions comprising a 12-hour light/dark photoperiod, adequate ventilation, and a room temperature of 22-25 °C Commercial rodent pellets and potable drinking water were provided without restriction for the entire duration of the experiment. Before any experimental procedure, all animals underwent a seven-day acclimatization period to minimize handling stress and ensure physiological stability. All procedures involving experimental animals were carried out in accordance with the NIH Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, revised 1985), and conformed to the ARRIVE 2.0 reporting guidelines. Ethical clearance was granted by the College of Medicine, University of Lagos Animal Care, Use and Research Ethics Committee (CMUL/ACUREC) under Approval No. CMUL/ACUREC/03/26/2213.

Chemicals and Reagents

Diagnostic kits for biochemical analyses were sourced from Thermo Fisher Scientific (Waltham, MA, USA) and Randox Laboratories Ltd (Crumlin, United Kingdom). Kits

for total protein (Cat. No. 981874), albumin (Cat. No. 981767), AST (Cat. No. 981771), ALT (Cat. No. 981769), ALP (Cat. No. 981833), total bilirubin (Cat. No. 981952), creatinine (Cat. No. 981811), HDL-cholesterol (Cat. No. 981955), and LDL-cholesterol (Cat. No. 981956) were obtained from Thermo Fisher Scientific, U.S.A. Blood urea nitrogen (Cat. No. UR220), glucose (Cat. No. GL2623), total cholesterol (Cat. No. CH200), and triglycerides (Cat. No. TR210) kits were obtained from Randox Laboratories Ltd. All reagents used were of analytical grade.

Acute Toxicity Study

The acute oral toxicity of the *Anthocleista djalonensis* hydroethanolic leaf extract (ADHE) was evaluated in accordance with OECD Guideline 423. Six (6) male swiss mice were randomly assigned to two groups of three. All animals were fasted overnight before dosing but had free access to water throughout. The control group received distilled water at 10 mL/kg orally, while the treated group received a single oral dose of the extract at 2000 mg/kg. Following administration, animals were observed for 24 hours for signs of toxicity, including behavioral changes, restlessness, salivation, diarrhea, tremors, convulsions, and mortality.

Subacute Toxicity Study

Fifteen (15) male Swiss mice were randomly assigned to three experimental groups of five animals each. The control group received distilled water at 10 mL/kg by oral gavage daily. The two treatment groups received ADHE at 400 mg/kg and 800 mg/kg, respectively, administered once daily by oral gavage for 28 consecutive days. We selected these doses as fractions of the 2000 mg/kg limit dose used in the acute toxicity study. The 28-day repeated-dose exposure period was selected to evaluate the effects of repeated oral administration of ADHE on hematological, biochemical, metabolic, organ-weight, and histopathological parameters. We observed animals daily for behavioral changes, signs of toxicity, and mortality. We recorded body weight every 3 days and adjusted dosing volumes accordingly. The area under the curve (AUC) for body weight changes over the 28-day administration period was calculated from the mean body-weight values of each experimental group. At the end of the treatment period, we fasted animals overnight before terminal blood collection. We then anesthetized the animals and collected blood via retro-orbital puncture into EDTA-containing tubes for hematological analysis and plain tubes for serum biochemistry. Animals were subsequently sacrificed and organs harvested for biochemical and histopathological examination. Biochemical and hematological analyses were conducted using samples from three mice per group (n = 3).

Relative Organ Weight

The liver, kidney, heart, and brain were carefully excised, blotted dry, and weighed. Relative organ weight was calculated as: Relative organ weight (%) = 100 × absolute organ weight (g) / body weight (g)

Hematological Analysis

Hematological parameters, including total and differential leukocyte counts, erythrocyte count, hemoglobin concentration, packed cell volume, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet count, were determined using an Automated Hematology Analyzer (XT- 1800i, Japan) on EDTA-anticoagulated blood samples.

Biochemical Analysis

Serum was obtained by centrifugation of blood samples collected in plain tubes at 3000 × g for 10 minutes. Liver function parameters, including ALT, AST, ALP, total protein, albumin, and total bilirubin, were determined using commercially available diagnostic kits (Thermo Fisher Scientific, USA). Renal function markers, including blood urea nitrogen (BUN) and creatinine, were assayed using Randox Laboratories Ltd and Thermo Fisher Scientific kits, respectively. Blood glucose and lipid profile parameters, including total cholesterol and triglycerides, were analyzed using Randox Laboratories Ltd kits, while HDL- and LDL-cholesterol were determined using Thermo Fisher Scientific direct assay kits, according to the manufacturers' instructions.

Histopathological Examination

The liver, kidney, and heart tissues from two animals per group were fixed in 10 % neutral buffered formalin, dehydrated, embedded in paraffin wax, and sectioned at 5 µm thickness. Sections were stained with hematoxylin and eosin (H&E) and examined under a light microscope by a qualified histopathologist (Dr. Timothy Jenkre), and representative tissue sections were assessed for treatment-related morphological changes. Slides were coded before examination to minimize observer bias. The assessment focused on the presence, extent, and distribution of observable histopathological lesions in the relevant organs, with findings compared with those of the control group. Lesion severity was evaluated qualitatively based on the degree of observed histopathological changes, including cellular degeneration, necrosis, inflammation, and other relevant tissue alterations.

Data Analysis

All data are presented as mean ± standard error of the mean (SEM). For the subacute study, we assessed differences among group means using one-way or two-way analysis of variance (ANOVA) where applicable, followed by Tukey's post hoc test for multiple comparisons. We set statistical significance at $p < 0.05$. All analyses were performed using GraphPad Prism version 8.0.2 (GraphPad Software, San Diego, USA).

RESULTS

Acute Toxicity Study

Administration of ADHE at 2000 mg/kg did not result in mortality or any observable signs of toxicity during the 24-hour observation period. Mice showed normal behavior, with no tremors, convulsions, salivation, diarrhea, or changes in food and water intake, suggesting low acute toxicity and an oral LD_{50} greater than 2000 mg/kg.

Subacute Toxicity Study of *Anthocleista djalonensis* Hydroethanolic Leaf Extract (ADHE)

Effect of Anthocleista djalonensis hydroethanolic leaf extract (ADHE) on relative organ weights of male Swiss mice

Administration of ADHE at 400 mg/kg caused a significant reduction [$F(2, 6) = 8.814$, $p = 0.0164$] in relative liver weight (3.129 ± 0.114 %) when compared to control (5.623 ± 0.619 %) (Table 1). However, this effect was not sustained at 800 mg/kg (5.739 ± 0.586 %), with the 800 mg/kg group showing values significantly higher than the 400 mg/kg group ($p < 0.01$), indicating a non-dose-dependent response. No significant alterations were observed in the relative weights of the brain [$F(2, 6) = 0.2572$, $p = 0.7813$], kidney [$F(2, 6) = 1.029$, $p=0.4128$], or heart [$F(2, 6) = 1.390$, $p = 0.3192$] (Table 1)

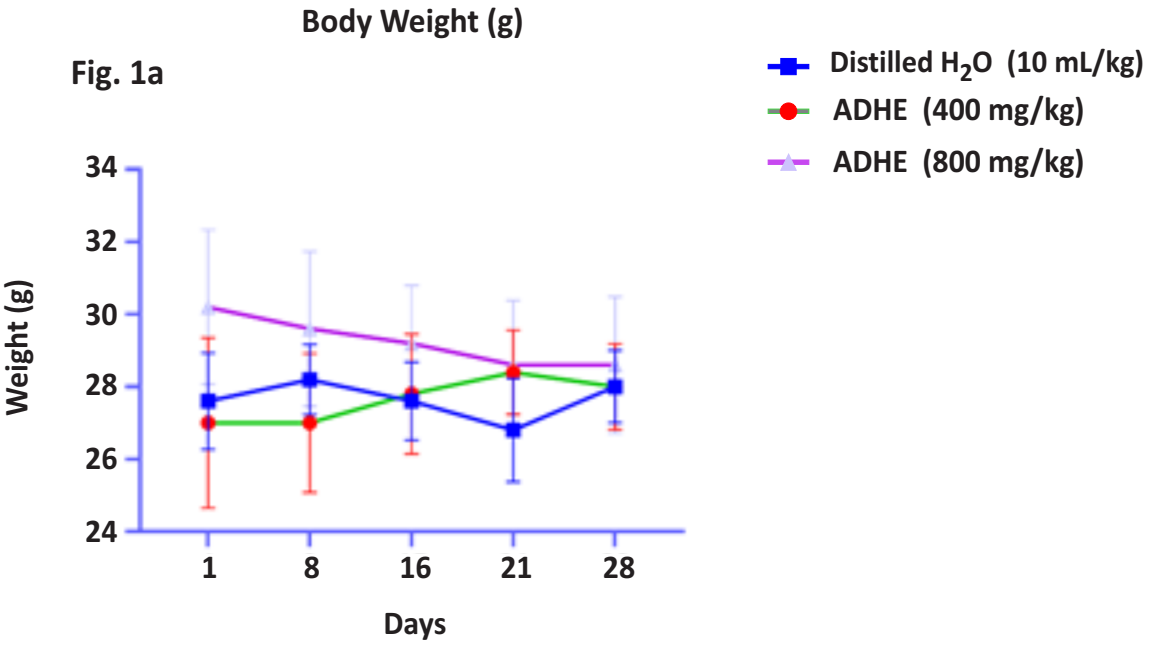
Table 1: Effect of sub-acute oral administration of *Anthocleista djalonensis* hydroethanolic leaf extract (ADHE) on relative organ weights (%) of male Swiss mice

Treatments	Relative Liver Weight (%)	Relative Brain Weight (%)	Relative Kidney Weight (%)	Relative Heart Weight (%)
Control (Distilled H_2O, 10 mL/kg)	5.623 ± 0.619	1.579 ± 0.156	0.547 ± 0.017	0.368 ± 0.031
ADHE (400 mg/kg)	3.129 ± 0.114*	1.448 ± 0.002	0.500 ± 0.017	0.448 ± 0.002
ADHE (800 mg/kg)	5.739 ± 0.586#	1.545 ± 0.171	0.5845 ± 0.004	0.438 ± 0.055

*Data are expressed as mean ± SEM (n=3). *p<0.05, **p<0.0001 compared to negative control; #p<0.05 compared to 400 mg/kg (Tukey's post - hoc test).*

Effect of *Anthocleista djalonensis* hydroethanolic leaf extract (ADHE) on body weight of male Swiss mice

Body weights recorded at baseline across all three groups were similar and showed little variation throughout the 28-day administration period (Figure 1a). Two-way ANOVA revealed no significant effects of treatment ($F(2,60)=1.602$, $p=0.2101$), time ($F(4,60)=0.02152$, $p=0.9991$), or treatment × time interaction ($F(8,60)=0.2040$, $p = 0.9891$) on body weight changes during the 28-day administration period. Analysis of the area under the body weight-time curve (AUC) (Figure 1b) equally showed no significant difference among the control (110.4 ± 3.72), ADHE 400 mg/kg (110.7 ± 5.30), and ADHE 800 mg/kg (116.8 ± 0.98) groups. One way ANOVA showed no significant difference [$F (2, 6) = 0.9124$, $p = 0.4508$] (Figure 1a and Figure 1b).



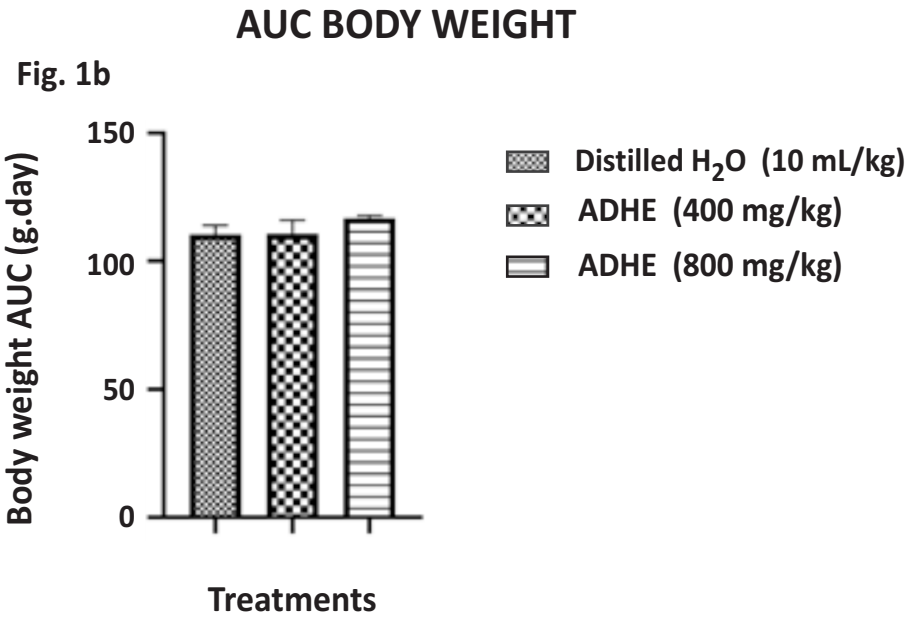


Figure 1: Effect of *Anthocleista djalonensis* hydroethanolic leaf extract (ADHE) on body weight of mice during the 28-days treatment period. (a) Mean body weight changes over time; (b) area under the curve analysis of body weight. Data are presented as mean ± S.E.M. (n = 5/group). *p < 0.05 compared with negative control; #p < 0.05 compared with 400 mg/kg group.

Effect of *Anthocleista djalonensis* hydroethanolic leaf extract (ADHE) on kidney function markers

As shown in Table 2, administration of ADHE produced a significant increase in BUN levels [F(2,6) = 24.88, p = 0.0012], with significant elevations recorded at 400 mg/kg (13.00 ± 0.11 mg/dl) and 800 mg/kg (14.17 ± 0.31 mg/dl) compared to the control (11.07 ± 0.42 mg/dl). Serum creatinine did not differ significantly between the treated groups and the control [F(2,6) = 2.400, p = 0.1715] (Table 2).

Table 2: Effect of sub-acute oral administration of *Anthocleista djalonensis* hydroethanolic leaf extract (ADHE) on kidney function markers in mice

Treatment	BUN (mg/dl)	CREAT (mg/dl)
Control (H_2O 10 mL/kg)	11.07±0.42	0.56 ± 0.03
ADHE (400 mg/kg)	13.00±0.11*	0.63 ± 0.03
ADHE (800 mg/kg)	14.17±0.31*	0.70 ± 0.05

*Data are expressed as mean ± SEM (n=3). *p<0.05, **p<0.0001 compared to negative control; #p < 0.05 compared to 400 mg/kg (Tukey's post-hoc test).*

Effect of Anthocleista djalonensis hydroethanolic leaf extract (ADHE) on liver function markers

Sub-acute administration of ADHE produced significant alterations in selected hepatic enzyme activities and serum protein levels, with liver function markers significantly increased relative to control at one or both doses tested (Table 3). TP levels were significantly elevated at 400 mg/kg (7.30 ± 0.05) and 800 mg/kg (7.63 ± 0.08) compared to control (6.53 ± 0.12) [F(2,6) = 37.35, p = 0.0004]. ALB was significantly elevated at 800 mg/kg (3.50 ± 0.11) compared to control (2.70 ± 0.11), with the 800 mg/kg group also significantly higher than the 400 mg/kg group (p = 0.0323) [F(2,6) = 17.44, p = 0.0032]. Total bilirubin did not differ significantly between the treated groups and the control [F(2,6) = 2.333, p = 0.1780]. AST was significantly elevated at 400 mg/kg (42.00 ± 0.57) and 800 mg/kg (44.00 ± 1.15) compared to the control (34.67 ± 1.45) [F(2,6) = 19.18, p = 0.0025]. ALT was significantly elevated at 400 mg/kg (30.33 ± 0.33) and 800 mg/kg (32.67 ± 1.20) compared to the control (26.00 ± 0.57) [F(2,6) = 18.18, p = 0.0028]. ALP was significantly elevated at 400 mg/kg (102.33 ± 1.20) and 800 mg/kg (109.00 ± 1.52) compared to the control (94.33 ± 0.66), with the 800 mg/kg group significantly higher than the 400 mg/kg group (p = 0.0172) [F(2,6) = 38.32, p = 0.0004] (Table 3).

Table 3: Effect of sub-acute oral administration of *Anthocleista djalonensis* hydroethanolic leaf extract (ADHE) on liver function markers in mice

Treatment	TP (g/dl)	ALB(g/dl)	T.BIL(mg/dl)	AST(U/L)	ALT (U/L)	ALP (U/L)
Control (Distilled H_2O 10 mL/kg)	6.53±0.12	2.70 ± 0.11	0.23 ± 0.03	34.67 ± 1.45	26.00 ± 0.57	94.33 ± 0.66
ADHE (400 mg/kg)	7.30 ± 0.05*	3.03 ± 0.03	0.27 ± 0.03	42.00 ± 0.57*	30.33 ± 0.33*	102.33 ± 1.20
ADHE (800mg/kg)	7.63 ± 0.08*	3.50 ± 0.11*#	0.33 ± 0.03	44.00 ± 1.15*	32.67 ± 1.20*	109.00 ± 1.52*#

*Data are expressed as mean ± SEM (n=3). *p<0.05, **p<0.0001 compared to negative control; #p < 0.05 compared to 400 mg/kg (Tukey's post-hoc test).*

Effect of *Anthocleista djalonensis* hydroethanolic leaf extract (ADHE) on metabolic and lipid profile

ADHE administration significantly affected blood glucose and lipid profile parameters (Table 4). Blood glucose was significantly elevated at 400 mg/kg (112.33 ± 2.84) and 800 mg/kg (188.33 ± 0.88) compared to control (97.33 ± 1.76), with the 800 mg/kg group significantly higher than the 400 mg/kg group (p < 0.0001) [F(2,6) = 595.1, p < 0.0001]. Total cholesterol was significantly elevated only at 800 mg/kg (77.67 ± 1.33) compared to control (67.66 ± 1.45), with no significant difference at 400 mg/kg [F(2,6) = 16.10, p = 0.0039]. Triglycerides were significantly elevated at 800 mg/kg (44.67 ± 1.85) compared to control (36.33 ± 0.33) and the 400 mg/kg group (38.33 ± 0.66), with no significant change at 400 mg/kg [F(2,6) = 14.19, p = 0.0053]. HDL cholesterol was significantly

elevated only at 800 mg/kg (41.33 ± 0.66) compared to control (32.67 ± 1.45) [F(2,6) = 15.84, p = 0.0040]. LDL cholesterol was significantly elevated at 800 mg/kg (28.67 ± 1.56) compared to control (24.53 ± 0.40), with no significant change at 400 mg/kg (25.70 ± 0.15) [F(2,6) = 5.150, p = 0.0499] (Table 4).

Table 4: Effect of sub-acute oral administration of *Anthocleista djalonensis* hydroethanolic leaf extract (ADHE) on blood glucose and lipid profile in male Swiss mice

Treatment	GLU (mg/dl)	CHOL (mg/dl)	TRIG (mg/dl)	HDL (mg/dl)	LDL (mg/dl)
Control (Distilled H_2O 10 mL/kg)	97.33 ± 1.76	67.67 ± 1.45	36.33 ± 0.33	32.67 ± 1.45	24.53 ± 0.40
ADHE (400 mg/kg)	112.33 ± 2.84*	72.33 ± 0.88	38.33 ± 0.66	37.00 ± 1.00	25.70 ± 0.15
ADHE (800 mg/kg)	188.33 ± 0.88**#	77.67 ± 1.33*	44.67 ± 1.85*#	41.33 ± 0.66*	28.67 ± 1.56*

*Data are expressed as mean ± SEM (n=3). *p<0.05, **p<0.0001 compared to negative control; #p<0.05 compared to compared to 400 mg/kg (Tukey's post-hoc test).*

Effect of Anthocleista djalonensis hydroethanolic leaf extract (ADHE) on full blood count

Administration of ADHE at 400 mg/kg and 800 mg/kg produced no significant effect on MCV [F(2,6) = 0.1429, p = 0.8697], MCHC [F(2,6) = 0.8000, p = 0.4921], and MCH [F(2,6) = 2.240, p = 0.1877] (Table 5). However, PCV was significantly reduced at 400 mg/kg (38.67 ± 2.40) and 800 mg/kg (32.66 ± 0.33) compared to control (46.00 ± 1.15) [F(2,6) = 18.52, p = 0.0027]. Hemoglobin was significantly reduced at 400 mg/kg (12.03 ± 0.42) and 800 mg/kg (10.60 ± 0.20) compared to control (14.53 ± 0.67) [F(2,6) = 17.51, p = 0.0031]. RBC count was significantly reduced at 400 mg/kg (6.02 ± 0.35) and 800 mg/kg (5.30 ± 0.05) compared to control (7.19 ± 0.22) [F(2,6) = 14.96, p = 0.0047]. Platelet counts showed no significant difference across treatment groups [F(2, 6) = 0.7269, P = 0.5216] (Table 5).

Table 5: Effect of sub-acute oral administration of *Anthocleista djalonensis* hydroethanolic leaf extract (ADHE) on Full Blood Count in male Swiss mice

Treatment	MCV (fL)	MCHC (g/dL)	MCH (pg)	PCV (%)	Hb (g/dL)	RBC (10^{12}/L)	Platelet (10^9/L)
Control (Distilled H_2O 10 mL/kg)	61.00 ± 0.57	33.00 ± 0.57	20.33 ± 0.33	46.00 ± 1.15	14.53 ± 0.67	7.19 ± 0.22	34.33 ± 5.54
ADHE (400mg/kg)	61.33 ± 0.33	32.33 ± 0.33	19.50 ± 0.25	38.67 ± 2.40*	12.03 ± 0.42*	6.02 ± 0.35*	41.67± 4.91
ADHE (800mg/kg)	61.00 ± 0.57	32.33 ± 0.33	17.87 ± 1.39	32.66 ± 0.33*	10.60 ± 0.20*	5.30 ± 0.05*	43.67± 6.69

*Data are expressed as mean ± SEM (n=3). *p<0.05, **p<0.0001 compared to negative control; #p < 0.05 compared to 400 mg/kg (Tukey's post-hoc test).*

Effect of *Anthocleista djalonensis* hydroethanolic leaf extract (ADHE) on differential blood count

Administration of ADHE at 400 and 800 mg/kg produced no significant effect on total WBC count [F(2,6) = 0.1881, p = 0.8332], lymphocyte percentage [F(2,6) = 0.4947, p = 0.6326], monocyte count [F(2,6) = 0.6000, p = 0.5787], or eosinophil count [F(2,6) = 0.6364, p = 0.5615] compared to control. All differential blood count parameters remained comparable across treatment groups (Table 6).

Table 6: Effect of sub-acute oral administration of *Anthocleista djalonensis* hydroethanolic leaf extract (ADHE) on Differential blood count in male Swiss mice

Treatment	Total WBC: cells/µL	Lymphocytes: %	Monocytes: %	Eosinophils: %
Control (Distilled H_2O 10 mL/kg)	8533.00 ± 497.80	69.67 ± 2.40	1.66 ± 0.33	2.33 ± 0.33
ADHE (400 mg/kg)	7933.00 ± 952.80	66.00 ± 2.08	2.00 ± 0.57	1.33 ± 0.88
ADHE (800 mg/kg)	8633.00 ± 1063.00	67.33 ± 3.28	1.33 ± 0.33	2.00 ± 0.57

*Data are expressed as mean ± SEM (n=3). *p<0.05, **p<0.0001 compared to negative control; #p<0.05 compared to 400 mg/kg (Tukey's post _hoc test).*

Histopathological Assessment of the Liver, Kidney, and Heart Following ADHE Administration.

Representative histological examination of the liver showed normal hepatic cord arrangement and patent sinusoidal spaces with no observable lesion in the control group (Figure 2a). At 400 mg/kg, moderate atrophy of the hepatic cords with accentuation of sinusoidal spaces was observed (Figure 2b). At 800 mg/kg, centrilobular hepatocellular degeneration with necrosis and inflammatory cell infiltration was observed (Figure 2c).

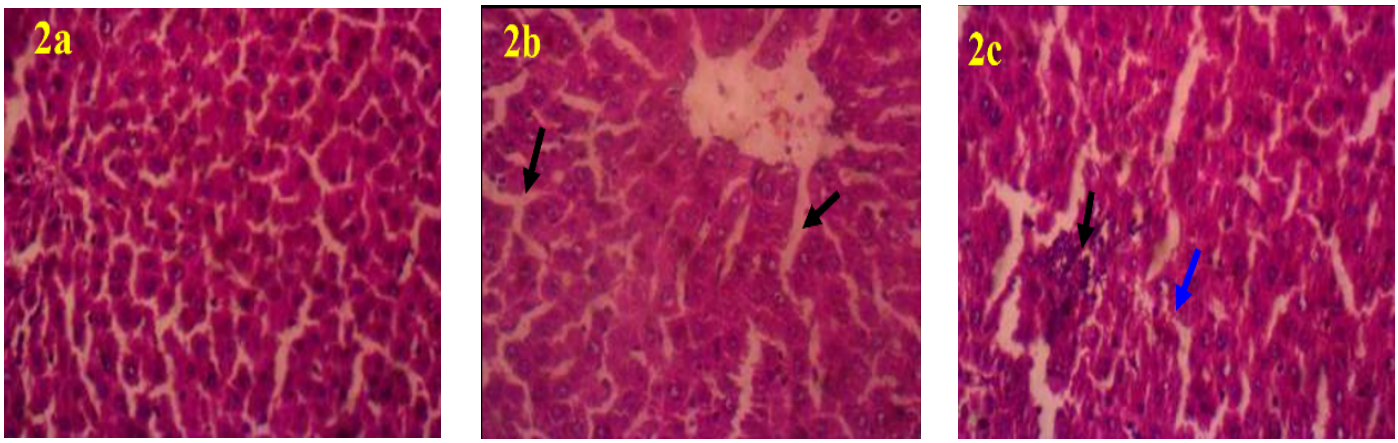


Figure 2 (a-c): Representative photomicrographs of liver sections from male Swiss mice following subacute oral administration of *Anthocleista djalonensis* hydroethanolic leaf extract (ADHE) for 28 days.

Figure 2a: Control group administered distilled water (10 mL/kg) showing normal hepatic architecture with no observable lesion. Figure 2b: ADHE 400 mg/kg showing moderate atrophy of hepatic cords and sinusoidal accentuation (arrows). Figure 2c: ADHE 800 mg/kg showing centrilobular hepatocellular degeneration, necrosis (blue arrow), and inflammatory cell infiltration (black arrow). H&E stain, ×400

Kidney section from the control group showed mild interstitial capillary congestion with preserved tubular architecture (Figure 3a). At 400 mg/kg, tubular epithelial coagulation necrosis with attenuation of tubular walls was observed (Figure 3b). At 800 mg/kg, patchy tubular epithelial coagulation necrosis, attenuation, and luminal ectasia were observed (Figure 3c).

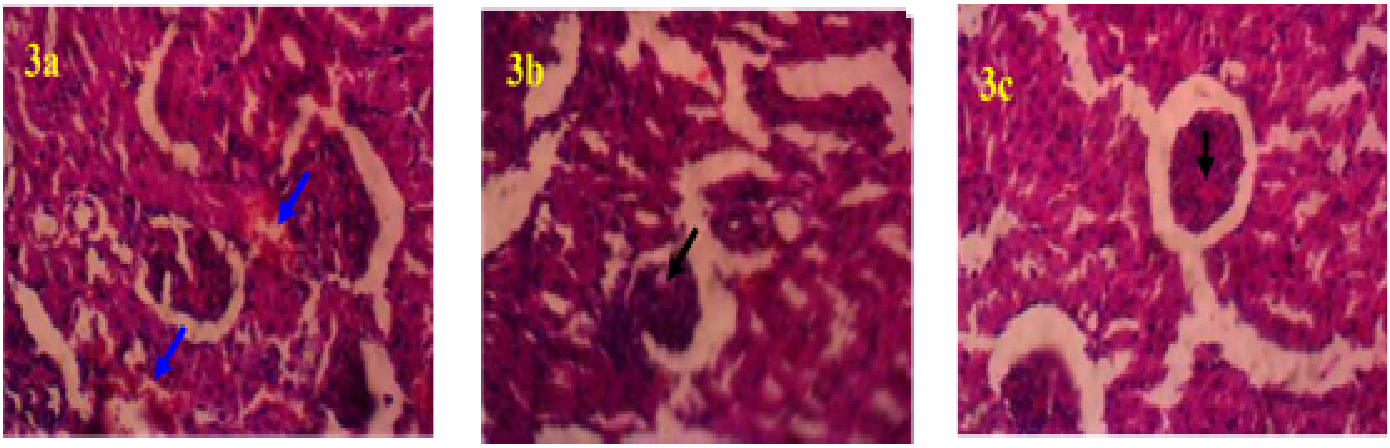


Figure 3 (a-c): Representative photomicrographs of kidney sections from male Swiss mice following subacute oral administration of *Anthocleista djalonensis* hydroethanolic leaf extract (ADHE) for 28 days.

Figure 3a: Control group administered distilled water (10 mL/kg), showing mild interstitial capillary congestion (arrows). Figure 3b: ADHE 400 mg/kg showing tubular epithelial coagulative necrosis (arrow) and attenuation. Figure 3c: ADHE 800 mg/kg showing patchy tubular epithelial coagulative necrosis (arrows) and attenuation. H&E stain, ×400

The cardiac section from the control group showed well-developed muscle fibers with uniform myocyte nuclear distribution (Figure 4a). At 400 mg/kg, cardiac muscle fiber architecture appeared normal (Figure 4b). At 800 mg/kg, overall cardiac architecture was preserved; however, some myocytes showed nuclear hypertrophy and an irregular distribution of myocyte nuclei (Figure 4c).

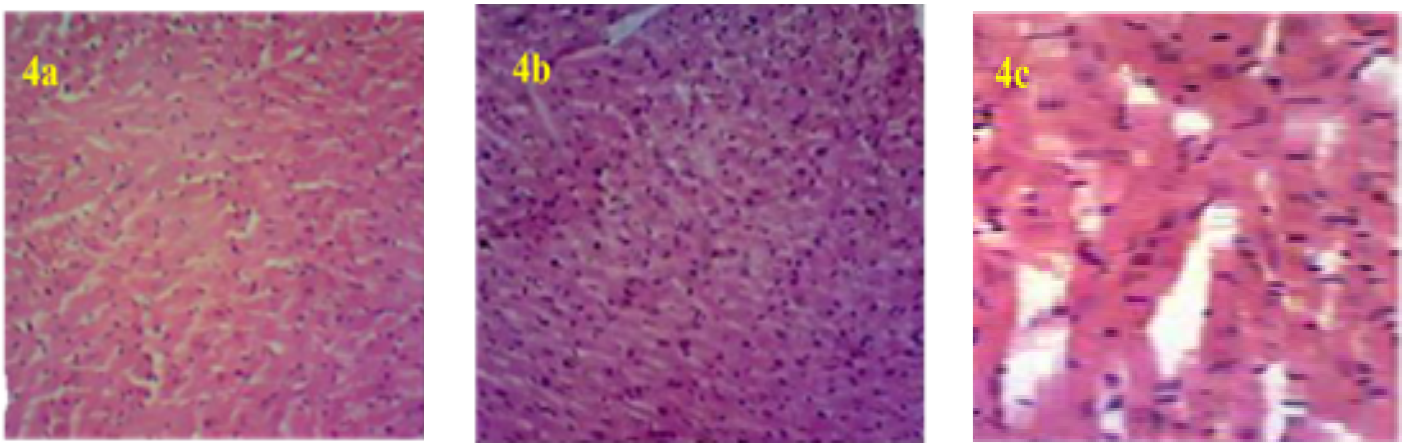


Figure 4 (a-c): Representative photomicrographs of cardiac sections from male Swiss mice following subacute oral administration of *Anthocleista djalonensis* hydroethanolic leaf extract (ADHE) for 28 days.

Figure 4a: Control group administered distilled water (10 mL/kg), showing well-developed cardiac muscle fibers with preserved myocardial architecture. Figure 4b: ADHE 400 mg/kg showing preserved cardiac muscle architecture without observable lesion. Figure 4c: ADHE 800 mg/kg showing generally preserved cardiac tissue architecture with hypertrophy of nuclei in some myocytes. H&E stain, ×400

DISCUSSION

A single oral dose of ADHE at 2000 mg/kg resulted in no mortality or observable signs of toxicity during the 24-hour observation period, indicating an oral LD_{50} greater than 2000 mg/kg. This finding is consistent with Erhunse *et al*.[18], who reported LD_{50} values exceeding 5000 mg/kg for aqueous and ethanolic stem-bark extracts of A. djalonensis, and corroborates reports of LD_{50} values exceeding 2000 mg/kg for hydroethanolic extracts of other African medicinal plants, including Lannea acida[19]

and Acacia senegal.[20] These findings indicate relatively low acute oral toxicity following single-dose exposure.

Repeated oral administration of ADHE at 400 and 800 mg/kg for 28 days did not significantly alter body weight gain in treated animals. Similar findings have been reported after administering other plant-derived hydroethanolic extracts.[21,22,23] The absence of significant changes in body weight suggests that ADHE administration did not influence overall growth parameters during the study period.

Administration of ADHE at 400 mg/kg significantly reduced relative liver weight, while histopathological examination revealed moderate atrophy of hepatic cords with accentuation of sinusoidal spaces, consistent with early hepatocellular injury. Alterations in liver weight have been associated with hepatotoxic responses in repeated-dose toxicity studies.[24] At 800 mg/kg, more pronounced hepatic alterations, including centrilobular hepatocellular degeneration, necrosis, and inflammatory cell infiltration, were observed. However, the relative liver weight was comparable with that of the control animals. These findings indicate that ADHE administration was associated with morphological alterations in the liver, particularly at the higher dose. Akachukwu *et al*. reported no observable histological alterations in the liver and kidney following short-term administration of an aqueous leaf extract of *A. djalonensis* in diabetic and malaria models.[25] The difference between the present findings and those of Akachukwu *et al*. may be due to the nature of the extract, the dose administered, the duration of treatment, and the experimental conditions employed. The hepatic alterations observed in the present study are similar to those reported after administering certain medicinal plant extracts.[26]

Relative kidney weight remained unaltered across treatment groups despite histologically confirmed coagulative necrosis of the tubular epithelium at both doses. In contrast to the liver, tubular injury in the kidney may occur without appreciable changes in organ weight, particularly when inflammatory enlargement is limited.[27,28] Nevertheless, the greater lesion severity observed at the higher dose indicates progressive tubular injury with increasing ADHE exposure.

Relative heart weight showed a slight, non-significant elevation in both treated groups compared with the control group. Preserved cardiac weight has been commonly reported in subacute toxicity studies of hydroethanolic plant extracts.[29,30,31] Histological examination revealed no appreciable alteration in myocardial structure at 400 mg/kg. At 800 mg/kg, enlargement of cardiomyocyte nuclei was observed, but no inflammatory changes, myofibrillar degeneration, or necrosis were present. Cardiac glycoside-containing plants such as Nerium oleander have been reported to cause marked myocardial degeneration and necrosis following exposure.[32] Such alterations were not observed in the present study, suggesting that ADHE did not produce significant cardiac injury at the administered doses.

Subacute administration of ADHE produced an increase in blood urea nitrogen, with values in both treated groups significantly higher than in the control group. Serum creatinine also increased across groups, although the change did not reach statistical significance. Creatinine is relatively insensitive to early tubular injury and may remain within normal limits until substantial nephron loss occurs.[33,34] The elevation in blood urea nitrogen corresponded with the histological findings. Coagulative necrosis of the tubular epithelium was observed at both doses. Similar elevations in blood urea nitrogen have been reported following subacute administration of the methanolic leaf extract of the related species Anthocleista vogelii.[35]

ADHE administration increased serum albumin and total protein concentrations, although histological examination showed hepatic changes. Although reduced albumin is commonly associated with severe hepatic dysfunction, mild hepatic injury may not immediately impair protein synthesis. Elevated serum albumin has also been associated with hemoconcentration and dehydration.[36] The activities of AST, ALT, and ALP were significantly increased in the treated groups compared with the control animals, with a greater increase in ALP observed at 800 mg/kg. These biochemical alterations were associated with the hepatic lesions observed microscopically, including centrilobular degeneration and necrosis at the higher dose. ALT is considered a useful indicator of hepatic injury, although AST activity may also arise from extrahepatic tissues.[37] Apiamu *et al* found no significant serum enzyme alterations following subacute administration of a methanolic leaf extract of the congener Anthocleista vogelii at lower doses, suggesting that solvent composition and dose magnitude influence the hepatotoxic expression of constituents within this genus.[35] Attemene et al reported elevated ALP and AST activities with hepatic histological abnormalities following chronic administration of a hydroethanolic

stem-bark extract of *A. djalonensis* at 1200 mg/kg.[38] The present findings demonstrate similar hepatic changes following administration of the leaf extract at lower doses over 28 days.

Blood glucose increased progressively across treatment groups, in contrast to previous reports of hypoglycaemic activity in *Anthocleista djalonensis*. Okokon *et al* and Akachukwu *et al* reported reductions in fasting blood glucose following administration of ethanolic root and aqueous leaf extracts, respectively.[10,25] However, those studies were conducted in diabetic models with altered glucose regulation. The increase in blood glucose observed following ADHE administration may be associated with the hepatic changes observed in the present study, as the liver plays an important role in glucose regulation.[39] Total cholesterol was significantly increased at 800 mg/kg, while triglyceride concentration was also elevated at the higher dose. Low-density lipoprotein and high-density lipoprotein concentrations were significantly increased in animals receiving 800 mg/kg of ADHE. These alterations were accompanied by centrilobular hepatocellular degeneration, necrosis, and increased hepatic enzyme activities at the same dose level. Previous studies have reported that lipid parameters remain largely unchanged in toxicity studies with no significant hepatic alterations.[29]

The hematopoietic system is highly sensitive to xenobiotic injury and is routinely evaluated in subacute toxicity studies as an index of systemic toxicity.[40] MCV, MCHC, and MCH showed no statistically significant differences across treatment groups, indicating that ADHE administration did not affect erythrocyte size or hemoglobin content per cell. However, PCV, hemoglobin concentration, and RBC count were significantly reduced in both treated groups, despite preserved erythrocyte indices and reduced red cell mass. These findings suggest a normocytic, normochromic anemia. Both the liver and kidney sustained significant injury in this study, and given their established roles in erythrocyte homeostasis, the anemia observed is consistent with the organ damage demonstrated biochemically and histopathologically. Previous subacute studies of other hydroethanolic plant extracts at moderate doses have reported preservation of erythrocyte indices.[41] Platelet counts showed a slight, non-significant increase in both treated groups. Thrombocytopenia is commonly associated with toxic suppression of hemopoiesis,[42] and its absence suggests that platelet homeostasis was not markedly affected at the doses examined. The hematological alterations observed following ADHE administration were less extensive than the hepatic and renal changes, suggesting that the extract's toxic effects were more pronounced in the liver and kidneys than in the hematopoietic system.

Subacute administration of ADHE produced no significant alteration in total white blood cell count or in the lymphocyte, monocyte, and eosinophil fractions relative to the control group. The absence of significant monocytic or eosinophilic responses suggests that repeated ADHE administration did not cause marked alterations in the leucocytic profile during the 28-day exposure period. Comparable leukocyte profiles have been reported in subacute studies of hydroethanolic plant extracts administered at similar doses.[43,44] In the present study, treatment-associated alterations were observed at both tested doses, including changes in selected biochemical parameters and histopathological findings in the liver and kidney. Therefore, a NOAEL cannot be established within the dose range evaluated in this investigation. Additional studies incorporating lower dose levels and a broader dose-response design are required to define the threshold for adverse effects and establish the safety margin of ADHE. A major limitation of this study is that only male Swiss mice were evaluated; therefore, the findings cannot be directly extrapolated to females. Given the traditional use of *A. djalonensis* among women, including reproductive-related applications, future studies involving female animals are warranted to determine potential sex-related differences in toxicity responses. In addition, the number of animals included in some repeated-dose toxicity assessments was limited. Future investigations using larger sample sizes, both sexes, and longer exposure durations would provide a more comprehensive assessment of the safety profile of ADHE. A further limitation of the histopathological assessment is that lesion incidence and semi-quantitative scoring were not performed. Future studies incorporating standardized histopathological scoring systems and larger sample sizes would provide a more comprehensive evaluation of lesion distribution and severity.

CONCLUSION

The present study demonstrates that repeated oral administration of ADHE altered hepatic and renal function in male Swiss mice, with the liver and kidney showing greater susceptibility to the extract's effects. The biochemical and histological changes observed

indicate that repeated administration of the extract at the doses examined may adversely affect organ function. These findings contribute to understanding the safety profile of *Anthocleista djalonensis* and provide a basis for further investigation of its effects after prolonged administration.

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ABBREVIATIONS

ADHE - *Anthocleista djalonensis* Hydroethanolic Extract
Ld_{50} - Median lethal dose
OECD - Organization for Economic Co-operation and Development
AST - Aspartate aminotransferase
ALT - Alanine aminotransferase
ALP - Alkaline phosphatase
BUN - Blood urea nitrogen
RBC - Red blood cell count
WBC - White blood cell count
MCV - Mean corpuscular volume
MCH - Mean corpuscular hemoglobin
MCHC - Mean corpuscular hemoglobin concentration
PCV - Packed cell volume
HDL - High-density lipoprotein
LDL - Low-density lipoprotein
SEM - Standard Error of Mean

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