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ORIGINAL RESEARCH

Acute and Chronic Oral Toxicity of Hydroethanolic Extract of *Sclerocarya birrea* (Anacardiaceae) in Wistar Rats

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Background: *Sclerocarya birrea* (A. Rich). Hochst, popularly known as Morula, is a plant in the Anacardiaceae family. The bark, fruits, and leaves have traditionally been used to manage a variety of health conditions, most especially diabetes. Unfortunately, there is a scarcity of data and publications on the toxicity and safety of this plant.

Purpose: The current study was designed to assess the acute and chronic toxicity of a hydro-ethanolic extract of *Sclerocarya birrea* in albino rats.

Materials and Methods: *Sclerocarya birrea* was extracted using an 80–20% hydro-ethanolic solution. For the acute toxicity study, female Wistar albino rats were treated with hydro-ethanolic leaf extract at a dose of 5000 mg/kg body weight and followed-up for 14 days. In the chronic toxicity study, 40 healthy Wistar albino rats were divided in 4 groups. The three treatment groups were administered the leaf hydro-ethanolic extract orally at dosages of 30, 150, and 1000 mg/kg once day for 90 days and the fourth group was a control group. Body and organs weights, haematological, serum biochemical, and histopathological parameters were measured at the end of the experiment.

Results: Single-dose oral administration of hydro-ethanolic leaf extract of *Sclerocarya birrea* at 5000 mg/kg produced no mortality indicating the LD50 is greater than 5000 mg/kg body weight. Following 90 days of administration of a hydro-ethanolic extract of *Sclerocarya birrea* leaves, there was no significant change in body and organs weights. Furthermore, biochemical, haematological and histopathological parameters did not vary significantly.

Conclusion: This data indicates neither acute or chronic toxicity in rats and is consistent with the widespread and long-term usage of *Sclerocarya birrea* in African traditional medicine.

Keywords: *Sclerocarya birrea*, acute toxicity, chronic toxicity, diabetes

Introduction

The use of plants for medicinal purposes has been practiced by all cultures since ancient times.[1] Herbal remedies are used by many millions of people worldwide and provide access to healthcare for many individuals. Herbal medication helps many individuals gain access to health care. In Africa, 65–80% of the population relies on medicinal plants for basic healthcare.[2–5] People choose herbal remedies for various reasons such as low cost, availability, and community trust.[5,6]

Sclerocarya birrea (A. Rich). Hochst (Anacardiaceae), sometimes known as Marula, is a well-known African wild tree found in numerous African countries. It belongs to the Anacardiaceae family[7] and is commonly used in traditional African medicine as food (fruits) and for treating various ailments.[8] Ethnomedical uses of Sclerocarya birrea include treating diabetes

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mellitus,[8–10] infections,[8,11] inflammation,[12–14] snake bite (Félix-Silva et al, 2017; Musa et al, 2020; Ojewole et al, 2010), diarrhea,[15] dysentery[8,16] and hypertension.[17–19]

Because *Sclerocarya birrea* is used to treat chronic diseases such as diabetes, long-term administration is required. Diabefla® (Phytofla, Burkina Faso) made from *Sclerocarya birrea* leaves, is prescribed to treat type 2 diabetes. Diabefla® has received market authorization for the treatment of diabetes mellitus and is available for purchase in pharmacies and para-pharmacies in Burkina Faso. It is therefore essential to investigate the safety of plants through acute and chronic toxicity studies. Despite the widespread use of *Sclerocarya birrea* leaves, our team found that the chronic toxicity of the leaves extract has not yet been evaluated.[9] To date, chronic toxicity studies of *Sclerocarya birrea* have predominantly focused on extracts derived from the stem bark and fruits. For instance, a sub-chronic study using a rat model found that doses of ≥1000 mg/kg of stem bark extract affected growth rate and liver and kidney function, emphasizing caution when using high doses.[13] Some other studies were conducted on acute and sub-chronic toxicity studies using *Sclerocarya birrea* stem bark extract in rats. In the acute study, various doses were administered orally, and no animal mortality occurred, suggesting a lethal dose likely higher than 2000 mg/kg. However, behavioural changes were observed at higher doses. During the sub-chronic study, rats receiving 1000 mg/kg and 2000 mg/kg doses showed significantly smaller growth rates. Liver and kidney toxicity was evident.[17,20] In two separate studies by Muhammad et al,[21,22] the toxicity of *Sclerocarya birrea* fruit components was investigated in rats. The first study examined the kernel extract, indicating minimal acute toxicity with no mortality observed at doses up to 3000 mg/kg. However, sub-chronic exposure (28 days) to higher doses (3000 and 4000 mg/kg) resulted in liver and kidney toxicity. Overall, while the kernel extract demonstrated potential toxicity at higher doses, the chronic effects of the peel extract remain inconclusive.[21,22]

Therefore, we undertook this study to evaluate the toxic effect of 80% ethanol extract of *Sclerocarya birrea* leaves on hepatic and renal histology, and biochemical and haematological parameters in rats, following short- and long-term administrations. The findings of this study might serve as a starting point for subsequent researches, such as a clinical trial on the anti-diabetic properties of *Sclerocarya birrea*, which our team is considering.

Materials and Methods

Plant Material

Fresh leaves of *Sclerocarya birrea* were collected at the orchard of Phytofla, Banfora, Burkina Faso. Botanical identification and authentication of the plant material carried out by the botanist of Phytofla and confirmed by Dr Ouaba Yempabou Hermann a botanist and cyto-ecologist from department of Botany, Faculty of Science, University of Nazi Boni, Burkina Faso.

Fresh leaves of *Sclerocarya birrea* were chopped into small pieces and air-dried until a consistent weight was reached. The fully dried material was grounded into fine powder with an electric blender resulting in 960 g of powdered material.

Extraction

Approximately 500 g of the powdered leaves of each plant were extracted with 2400 mL of ethanol/water (80:20) by maceration for 48 h under constant shaking at 25°C. The solution was filtered two times through cotton wool and centrifuged at 3000 rpm for 10 min. The extract was collected and concentrated in a rotary evaporator (Büchi R-205, Flawil, Switzerland) at 50°C under reduced pressure. The concentrated extracts obtained were frozen for 24 hours and then lyophilized for 96 hours. The solid extract was dissolved in distilled water to achieve varying concentrations for different doses needed for the administration to study animals.[23]

Laboratory Animals

The male and female Wistar albino rats (100–150 g) used in this study were obtained from the International Centre for Research and Development on Livestock in Sub-humid Zones (CIRDES, Bobo-Dioulasso, Burkina Faso). The animals were kept in plastic cages with wood chips as bedding, under standard environmental conditions (23–25 °C, 12-hour

light/dark cycle), given a standard rodent diet (Livestock Feed from the Ministry of Livestock, Burkina Faso), and had unlimited access to water. They were allowed to acclimate for 7 days before the experiment.

The protocol employed in this research was compliant with the approval of the Research Grants and Experimentation Ethics Committee of Burkina Faso's higher education (number 2021–06-148) and the National Institute of Health Guidelines for Care and Use of Laboratory Animals in Biochemical Research.[24]

Acute Toxicity Test

Study procedure was adapted from OECD 425. During the experiment, 10 Wistar albino rats were given the extract orally after fasting for 12 hours. Half received 5000 mg/kg of extract and the other half received distilled water. They were observed for 2 hours post-administration for any behavioural changes or signs of toxicity. Mortality was monitored for 24 hours, while surviving rats were watched for any delayed toxicity for 14 days.[25]

Chronic Toxicity Test

Forty Wistar albino rats were randomly divided into 4 groups (n = 10/group) using OECD. Test No. 452.[26] The rats were divided into the treatment groups as follows:

- Group I received sterilized distilled water as a normal control,
- Group II received a low dose of 30 mg/kg body weight,
- Group III received a medium dose of 150 mg/kg body weight,
- and Group IV received a high dose of 1000 mg/kg body weight.

For 90 days, each group received a unique daily dosage. The dosage of 25 to 30 mg/kg body weight aligns with the recommendation provided by Phytofla in the Diabafla product leaflet for the treatment of type 2 diabetes in humans. Consequently, the estimated human equivalent dose, based on published data, corresponds to approximately 150 mg/kg of rat body weight.[27] Animals were weighed on days 0 and every week until day 90.

Laboratory technicians and pathologists who read slides from toxicity experiments were blinded with respect to treatment group using alphanumeric coding.

Blood and Tissue Collection

Animals were sacrificed twenty-four hours following the last dose. The animals were sedated with 1% chloralose in 25% urethane (w/v, 5 mL/kg, intra peritoneal) and later euthanized by cervical dislocation before dissection (Adeyemi et al, 2008). Blood samples were taken for haematological and biochemical tests. The animals were dissected, and organs including lungs, spleen, pancreas, brain, heart, liver, kidneys, and testicles were identified, removed from surrounding tissues, and collected. The organs were washed with saline, dried on filter paper, carefully inspected for abnormalities, and weighed (Mettler-Toledo GmbH digital weighing balance, Greifensee, Zürich, Switzerland; Type BD202, SNR 06653). Key harvested organs were used for the histopathological assessment. Organs for histopathological assessment were preserved with 10% formal saline in properly labelled containers.[28]

Haematological Assessment

Sysmex® Automated Hematology Analyzer KX-21N was used to conduct a Full blood count (FBC) on rats. The measures parameters included Erythrocyte (RBC), haemoglobin (HBC), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), platelet count, mean platelet volume (MPV), platelet distribution width (PDW), red cell distribution width (RDW) and total and differential leukocyte count (WBC).

Biochemical Assessment

Serum biochemical analysis was performed on serum to determine the effect of the extracts on lipid metabolism, liver, and kidney functions. Lipid profile parameters such as total cholesterol, triglyceride, high-density lipoprotein (HDL), and

low-density lipoprotein (LDL) were measured. Liver function tests included total protein (TP), albumin (ALB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), and bilirubin, while kidney function was assessed through urea and creatinine levels, using a fully automated chemistry analyser (HumaStar 200).

Organ Weight and Macroscopic Examination

The liver, kidney, and brain were inspected for any gross morphological defects. Their weights were also measured.

Histopathological Assessment

A comprehensive post-mortem examination of the organs from albino rats was carried out, involving both macroscopic and microscopic analyses. The histopathologist was not part of the study team and was blinded to the group of each animal.The tissue preparation process included fixation in 10% formal saline, dehydration in graded alcohol concentrations, embedding in paraffin, and sectioning into 4–5 µm thick slices. The sections were stained using hematoxylin, mounted on microscope slides, and examined for morphological changes. The slides were examined for morphological changes and photographed with an Olympus CX 21 (Japan) microscope.

Statistical Analysis

In our study, all statistical analyses were conducted using R (version 4.0.2, R Foundation for Statistical Computing, Vienna, Austria). We performed a one-way analysis of variance (ANOVA) to assess whether the means of variables differed across treatment groups. While ANOVA provided overall group differences, it did not pinpoint specific pairwise comparisons. To address this, we employed the Newman-Keuls test, which allowed us to compare treatment groups in a pairwise manner, but only for statistically significant ANOVA results. Additionally, we utilized Student's *t*-test for comparing two groups. Our threshold for statistical significance was set at $p < 0.05$.

Ethical Consideration

The experimental animals were handled ethically following the OECD guidelines. For this research, ethical approval and clearance with the protocol number 2021–06-148 was obtained from ethics committee for health research, Ministry of Higher Education, Research and Innovation, Burkina Faso.

Results

Acute Toxicity

Clinical Observation Result

Rats treated with a single dose of 5000 mg/kg body weight ethanolic leave extract did not die or display significant behavioural changes such as hair erection, appetite loss, vomiting, diarrhoea, sleep impairment, or tremors. Thus, the LD50 of the studied plants is higher than 5000 mg/kg, making our preparation non-toxic according to OECD/OCDE.[29]

Effect on Weight Gain

Following the administration of a single dose of 5000 mg/kg body weight of ethanolic leaf extract from *Sclerocarya birrea* to rats, no statistically significant difference in weight was observed between the treated and control groups (p-value = 0.99). Specifically, the mean weights were 212.4 g (±11.2) to 214.0 g (±9.12) in the control group and 212.4 g (±9.64) to 213.6 g (±7.67) in the treated group.

Biochemical Parameters

After administering a single dose of 5000 mg/kg body weight of ethanolic leaf extract of *Sclerocarya birrea* to rats the enzymes aspartate aminotransferase (AST) and alanine aminotransferase (ALT) have been measured for an assessment of liver function (see Table 1). The mean difference between the treated and control groups was not statistically significant (P>0.05). The renal parameters assessed for renal function were uraemia and creatinine. There was no significant difference in mean the two groups (P>0.05). Similarly, there was no significant difference between treated and control groups when it comes to globulin, albumin and TP levels (P>0.05).

Table 1 Biochemistry Profile of Rats Treated with Single Dose of 5000 Mg/Kg Hydroethanolic Leaf Extract of Sclerocarya Birrea

Parameter Mean (SD)	Control (N = 5)	Hydroethanolic Extract of *Sclerocarya birrea* 5000 mg/Kg	p-value
TP	72.06 (0.89)	72.18 (1.17)	0.697
ALB	32.82 (0.84)	32.74 (1.06)	0.233
AST	193.2 (45.07)	227.4 (37.33)	0.203
ALT	105.4 (48.42)	91.0 (7.28)	0.958
ALP	105.4 (48.42)	117.4 (49.34)	0.783
Urea	5.98 (2.5)	6.77 (1.88)	0.816
Creat	62.8 (4.55)	51.0 (19.02)	0.552
GLB	39.24 (0.64)	39.44 (1.15)	0.0366*
ALT/AST	0.48 (0.11)	0.44 (0.06)	0.475
A/G	0.84 (0.03)	0.83 (0.031)	0.0073**

Abbreviations: WBC, White blood cells; RBC, Red blood cells; HBC, Haemoglobin; PTL, Platelet; TP, total protein; ALB, albumin; AST, Aspartate aminotransferase; ALT, Alanine aminotransferase; ALP, Alkaline phosphatase; Creat, creatinine; GLB, total globulin; AG, albumin/globulin ratio.

Haematological Parameters

Table 2 summarizes the results of the haematological tests following a single dose administration with 14 days of follow-up. When the treatment group (5000 mg/Kg) was compared to the control group, no significant differences in red and white cell lines or platelets were identified.

Chronic Toxicity

Weight Evolution Under Treatment

After 90 days of daily administering hydroethanolic extracts derived from *Sclerocarya birrea* leaves, there were no significant differences in weight gain between the treated rats and the control groups for both male and female rats (Figure 1).

In this study, the absolute weight of the liver, kidney, lung, spleen, and heart did not differ significantly when hydroethanolic extracts from *Sclerocarya birrea* leaves treated were compared to the control group (Figure 2).

Haematological Parameters

Following 90 days of administration of a hydro-ethanolic extract of *Sclerocarya birrea* leaves, when treatment groups (30, 150, and 1000 mg/kg) were compared to the control group, no significant differences in red cells, white cells and platelets counts were found. Furthermore, there was no significant difference in any of the measured parameters (Table 3).

Table 2 Haematological Profile of Rats Treated with Single Dose of 5000 Mg/Kg Hydroethanolic Leaf Extract of Sclerocarya Birrea

Parameter Mean (SD)	Control (N = 5)	Hydroethanolic Extract of *Sclerocarya birrea* 5000 mg/Kg	P-value
WBC	6.55 (1.04)	5.39 (1.47)	0.817
RBC	8.71 (0.25)	8.40 (0.22)	0.661
HBC	14.78 (1.24)	13.94 (1.36)	0.254
PLT	1137.2 (297.26)	1054.2 (210.8)	0.918

Abbreviations: WBC, White blood cells; RBC, Red blood cells; HBC, Haemoglobin; PTL, Platelet.

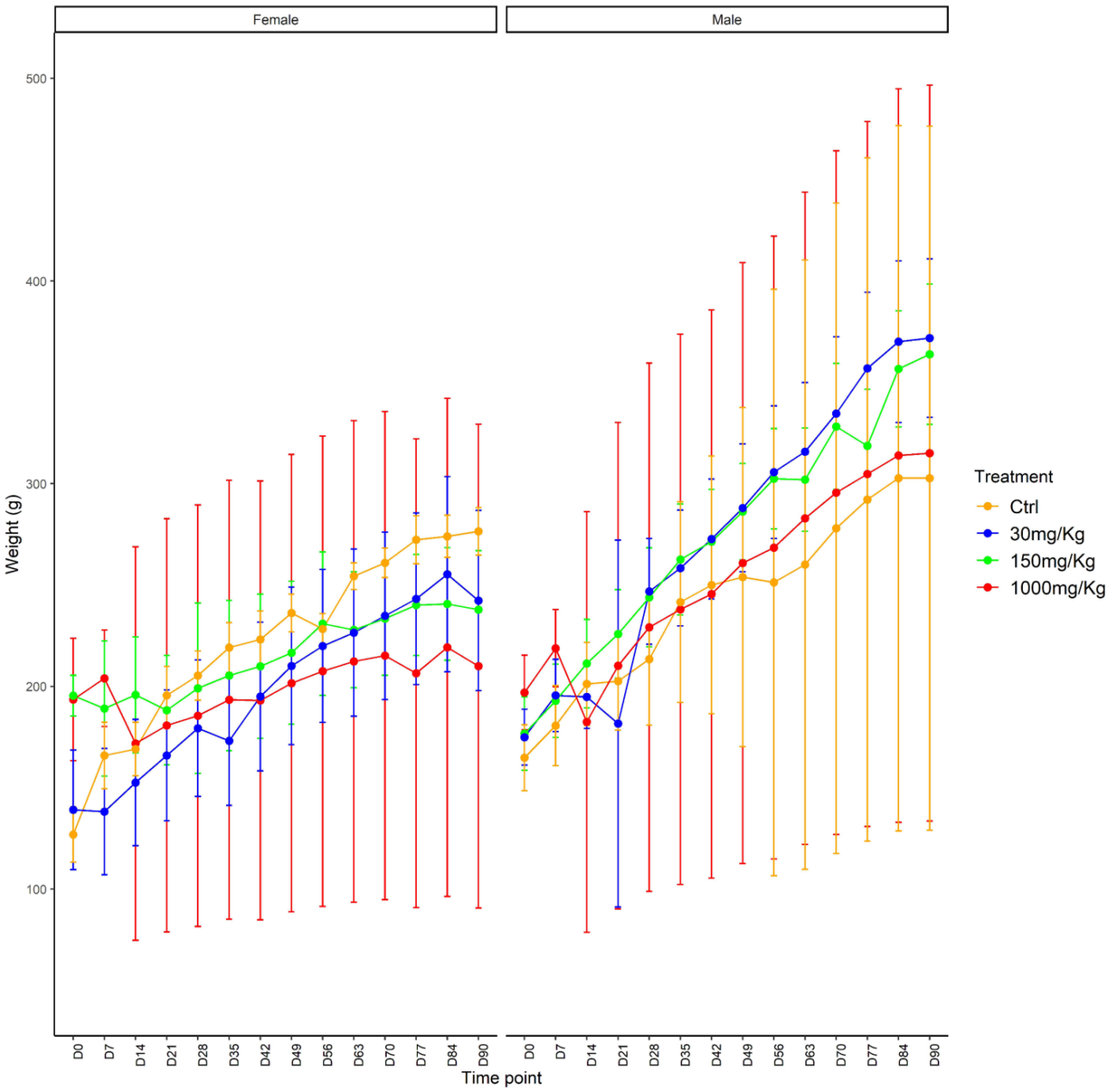

Figure 1 Mean body weights of treated (30, 150 and 1000 mg/kg/day; n = 10) and control groups following a daily administration of hydroethanolic extracts from Sclerocarya birrea leaves by gavage for 90 days. The right and left panels represent female and male, respectively. One way ANOVA was used.

Biochemical Parameters

Table 4 summarizes the results of the biochemistry tests after 90 days of daily administering hydroethanolic extracts derived from *Sclerocarya birrea* leaves. When treatment groups (30, 150, and 1000 mg/kg) were compared to the control group, no significant differences in any of the measured parameters (Glycaemia, Creatinine, AST, ALT, cholesterols and triglycerides) were identified.

Histopathological Parameters

Twenty-four (24) tissue samples, comprising 8 livers and 16 kidneys, were collected from 8 laboratory rats, with 1 male and 1 female rat per group. No abnormalities were detected upon examination, except in the kidney and liver of a male rat from the 30 mg/kg group.

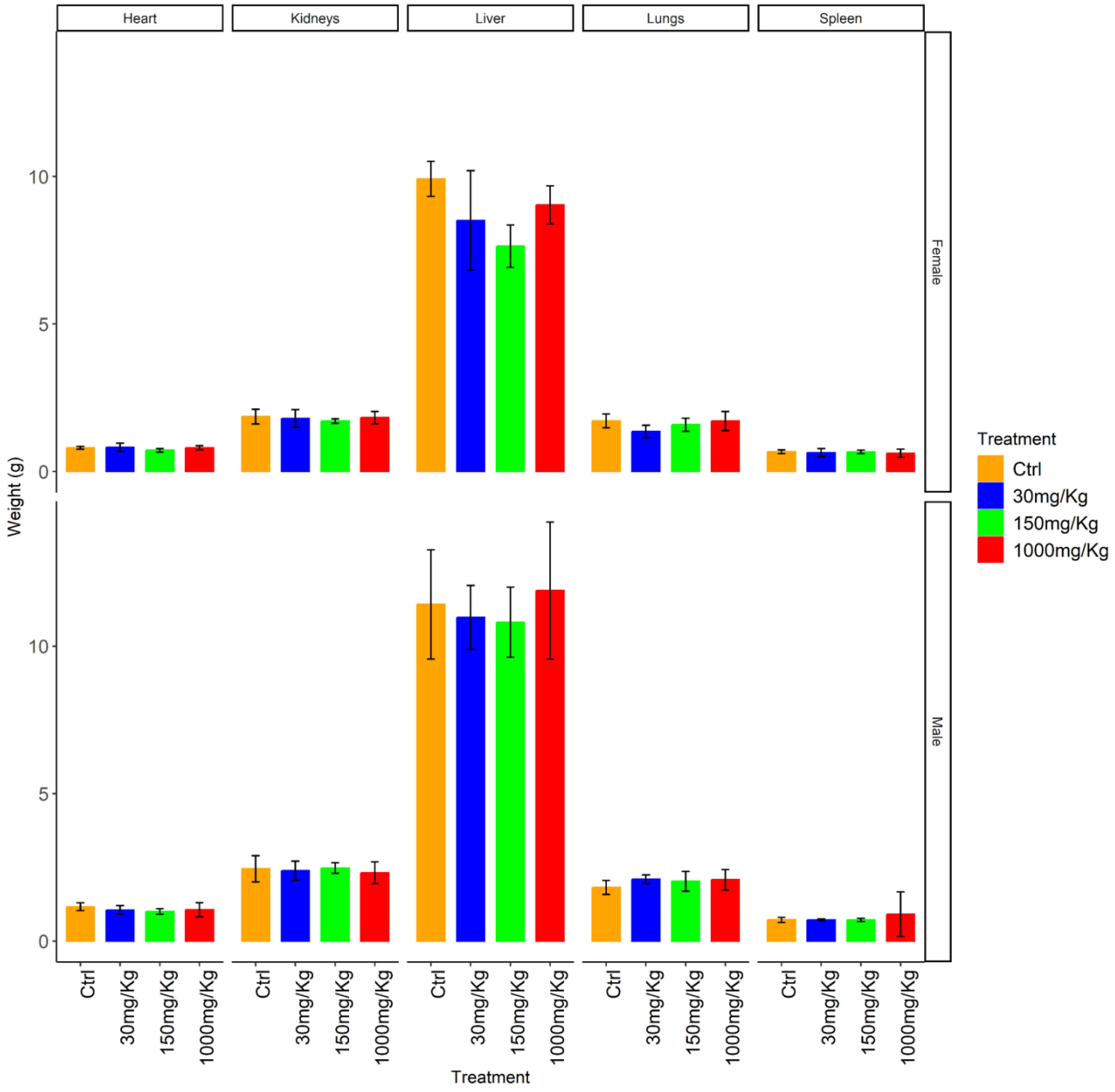

Figure 2 Organ weights of control and treated rats with daily doses of hydroethanolic extracts from Sclerocarya birrea leaves by gavage for 90 days. The top and bottom panels represent female and male, respectively. One way ANOVA was used.

Upon inspection of the organs from the male weighing 30 mg/Kg, our examination revealed a notable amount of apoptotic (isolated necrotic) liver cells and congestive kidney tissue.

There was no inflammation, haemorrhage, congestion, steatosis, or malignant cell proliferation on any of the pieces (Figure 3).

Discussions

In many African countries, herbal medicines face less stringent regulatory requirements compared to conventional pharmaceuticals. Clinical study evidence is often not mandatory for obtaining marketing authorization or for selling these products within the country. Consequently, there is a risk that unsafe herbal medicines may be used by the population, emphasizing the need for robust studies on toxicological effects of these herbal medicines.[30,31] The safety of

Table 3 Haematological Parameters of Rats Treated for 90 Days with Different Doses (30, 150 or 1000 Mg/Kg) of Hydroethanolic Extracts from Sclerocarya Birrea Leaves

Characteristic	Control, N = 10	Group 1 (30 mg/Kg), N = 10	Group 2 (150 mg/Kg), N = 10	Group 3 (1000 mg/Kg), N = 10	p-value
RBC ($\times 10^{12}$/L)	7.62 (0.76)	7.70 (0.85)	7.52 (0.81)	7.70 (0.34)	>0.9
HBG (g/dL)	14.97 (1.24)	15.05 (1.10)	14.75 (1.14)	15.09 (0.47)	>0.9
HCT (%)	41.29 (4.31)	41.47 (4.23)	40.57 (4.04)	41.30 (1.70)	>0.9
MCV (fL)	53.83 (1.83)	54.33 (1.86)	54.22 (2.39)	53.80 (1.92)	>0.9
MCH (pg)	19.88 (0.84)	20.15 (0.69)	19.73 (0.98)	19.86 (0.96)	0.8
MCHC (g/dL)	36.80 (0.95)	37.07 (0.84)	36.49 (1.18)	36.90 (0.60)	0.7
PTL ($\times 10^9$/L)	829.00 (107.60)	773.50 (108.87)	792.24 (320.86)	658.00 (177.48)	0.4
MPV (fL)	6.52 (0.28)	6.49 (0.58)	6.58 (0.42)	6.63 (0.73)	>0.9
WBC (10^9/L)	5.60 (2.64)	3.66 (2.02)	3.84 (1.88)	3.71 (2.54)	0.2
NEU (10^9/L)	8.39 (3.08)	9.63 (4.19)	10.82 (3.76)	10.67 (4.49)	0.5
LYM (10^9/L)	84.72 (6.62)	84.12 (6.27)	83.46 (4.19)	82.61 (5.42)	0.9
MON (10^9/L)	5.76 (3.85)	4.87 (2.78)	4.48 (1.49)	5.54 (1.56)	0.7
EOS (10^9/L))	0.32 (0.29)	0.40 (0.29)	0.38 (0.19)	0.39 (0.18)	>0.9
BAS (10^9/L)	0.81 (0.57)	0.98 (0.51)	0.79 (0.61)	0.79 (0.27)	0.8

Abbreviations: SD, Mean; RBC, Red blood cell count; HGB, hemoglobin concentration; HCT, hematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular hemoglobin; MCHC, hemoglobin concentration, RDW, red cell volume distribution, PLT, platelet count; WBC, white blood cell count; Neut, Neutrophiles; Bas, Basophil; Eos, Esinophil; Lymph, Lymphocytes; Mon, Monocytes.

Table 4 Biochemical Parameters of Rats Treated for 90 Days with Different Doses (30, 150 or 1000 Mg/Kg) of Hydroethanolic Extracts from Sclerocarya Birrea Leaves

Characteristic	Control, N = 10	Group 1 (30 mg/Kg), N = 10	Group 2 (150 mg/Kg), N = 10	Group 3 (1000 mg/Kg), N = 10	p-value
Gluc (mmol/L)	4.83 (0.68)	4.65 (0.87)	4.72 (0.86)	4.59 (0.62)	>0.9
Creat (µmol/L)	22.83 (9.13)	18.50 (7.68)	23.90 (4.84)	19.86 (8.51)	0.4
ALT (U/L)	60.42 (31.26)	71.00 (18.37)	52.30 (13.97)	60.86 (28.90)	0.4
AST (U/L)	188.38 (113.30)	293.20 (116.27)	196.50 (72.22)	192.71 (152.58)	0.2
Chol (mmol/L)	3.56 (5.84)	1.52 (0.35)	1.17 (0.19)	1.44 (0.24)	0.3
HDL (mmol/L)	10.34 (27.34)	0.60 (0.21)	0.59 (0.17)	0.63 (0.27)	0.3
LDL (mmol/L)	14.11 (39.15)	0.24 (0.08)	0.23 (0.08)	0.26 (0.06)	0.3
Trigly (mmol/L)	49.74 (139.91)	0.35 (0.11)	0.29 (0.15)	0.30 (0.08)	0.3

Abbreviations: SD, Mean; Gluc, Glycemia; Crea, creatinine; ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; Chol, Cholesterol; HDL, High-density lipoprotein cholesterol; LDL, Low-density Lipoprotein-cholesterol.

herbal medications has become a public health concern as demand has increased. Toxic phytochemical components of plants can sometimes limit the usage of herbal medications. Furthermore, excessive or long-term use of herbal medications for the treatment of chronic disorders such as diabetes may result in irreversible organ damage. *Sclerocarya birrea* is a widely used medicinal plant in sub-Saharan Africa, employed for the treatment of various conditions, including diabetes, which is currently under investigation by our team. Given that diabetes necessitates prolonged medication use, it is crucial to elucidate its chronic toxicity. *Sclerocarya birrea* is also utilized in the management of various chronic conditions that necessitate long-term use.[8,32] Therefore, this study aimed to evaluate the acute and chronic toxicity of Sclerocarya birrea through oral administration in rats.

The acute toxicity study is used to determine the adverse effects of a substance on the body after a single or brief exposure.[33] The study primarily assesses mortality, behavioural changes, body weight and other spontaneous changes in general well-being of rats. In the present study, the acute toxicity assessment showed that the hydroethanolic extract of *Sclerocarya birrea* at a dose up to 5000 mg/kg exhibited no significant evidence of toxicity in rats and no animals have died within 14 days post-administration. These findings can be compared with the results reported by Baba et al

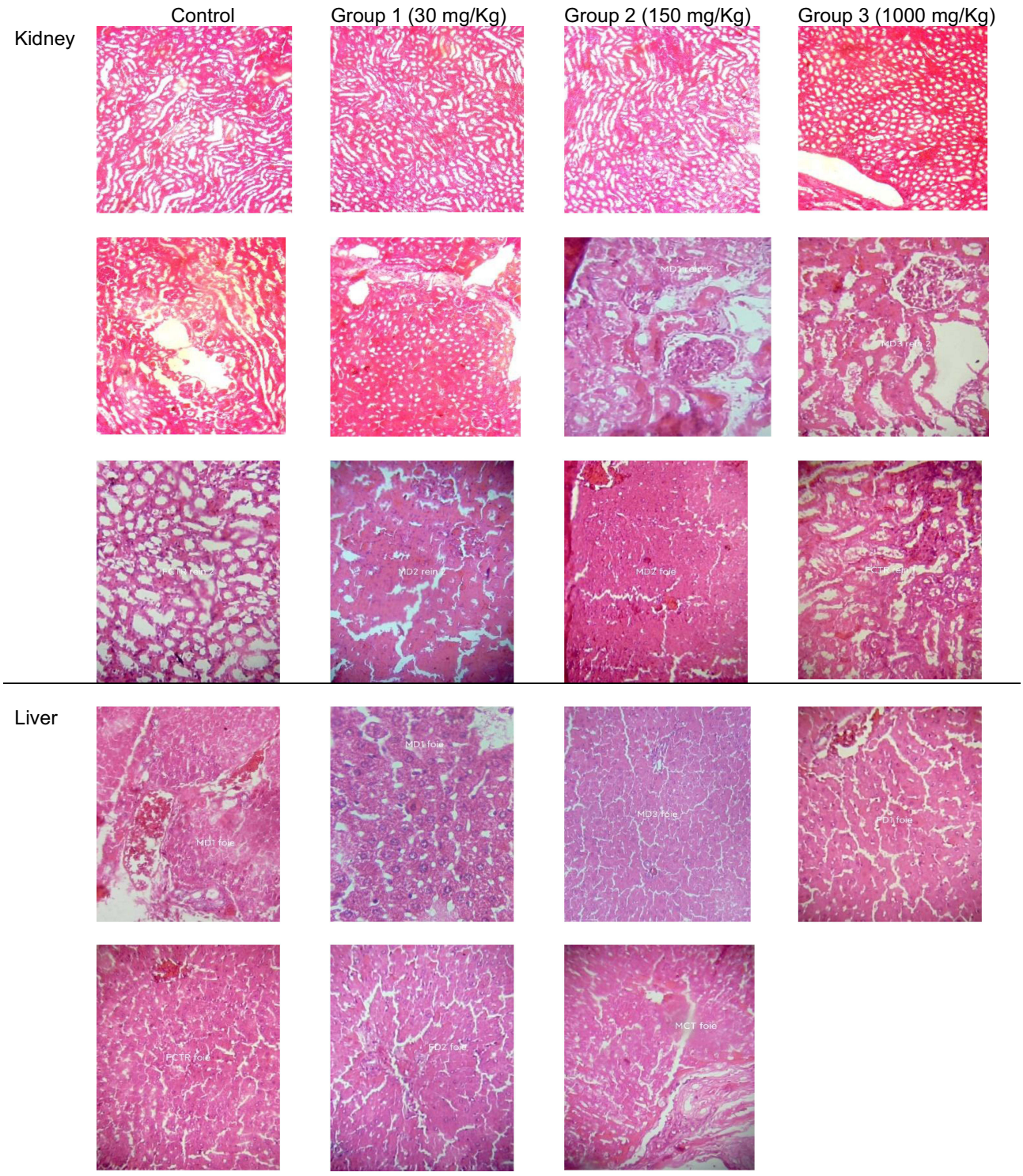

Figure 3 Photomicrographs of stained sections of various organs in control and experimental groups treated with *Sclerocarya birrea* leaves extract daily for 90 days (x 200 magnification). One way ANOVA was used.

who found that *Sclerocarya birrea* extracts found LD50 values of aqueous and ethanolic extracts are 566 and 800 mg/kg using intra-peritoneal (vs gavage in our study).[34] Despite variations in the extraction technique, administration route, and animal of study (rats vs mice), the researchers found that the leave extracts could be categorized as relatively non-toxic.

The chronic toxicity study provides information on potential health hazards that could result from repeated exposure for much of the life of the species used.[26] Thus, we examined the potential harmful effects of *Sclerocarya birrea* extracts at different levels of the rat body system to have an insight into the chronic toxicity of the plant extracts.

First, we found that *Sclerocarya birrea* did not induce alterations on biochemical and haematological parameters of rats. In addition, in the 3-month chronic toxicity study, Sclerocarya *birrea* extract, regardless of dose, did not appear to affect rat bodyweight or behaviour and did not result in significant changes in food intake, showing normal metabolism in the animals. This also suggests that, at the oral doses administered, *Sclerocarya birrea* extract did not impede rat growth. Although chronic toxicity study on leaves is not available, the sub-chronic toxicity studies of other parts of *Sclerocarya birrea* reveal differential effects on animal growth and organ health. While stem-bark extract at doses of 1000 mg/kg and 2000 mg/kg significantly reduced growth rates and altered liver and kidney organ-to-body weight ratios at doses of 800 mg/kg and above, kernel extract caused a non-significant increase in body weight at lower doses but led to significant weight reductions at 3000 mg/kg and 4000 mg/kg, suggesting dose-dependent toxicity.[21,35]

Interestingly, although *Sclerocarya birrea* has been shown to lower glycemia,[9] there is no significant reduction in blood glucose levels. Only a tendency to decrease blood glucose levels was noticed as the extracts dose increases. While a more significant effect could need to be demonstrated, this only tends to confirm that *Sclerocarya birrea* has anti-hyperglycaemic proprieties, which acts in a dose-dependent manner (and not simple hypoglycaemic effect). The leave has been shown to content components that increase glucose uptake[36] and inhibit the activities of α-amylase and α-glucosidase.[37]

In both humans and animals, the hematopoietic system is one of the most sensitive targets for toxic compounds and an essential indicator of physiological and pathological status.[38] This study found no significant variation in haematological parameters, suggesting that hydroethanolic extract of *Sclerocarya birrea* is not haemato-toxic. The lack of prior chronic and sub-chronic toxicity studies on the leaves of the plant extract limits the comparison of the results of this study.

Furthermore, we examined the toxic effects of *Sclerocarya birrea* on the liver and the kidneys, which are key body organs for the biotransformation and excretion/elimination drugs and toxics. The liver is an important organ in drug biotransformation, and its normal function is monitored by blood biomarker enzymes.[39] This study found no significant differences in liver biomarkers, suggesting that *Sclerocarya birrea* extract is not hepatotoxic. However, the anatomopathological examinations revealed necrotic liver cells in one male rat at the extract's dose of 30 mg/kg. This observation could raise some level of concerns and more studies are needed to dispel any ambiguity. It is unlikely that the observed adverse effect is directly related to the experimental treatment, as it appears to be a singular occurrence in the lower dose group and is not supported by biochemistry results. A prior investigation into the kernel extract revealed no impact on liver function, whereas the analysis of the stem bark demonstrated inconsistent alterations.[21,35]

Because of the huge amount of blood flowing through the kidneys, they constitute a crucial organ for the harmful effects of drugs, and they are extremely sensitive to toxic compounds. The kidneys filter a wide range of toxins that can accumulate in the tubules.[40] Creatinine is recognized as a sensitive biomarker of renal dysfunction.[40] In our toxicity study, there were no significant variations in creatinine levels ($P > 0.05$) between the *Sclerocarya birrea* treated groups when compared to the control groups. These data can reasonably be taken as evidence that the extract did not cause kidney injury or alterations in renal function at the tested doses, notwithstanding the observed congestion in one unique male rat (the same as above). Additionally, this singular occurrence could be regarded as a singular incident level since no other toxicological signs or biochemical changes in the liver and kidneys were observed in higher doses. However, the possibility of reno-hepatic toxicity cannot be unequivocally excluded.

Finally, Hyperlipidaemia is well established as one of the primary risk factors for atherosclerosis, which leads to coronary artery disease.[41] Oral administration of *Sclerocarya birrea* extract to rats over 90 days resulted in no significant changes in lipid profile, however there is a lowering tendency for triglycerides, total, LDL, and HDL-cholesterol levels. These findings are consistent with those of an as-yet unpublished study conducted by our team, which was carried out on human subjects. Pending a validation of this tendency in further studies, *Sclerocarya birrea* could be a prospective drug for lipid profile improvement, a process much desired in atherosclerotic, coronary heart disease, and/or diabetic conditions.

Conclusion

In essence, this study provides preliminary data on the toxicity of *Sclerocarya birrea*, which will be useful in designing future pre-clinical and clinical trials of this plant. The results indicate that the hydro-ethanolic extract of *Sclerocarya birrea* at a single dose of 5000 mg/kg did not cause mortality, suggesting an LD50 greater than 5000 mg/kg body weight. Additionally, biochemical and hematological parameters remained unaltered and were comparable to those of the control group. In the chronic study, following 90 days of daily oral administration, doses of 30, 150, and 1000 mg/kg were determined to be relatively non-toxic. There were no significant changes observed in weight, biochemical, hematological, and histopathological parameters, which were comparable to those of the control group. However, additional studies are needed to fully assess the safety of *Sclerocarya birrea*, including evaluating its effects on the foetus in pregnant animals, reproductive function, genetic system, and potential to cause cancer.

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Disclosure

The authors report no conflicts of interest in this work.

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