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Original Research Article

Sub-acute toxicological assessment of *n*-hexane fraction of *Anogeissus leiocarpus* stem bark extract in rats

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ABSTRACT

Anogeissus leiocarpus is traditionally used in Africa for treating diabetes and gastrointestinal disorders; and pharmacologically validated for its anti-inflammatory and antioxidant properties. However, data on the toxicological profile of its fractions remain limited. This study evaluated the acute toxicity of *A. leiocarpus* stem bark hydromethanolic extract (ALBHE) and subacute toxicity of its *n*-hexane fraction (n-hex). Acute toxicity was assessed by administering single oral doses of ALBHE (2000 and 5000 mg/kg body weight) to female rats (n = 5/group) and monitored for 14 days. For the subacute study, male and female rats (n = 10/group) received repeated oral doses of n-hex (40 or 80 mg/kg) for 28 days. The animals were sacrificed on day 29 following overnight fasting, and blood and essential organs (liver, kidney, heart, spleen, brain, uterus, and testes) were collected for haematological, biochemical, and histological assessments. Chemical profiling of n-hex was performed using high-performance liquid chromatography (HPLC), revealing 12 distinct compounds. No mortality or clinical signs of toxicity were observed in the course of acute toxicity. Haematological and biochemical parameters were not significantly altered ($p > 0.05$) compared to controls except for the mean corpuscular volume which was significantly decreased ($p < 0.05$) in male rats compared to control, and histological examination revealed well-preserved organ architecture. Taken together, the oral median lethal dose (LD50) of ALBHE was greater than 5000 mg/kg and the results indicate that both ALBHE and its *n*-hexane fraction are non-toxic at the tested doses and duration, supporting their potential safety in therapeutic applications.

Keywords: Hematology, Histopathology, Medicinal biochemistry, Phytomedicine, Safety assessment, Wistar rat .

Introduction

Plants have been the cornerstone of healthcare in many indigenous cultures for ages serving as sources of food and medicaments. The widespread acceptance of herbs is due to the ready accessibility and affordability of plant-based medicine compared to orthodox medicine and the deep rooted cultural and spiritual connection to plants in many societies.[1] Despite advancement in allopathic medicine, herbal medicine use in developing countries still supports the health needs of almost 80% of people globally in the management of chronic diseases, infectious diseases and wound healing.[2, 3] Medicinal plants undoubtedly play an important role in modern drug development. This is considerably due to their robust phytochemical bioactive constituents and minimal undesirable effects of plant-based natural product in the treatment and prevention of diseases.[4] However, some medicinal herbs have been reported to induce toxic effects, including hepatotoxic, nephrotoxic, cardiotoxic, hematoxic and splenotoxic effects.[5] *Anogeissus leiocarpus* (DC.) Guill. & Perr. (*A. leiocarpus*), a deciduous tree belonging to the Combretaceae family, is prominently known as the African birch.[6] It is rather common in tropical regions of Africa and Asia (including the northern part of Nigeria). *A. leiocarpus* holds significant cultural value among the Malian and Burkinababé people where dyes obtained from the plant are used for Bogolian textile artistry.[7] The folkloric and contemporary use of *A. leiocarpus* in treating several diseases by traditional healers in tropical Africa and Asia is noteworthy. Locals have reported the use of stem bark as a remedy for diabetes, dysentery, malaria, cough and giardiasis.[8,9] Ethno-medicinal records in southern Nigeria show the effectiveness of its root as an antimalarial remedy,[10] and the potency of its leaves in treating skin diseases such as psoriasis.[11] Furthermore, the aerial parts of *A. leiocarpus* have been exploited locally for treating myriads of veterinary ailments; helminthic infections, gastrointestinal disorders, and bites thus improving livestock production.[12] The biological potential of *A. leiocarpus* such as its antioxidant,[13] antidiabetic,[14] hepatoprotective,[15] antimicrobial,[11] and anti-inflammatory[16] activities has been pharmacologically validated in several studies. The several biological activities reported in *A. leiocarpus* are attributed to its robust phytochemical profile. These phytochemicals, including tannins, alkaloids, saponins, flavonoids, steroids, ellagic acid and anthraquinone, are reported to be present in different parts of *A. leiocarpus* [11].

There are few reports on the acute and short-term toxicity of the stem and root bark of *A. leiocarpus* crude extract[17, 18] and the ethanolic fraction of the root bark.[19] However, there are still some obscure areas in terms of holistic toxicity and safety profiles that need to be unveiled. Therefore, it is imperative to assess the plant stem bark toxicity effects through an *in vivo* model that simulates local practice in order to obtain accurate data that may be extrapolated to humans. This study seeks to evaluate the safety profile of *A. leiocarpus* stem bark hydromethanolic

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extract (ALBHE) and its *n*-hexane fraction through acute and sub-acute oral toxicity respectively.

Materials and Methods

Collection of Plant, Extraction and Fractionation

The stem bark of *A. leiocarpus* was obtained in July 2022 from the Botanical Garden of the University of Ilorin, which is located in Ilorin South Government Area, Nigeria. The plant was taxonomically verified and authenticated as *Anogeissus leiocarpus* and a voucher specimen (UIL/001/167/2023) was deposited for future reference at the Herbarium of Plant Biology Department, University of Ilorin, Nigeria. (The name of the plant has also been checked with http:// http://www.worldfloraonline.org on 22nd October, 2024). The plant sample was rinsed under fresh water, and air-dried under ambient conditions for about 4 weeks until a constant weight was achieved. The plant material was subsequently milled using a mechanical blender and 1 kg of pulverized material was cold macerated in 3 L of 70% v/v methanol in water for 72 hours, with intermittent stirring and agitation on an orbital shaker. The extract was filtered via a vacuum pump using no 1 Whatman filter paper, and the resulting hydro-methanolic extract was first concentrated using a rotary evaporator (Stuart RE300, Bibby Scientific Ltd., Starffordoshine, UK). The concentrated extract was further dried in a water bath at 45°C and stored in glass vials at -4°C. Three solvents with increasing polarity—*n*-hexane, ethyl acetate, and *n*-butanol, were used to successively partition *A. leiocarpus* hydromethanolic stem bark extract (ALHBE).[20] Ten (10) grams of crude extract was dissolved in 100 ml of distilled water and transferred into a clean and dry separating funnel. Starting from the least polar solvent, 150 ml of *n*-hexane was introduced into the separating funnel and shaken vigorously to mix with the extract. The separating funnel with the two immiscible solvents was suspended on a tripod stand and clearly partitioned over time. The bottom aqueous layer was collected into a beaker, and then the floating *n*-hexane layer was also collected. The extraction cycle was repeated for ethyl acetate and *n*-butanol using the collected aqueous layer. The resulting fractions of *A. leiocarpus* were concentrated in a rotary evaporator, further dried in a water bath at 45°C and stored in glass vials at -4°C. For this experiment, only the *n*-hexane fraction was utilized.

High-performance liquid chromatography (HPLC) analysis of n-hexane fraction of A. leiocarpus stem bark hydromethanolic extract

HPLC analysis of the *n*-hexane fraction of *A. leiocarpus* stem bark extract was carried out according to an earlier reported protocol with slight alterations.[21] The HPLC system (Shimadzu, Nexera MX) was equipped with a degasser, a 15 MPa pump, and an ultraviolet detector. A µBondapak C18 column (7 µm, 100 mm × 4.6 mm) was used, with the column temperature maintained at 25°C. The mobile phase was delivered at a flow rate of 1 mL/min and was composed of water and methanol. A sample volume of 10 µL was introduced into the system, and eluent detection was monitored at a wavelength of 254 nm. Briefly, 10 mL of acetonitrile/methanol was added to 500 mg of sample placed in an amber bottle. To facilitate the release of the constituents in the sample, the mixture was vigorously agitated for approximately half an hour. The organic solvent was subsequently collected in a 25 mL volumetric flask and made up to mark in preparation for chromatographic analysis. Standard forms of analytes were initially injected into the HPLC system to generate a standard chromatographic profile. These standards were used to set up a detection window on the HPLC for subsequent analysis. A 10 µL aliquot of the extracted sample was then injected into the HPLC, producing a chromatogram with defined peak areas and heights. The sample's peak area was compared to that of the standard, and the sample concentration was determined on the basis of the standard's concentration.

Experimental animals

Thirty (30) female and fifteen (15) male Wistar rats (6 to 8 weeks) weighing 120 to 150 g used for the study were sourced from the Experimental Animal House, Landmark University Omu-Aran, Kwara, Nigeria and the experiments were conducted there. The rats chosen for the study were thoroughly examined to ascertain the robustness of their apparent health. The rats were sheltered in a standard wooden cage with a mesh wire base under the following conditions: temperature, 24 ± 2°C; 12-hour day/night cycle and freely provided adequate water and pelleted feed (Chikun feed). Before the commencement of each experiment, the rats were acclimated for 7 days. The acute toxicity test was performed on 15 female rats out of the 30 female rats for the entire experiment, whereas the sub-acute toxicity test was performed on the remaining 15 female rats and 15 male rats.

Acute Toxicity Study

The study design and execution scheme for the acute toxicity of ALBHE was carried out according to the Organization for Economic Co-operation and Development (OECD) guidelines 425.[22] Initially, a one-time oral dose of 2000 mg/kg bw ALHBE was given to five female Wistar rats. The rats were closely observed in the first 30 minutes and through to the 4th hour. The animals were subsequently observed daily for two weeks to identify possible clinical signs such as toxicity, mortality, behavioural patterns, and physical appearance, as well as other adverse effects, such as diarrhoea, tremors, lethargy and salivation. There was no record of death for the 2000 mg/kg bw dose. A higher dose of 5000 mg/kg bw ALBHE was subsequently given to another batch of five (5) female Wistar rats. They were likewise closely monitored as the first batch of rats for two weeks. The rats (5) in the control group received distilled water only. After treatment, on day 15, each batch of animals was euthanized under mild diethyl ether vapour and sacrificed to collect selected organs (liver, kidney, heart, brain, ovary, stomach and spleen) for histopathological analysis (results not included).

Sub-Acute Oral Toxicity Study

The study design and execution scheme for the sub-acute toxicity of *n*-hexane fraction of ALBHE was carried out according to OECD guidelines 407.[23] Thirty Wistar rats of both sexes were assigned to three groups of ten (10) rats each. The male (5) and female (5) in each group were separated into different compartments of the cage. For a period of 28 days, groups one to three once daily orally received vehicle (0.5% dimethylsulfoxide (DMSO)), and *n*-hexane fraction of ALBHE (40 and 80 mg/kg bw respectively). The dose selected for the low dose fraction (40 mg/kg bw) was equivalent to 125 mg/kg bw of crude extract on the basis of a preliminary study in our laboratory (unpublished) that revealed that 125 mg/kg bw of ALBHE to be most potent hepatoprotective extract in rats. Throughout the experiments, observations and recordings were made weekly for parameters such as body weight and daily for behavioral characteristics and possible signs of toxicity. On the 29th day after an overnight fast, the rats were euthanized under mild diethyl ether vapour., The jugular vein of each rat was subsequently slit with a razor, and approximately 0.5 mL and 2mL of blood per animal was collected into two separate ethylenediaminetetraacetic acid (EDTA) tubes for haematological and biochemical analysis respectively. Furthermore, target organs (liver, kidney, brain, heart, spleen, testes and uterus) were collected for histopathological examination. The experimental animals received humane treatment in compliance with the institution's principles and criteria as outlined by the National Institute of Health (NIH). Furthermore, the protocol for the research was authorized by the Landmark University Animal Care and Use Committee (LUAC/BCH/2023/003A).

Organ body weight ratio

The brain, heart, liver, kidney, spleen, uterus, and testes were among the organs that were carefully excised. The samples were then bathed in physiological saline, blotted with filter paper, and weighed. Each animal's organ-to-body weight ratio was determined using the following formula:

$$\frac{\text{Organ weight (g)}}{\text{Body weight (g)}} \times 100\%$$

Haematological Analysis

The blood samples were processed and analysed in an automated Haematological analyser (KX-21N, Sysmex, Japan) as previously described.[24] The parameters evaluated in the blood sample included the

level of haemoglobin (HGB), the red blood cell (RBC) count, and the white blood cell (WBC) count. Furthermore, the hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), and mean corpuscular haemoglobin concentration (MCHC) were evaluated, providing further insight into the cellular features of blood.

Biochemical Analysis
The manufacturer's instructions were strictly followed for the biochemical assays using an autoanalyser. The biochemical indices included total protein, globulin, albumin, and total bilirubin in rat plasma; kidney function markers such as urea, creatinine and electrolytes (sodium and potassium); and liver function indicators such as aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP) and gamma-glutamyl transferase (GGT). Lipid profile including total cholesterol, triglyceride, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C), were also determined.

Histopathology
The harvested organs (liver, kidney, brain, heart, spleen, uterus, and testes) were fixed in 10% buffered normal formalin and processed for histological assessment as previously described.[25] Thin 0.3 µm slice were cut from each sample. The tissues were then dehydrated in increasing concentrations of alcohol (70, 80, 90, and 100%), cleared in xylene, and then firmly embedded in paraffin wax. Afterward, each section was stained with hematoxylin-eosin. A histopathologist unaware of the treatments captured the photomicrograph using a camera mounted on a light microscope (MU1803, Amscope, Zhejiang, China).

Data analysis
Data analyses were conducted with GraphPad Prism Version 9.0.1 (San Diego, California, USA). The data are presented as mean ± standard error of mean (SEM) One-way analysis of variance (ANOVA) was used to compare groups in the experiment. Significance for the organ body weight ratio; haematology and plasma biochemistry analyses was considered at $p < 0.05$.

Results and Discussion

The use of medicinal plants and their therapeutic constituents may be unsafe and result in diverse modes of toxicity at the cellular, tissue, organ or organismal level. This is because the production and use of these materials are not sufficiently regulated in several parts of the world.[26] According to a WHO report, more than 80% of the global population, especially in developing countries, depend on traditional herbal remedies to address a variety of diseases and primary health needs.[3]

HPLC fingerprint of n-hexane fraction of A. leiocarpus stem bark hydromethanolic extract
Qualitative analysis of the *n*-hexane fraction of *A. leiocarpus* stem bark hydromethanolic extract was accomplished using HPLC-UV. The chromatogram revealed the presence of twelve phytocompounds (Figure 1, Table 1) namely; ellagic acid, gallic acid, anogeissinin, anolignan A, flavellagic acid, ampelopsin, quercetin, rutin, colilagin, castalagin, pinosylvin, and punicalagin. The 13th compound zidovudine is a likely contaminant. The retention time for each compound and their respective area and peak height were also reported. Anogeissinin had the highest concentration, followed by anolignan A and rutin. Because the phytoconstituents in herbal plants are derived from nature or obtained from natural sources, many people mistakenly conclude that they are safe and without any adverse effects or reactions.[27] Therefore, exhaustive methodical toxicological investigations are imperative prior to the use of any botanical drug.

Acute toxicity of A. leiocarpus stem bark hydromethanolic extract
One-time oral administration of ALBHE at both doses (2000 and 5000 mg/kg bw) did not result in any observable toxicity, morbidity or mortality. No major changes occurred in the movement pattern, behaviour and overall appearance of the rats. This outcome confirms the safe use of the extract at 2000 and 5000 mg/kg bw. Hence, the median lethal dose (LD_{50}) of ALBHE exceeded 5000 mg/kg bw.

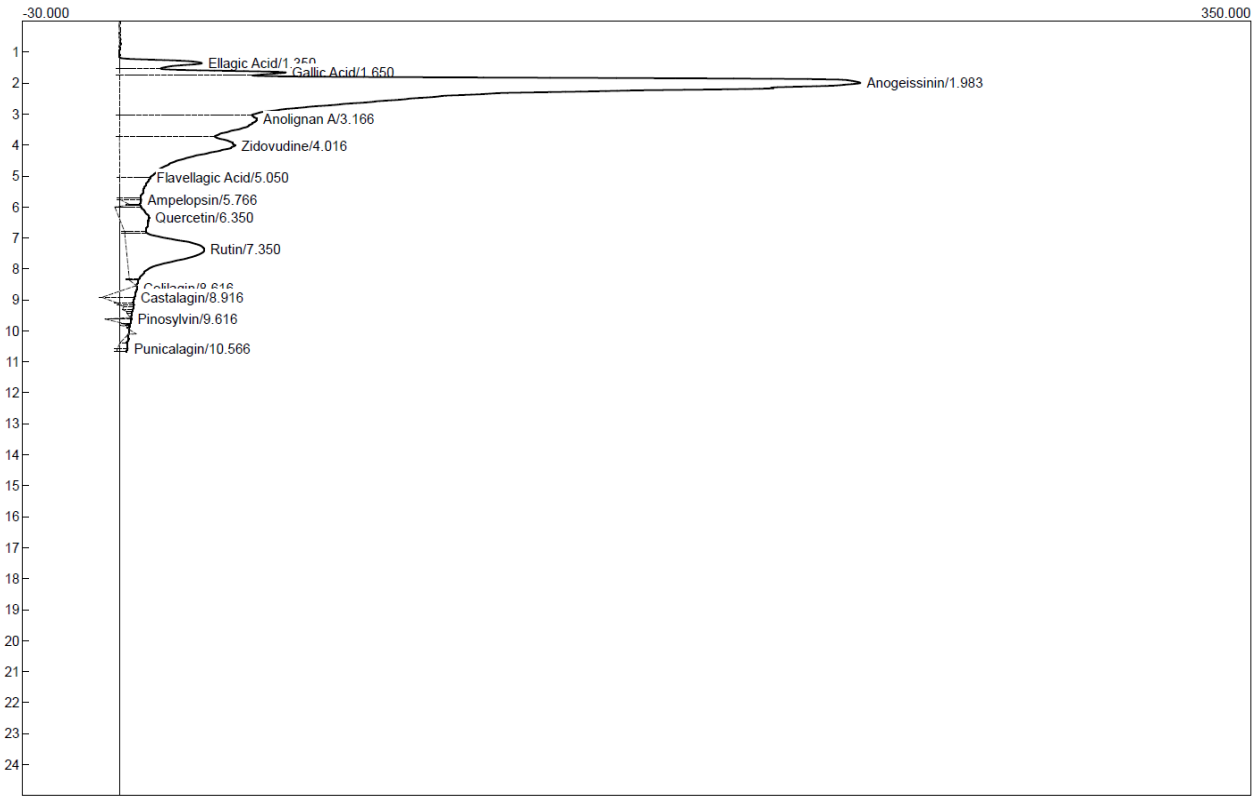

Figure 1: HPLC chromatogram of the *n*-hexane fraction of *A. leiocarpus* stem bark hydromethanolic extract.

The tested substance exhibited low toxicity, placing it in category 5 according to the OECD Guideline 425 for acute oral toxicity.[22] In a related study, it was reported that the median lethal dose (LD50) of the aqueous and ethanolic extract *A. leiocarpus* bark exceeded 5000 mg/kg.[17]

Effect of oral subacute exposure to the n-hexane fraction of A. leiocarpus on organ body weight ratio

In the sub-acute toxicity study, the oral administration of the *n*-hexane fraction of ALBHE did not result in any clinical alterations, morbidity or death throughout the 28 days. In general, one of the most important markers of negative side effects in animals after exposure to any harmful agent is fluctuations in body weight. Likewise, alteration in absolute organ weight and relative organ weight are typically significant markers for evaluating the potential toxic effects of an administered drug.[28] The administration of the *A. leoicarpus* fraction did not elicit any significant alterations ($p > 0.05$) in the relative organ body weight ratio in both sexes at the administered doses compared to their respective control (Table 2), in all the organs examined except in the uterus which was significantly elevated ($p < 0.05$) at 40 mg/kg bw compared to its control.

Effects of oral subacute exposure to the n-hexane fraction of A. leiocarpus on haematological parameters

Haematological parameters are key indicators for understanding overall human health. The findings revealed no significant difference ($p > 0.05$) in all the blood components and related indices between the groups treated with the *n*-hexane fraction of *A. leiocarpus*, in both male and female rats and the corresponding control groups and (Table 3).

Table 1: Compounds detected in the *n*-hexane fraction of *A. leiocarpus* stem bark hydromethanolic extract

S/No	Retention time (min)	Area	Height	Compounds
1	1.350	340.0715	25.550	Ellagic acid
2	1.650	475.5230	51.511	Gallic acid
3	1.983	9236.5595	229.238	Anogeissinin
4	3.166	1566.3170	42.555	Anolignan A
5	4.016	1813.3890	35.747	Zidovudine
6	5.050	315.1840	9.558	Flavellagic Acid
7	5.766	50.0840	6.381	Ampelopsin
8	6.350	399.6870	9.363	Quercetin
9	7.350	1248.1720	24.165	Rutin
10	8.616	120.8390	2.572	Colilagin
11	8.916	82.2980	10.118	Castalagin
12	9.616	44.6260	8.224	Pinosylvin
13	10.566	32.4910	3.183	Punicalagin

Table 2: Effects of the *n*-hexane fraction of *A. leiocarpus* extract on the organ-body weight ratio in female and male rats

Parameters (%)	Sex	Control	n-hex 40 mg/kg bw	n-hex 80 mg/kg bw
Liver	Female	2.682 ± 0.05	2.838 ± 0.10	2.869 ± 0.08
	Male	2.904 ± 0.16	2.661 ± 0.22	2.667 ± 0.10
Kidney	Female	0.599 ± 0.02	0.668 ± 0.01	0.580 ± 0.06
	Male	0.648 ± 0.07	0.626 ± 0.08	0.656 ± 0.03
Brain	Female	0.799 ± 0.11	0.806 ± 0.03	0.931 ± 0.02
	Male	0.756 ± 0.02	0.836 ± 0.11	0.748 ± 0.04
Heart	Female	0.362 ± 0.03	0.408 ± 0.02	0.424 ± 0.04
	Male	0.406 ± 0.04	0.435 ± 0.02	0.407 ± 0.03
Spleen	Female	0.329 ± 0.03	0.358 ± 0.05	0.461 ± 0.05
	Male	0.375 ± 0.03	0.472 ± 0.05	0.396 ± 0.04
Uterus/Testes	Female	0.108 ± 0.01	0.210 ± 0.01**	0.168 ± 0.03
	Male	2.119 ± 0.13	2.228 ± 0.14	2.074 ± 0.17

The data are presented as the mean of five replicates ± SEM. A value with ** is statistically significant at $p < 0.01$ across the row

Table 3: Effects of the *n*-hexane fraction of *A. leiocarpus* extract on haematological indices in female and male rats

Parameters	Sex	Control	40 mg/kg bw	80 mg/kg bw
HGB[a] (g/dL)	Female	10.23 ± 0.30	10.37 ± 0.50	10.6 ± 0.40
	Male	9.83 ± 0.90	9.60 ± 0.40	10.2 ± 0.60
RBC[b] (10^6/µL)	Female	6.87 ± 0.06	6.80 ± 0.30	7.23 ± 0.25
	Male	6.93 ± 0.40	6.99 ± 0.27	7.06 ± 0.40
WBC[c] (10^3/µL)	Female	4.87 ± 0.12	3.83 ± 0.12	6.47 ± 1.40
	Male	8.33 ± 1.47	7.37 ± 0.86	4.97 ± 0.87
HCT[d] (%)	Female	38.53 ± 1.30	38.63 ± 1.53	40.00 ± 1.46
	Male	39.63 ± 1.87	37.97 ± 1.27	40.07 ± 1.83
MCV[e] (fL)	Female	56.13 ± 1.74	56.83 ± 1.42	55.30 ± 0.80
	Male	57.20 ± 0.75	54.30 ± 0.46*	57.03 ± 0.54
MCH[f] (pg)	Female	14.90 ± 0.40	15.27 ± 0.19	14.67 ± 0.28
	Male	14.17 ± 0.86	13.77 ± 0.80	14.50 ± 0.30
MCHC[g] (g/dL)	Female	26.60 ± 0.17	26.80 ± 0.46	26.47 ± 0.13
	Male	24.80 ± 1.72	25.33 ± 1.23	25.43 ± 0.66
PLT[h] (10^3/µL)	Female	875.00 ± 49.4	705.00 ± 65.4	761.00 ± 31.3
	Male	633.00 ± 31.2	527.00 ± 62.1	612.67 ± 35.8

[a]Haemoglobin; [b]red blood cell; [c]white blood cell; [d]hematocrit; [e]mean corpuscular volume; [f]mean corpuscular haemoglobin concentration; [g]mean corpuscular haemoglobin concentration; [h]platelets. The data are presented as the mean of five replicates ± SEM. The value with * is statistically significant at $p < 0.05$ across the row.

However, the mean corpuscular volume was significantly elevated ($p < 0.05$) in male rats at 40 mg/kg bw but not at 80 mg/kg bw. However, the same parameter was not significantly altered ($p > 0.05$) in the female rats. Exposure to substances or drugs at lethal doses can potentially disrupt the normal state of blood indices, hence serving as indicators of pathologic and physiologic states in both humans and animals.[29] In the present study, the haematological parameters of the groups treated with the *n*-hexane fraction of *A. leoicarpus* remained the same as the control group in terms of almost all the blood parameters, indicating that the fraction might not be a blood-modulating agent.

Effects of oral subacute exposure to the n-hexane fraction of A. leiocarpus on renal function parameters

There was no significant change ($p > 0.05$) in the creatinine, urea, sodium, and potassium concentrations following treatment with the *n*-hexane fractions of *A. leiocarpus* in the female and male rats compared with their respective controls (Figure 2).

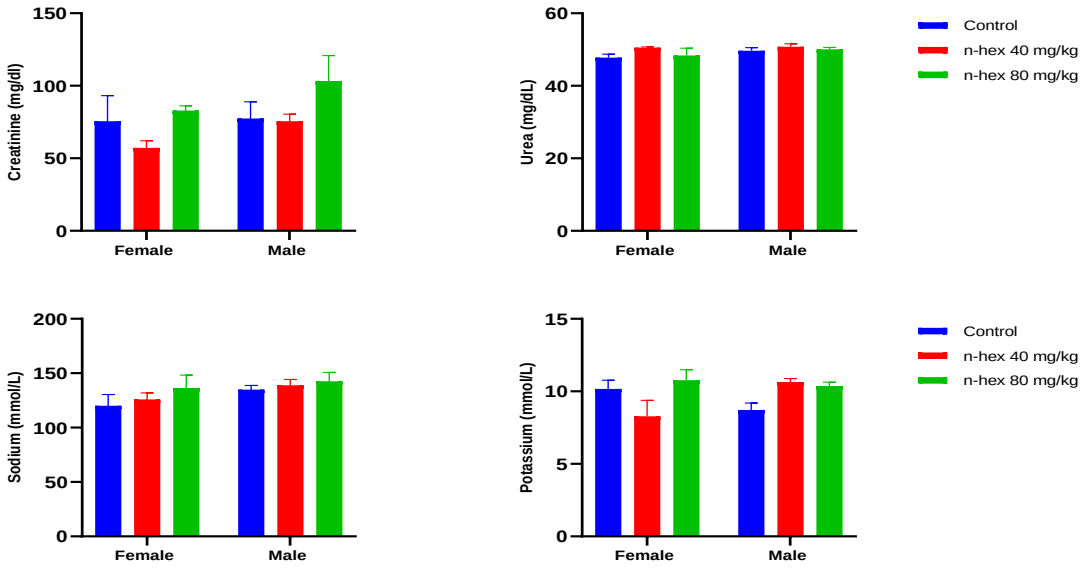

Figure 2: Effects of *n*-hexane fraction of *A. leiocarpus* (40 and 80 mg/kg bw) on some renal function parameters in a sub-acute toxicity study. n-hex: *n*-hexane. The data presented as the mean of five replicates ± SEM

Alterations in biochemical parameters are vital measures of organ damage or dysfunction. Owing to their detoxification functions, the liver and kidney are often the organs most susceptible to drug-induced toxicity. Renal damage is usually evaluated as a function of modifications in the levels of plasma/serum creatinine, urea, and electrolytes such as sodium and potassium.[30] Creatinine is a degradation product of muscle creatinine phosphate; its value also depicts the efficiency of renal glomerular filtration.[31] Urea is a nitrogenous waste product of amino acid and protein degradation. It is filtered out of the blood by the glomeruli of the kidney and, to some extent, is reabsorbed with water. The kidney plays a pivotal role in the elimination of creatinine and urea from the body.[32] The findings of this study revealed normal renal clearance, as the levels of creatinine and urea were physiologically stable, as they were not significantly altered in the treatment groups or the control. Likewise, the level of electrolytes (sodium and potassium) are important indicators of kidney function.[33] Major changes in their concentrations can suggest that the kidneys may be damaged. However, their levels remained consistent in the treatment groups relative to those in the control. Thus, this study highlights the safe use of the plant.

Effects of oral subacute exposure to n-hexane fraction of A. leiocarpus on total protein, globulin, albumin and total bilirubin concentrations

There was no significant change ($p > 0.05$) in the total protein, globulin, albumin and total bilirubin concentrations in female and male rats following treatment with the *n*-hexane fraction of ALBHE (Figure 3).

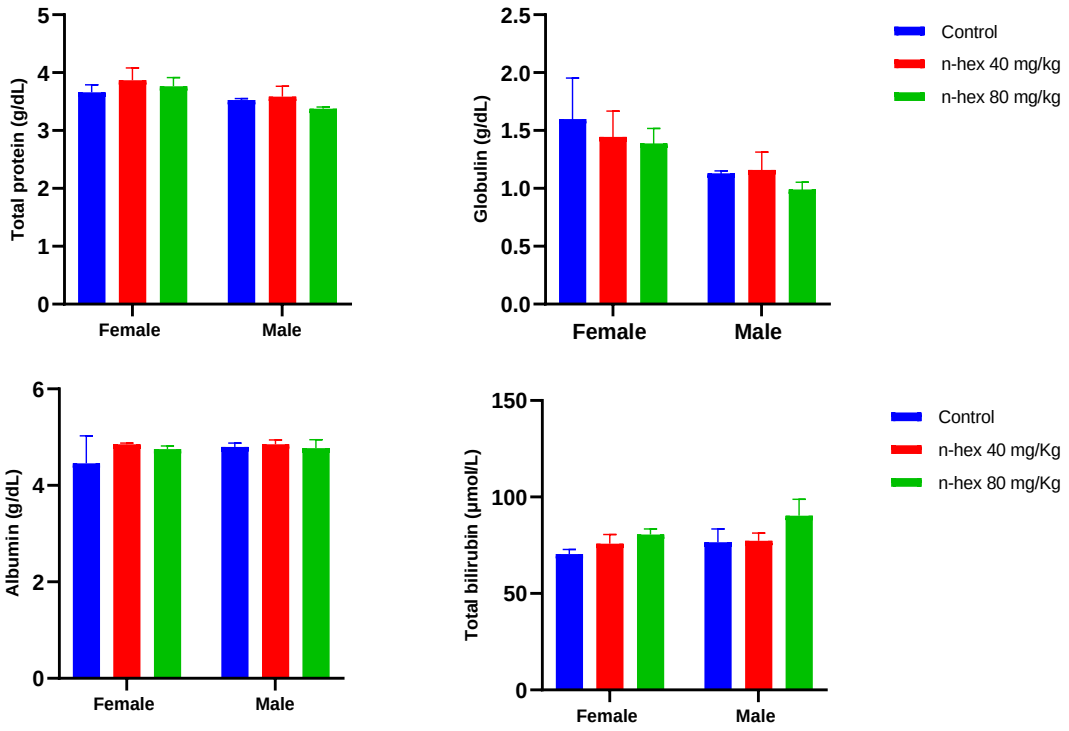

Figure 3: Effects of the *n*-hexane fraction of *A. leiocarpus* (40 and 80 mg/kg bw) on total protein, globulin, albumin and total bilirubin concentrations in sub-acute toxicity study. n-hex: *n*-hexane. The data presented as the mean of five replicates ± SEM.

The total protein, albumin, globulin and bilirubin are universally approved routine biomarkers of liver health status. The liver is a core site for protein, albumin and globulin synthesis and their plasma/serum levels can automatically reflect the physiologic or pathologic state at any point in time. Bilirubin is primarily a degradation product of haemoglobin, and its plasma concentration can indicate hepatic excretory function.[39] Compared to the control, oral administration of the *n*-hexane fraction of *A. leiocarpus* did not significantly affect the plasma concentration of these metabolites in male and female rats at either dose.

Effects of oral subacute exposure to n-hexane fractions of A. leiocarpus on some hepatic function indices

AST, ALT, GGT and ALP levels in both male and female rats did not significantly differ across the treatment groups ($p > 0.05$) (Figure 4). Liver function enzymes (AST, ALT, ALP and GGT) were evaluated to detect any possible hepato-cellular injury caused by the *n*-hexane fraction of the *A. leiocarpus* extract. ALT and AST are widely known as biomarkers of liver damage, with ALT having greater specificity.[34] The leakage of these enzymes into the blood could suggest compromised integrity of the hepatocytes. Likewise, abnormally high ALP levels in blood could indicate threatened hepatic health (such as blocked bile duct) or certain bone related diseases.[35] An increase in ALT coupled with GGT could further confirm the likelihood of biliary obstruction or certain liver diseases.[36] These marker enzymes (AST, ALT, ALP and GGT) remained largely unaltered in the extract treated groups compared with to the control group in the female and male rats thereby supporting the reported hepatoprotective effect of *A. leiocarpus*[37,38]; however, another report suggested a mild hepatotoxic effect.[18]

Effect of oral subacute exposure to n-hexane fractions of A. leiocarpus on lipid profile

There was no significant difference ($p > 0.05$) in total cholesterol, triglyceride, HDL-C, or LDL-C levels between the *A. leoicarpus*-treated groups and the control groups, in both male and female rats (Figure 5). The lipid profile is a worthwhile measure of cardiovascular health, and unusual levels of plasma cholesterol and triglyceride could lead to complications in the renal and hepatic systems. Cholesterol and triglyceride are important components of the biological membrane and cholesterol serve as a precursor of crucial substances, such as adrenal and gonadal steroid hormones. A high level of LDL-C potentiates the risk of cardiac disease, as it builds up on the blood vessel walls whereas a high level of HDL-C reduces the risk of a cardiac disease.[40] The integration of the values of total cholesterol, triglyceride, HDL-C and

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LDL-C measured indicate the lipid profile pattern. Since there was no considerable alteration in the lipid profile of rats given the *n*-hexane

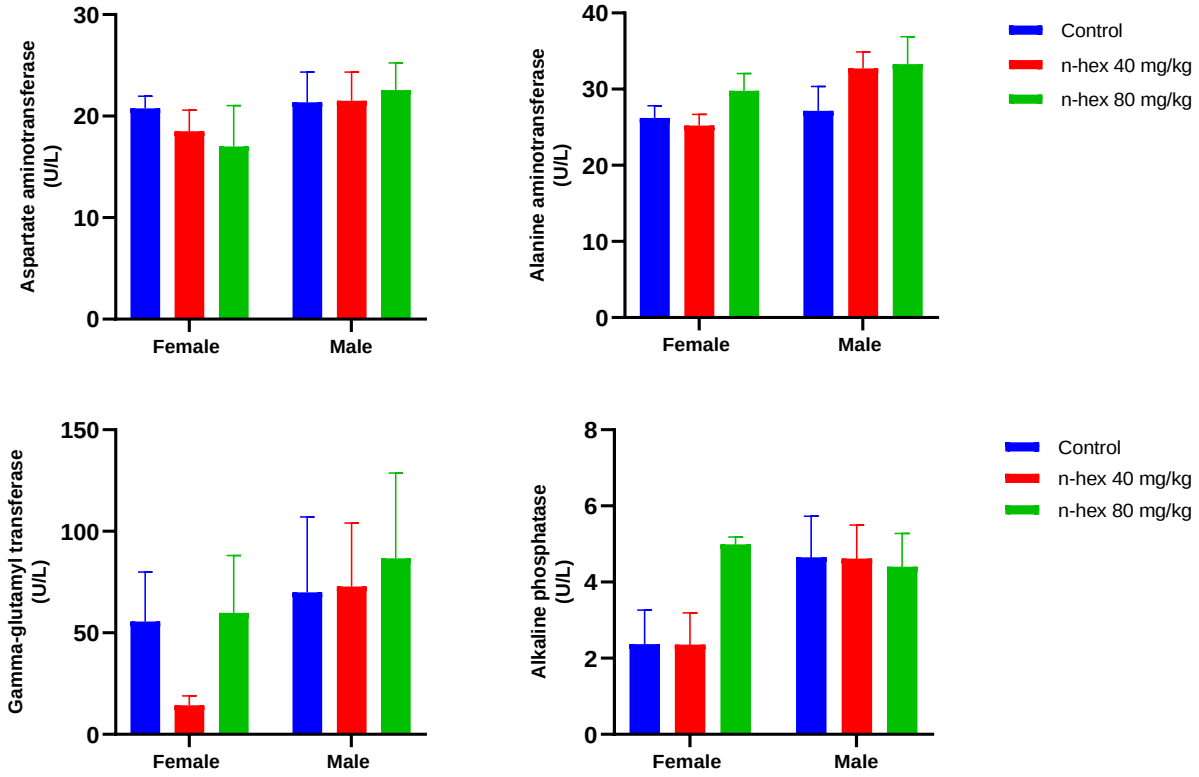

Figure 4: Effects of *n*-hexane fraction of *A. leiocarpus* (40 and 80 mg/kg bw) on some hepatic function indices in sub-acute toxicity study. n-hex: *n*-hexane. The data presented as the mean of five replicates ± SEM.

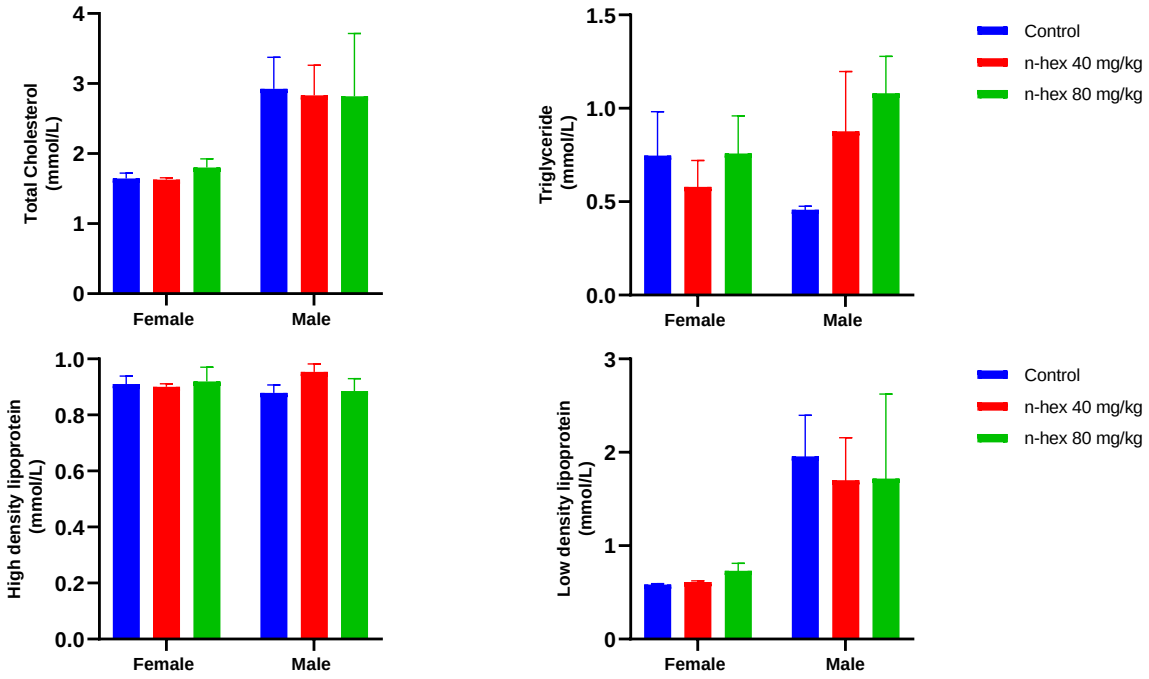

Figure 5: Effects of *n*-hexane fractions of *A. leiocarpus* (40 and 80 mg/kg bw) on the lipid profile of Wistar rats following 28-days of oral administration. n-hex: *n*-hexane. The data presented as the mean of five replicates ± SEM.

fraction of *A. leiocarpus* at either dose compared with the control, it can be inferred that the fraction was experimentally safe and tolerable within the span of the administered dose.

Effects of oral subacute exposure to n-hexane fractions of A. leiocarpus on rats' histoarchitecture

Gross pathological examination was carried out on the following organs: liver, kidney, heart, spleen, brain, uterus and testes (Figures 6 to 11). In the control and treatment groups (n-hex 40 and 80 mg/kg) of both sexes, hepatic sections showed no signs of significant inflammation or damage. The histoarchitecture of hepatic tissue, which is composed of normal hepatocytes, portal tracts, and central veins is intact and undamaged. (Figure 6). In the control and treatment groups (n-hex 40 and 80 mg/kg) of both sexes, the sections revealed renal tissue with preserved architecture, consisting of typical tubules, glomeruli, and an unremarkable interstitium. There were no discernible vascular lesions. There was no evidence of damage (Figure 7).

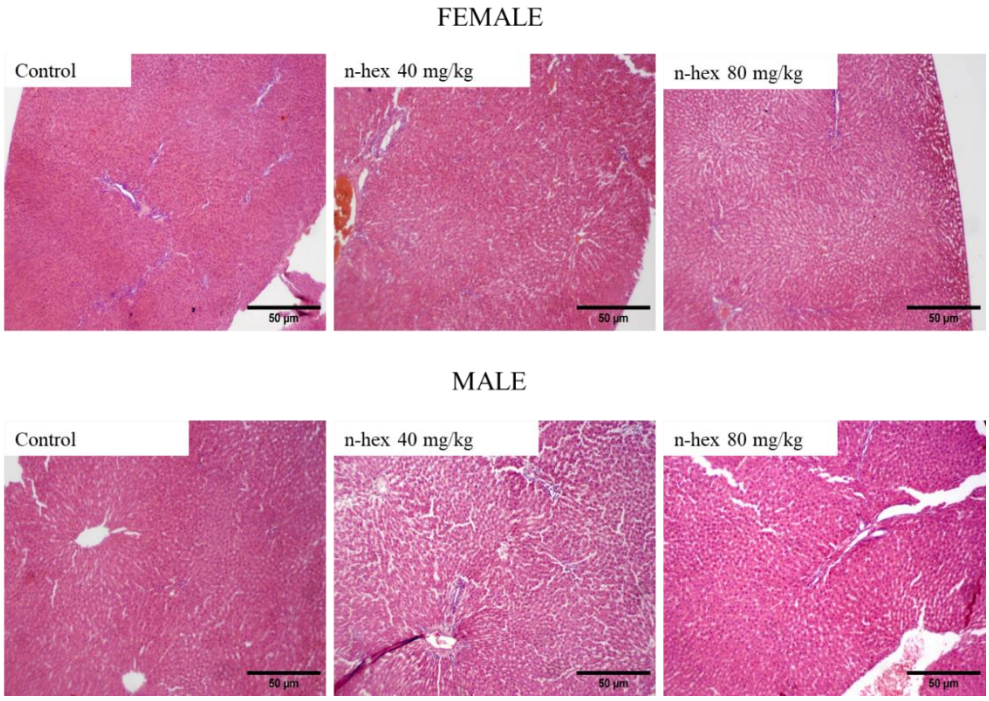

Figure 6: Photomicrographs of the liver in female and male rats given repeated oral treatment with the *n*-hexane fraction of *A. leiocarpus* (40 and 80 mg/kg bw) for 28 days. n-hex: *n*-hexane; magnification-40x; scale bar-50 µm.

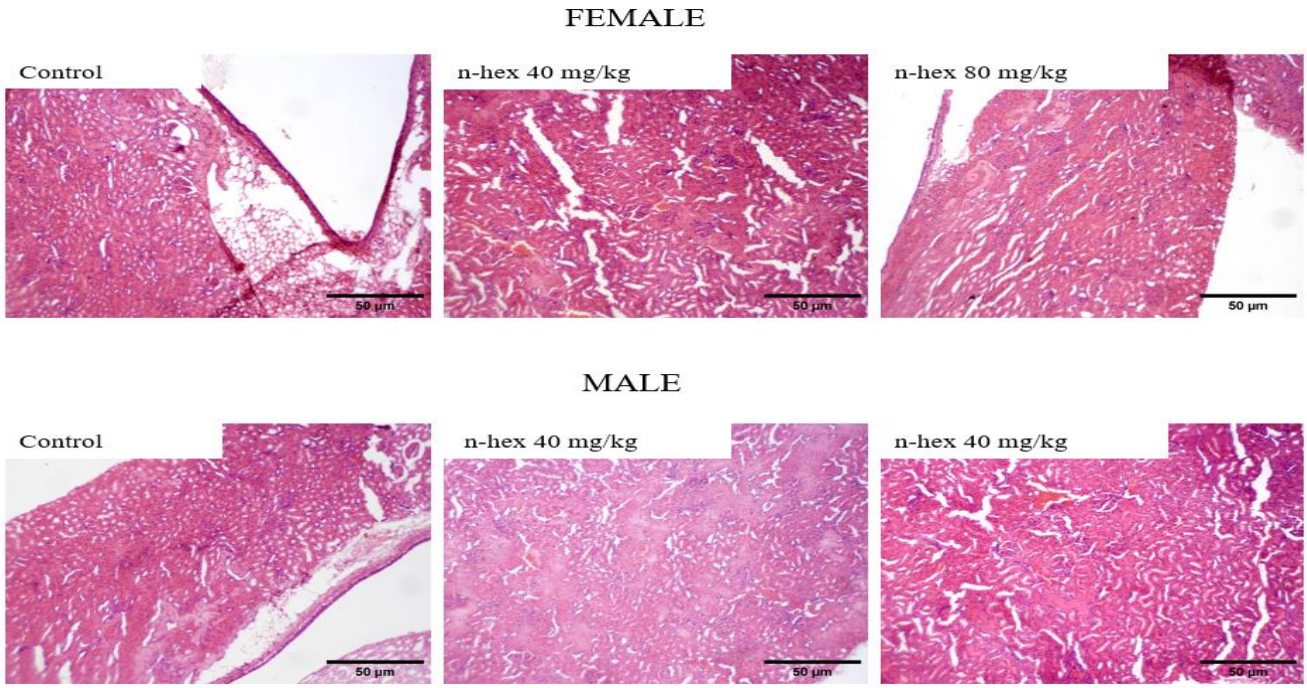

Figure 7: Photomicrographs of kidney in female and male rats given repeated oral treatment with the *n*-hexane fraction of *A. leiocarpus* (40 and 80 mg/kg bw) for 28 days. n-hex: *n*-hexane; magnification-40x; scale bar-50 µm.

The histopathological section from the control and treatment groups (n-hex 40 and 80 mg/kg) of both sexes revealed heart tissue composed of normal endocardial lining and myocardial muscle fibre bundles with interspersed vascular channels. There were no atherosclerotic lesions or features of inflammation or infarction (Figure 8). The histologic section in the control and treatment groups (n-hex 40 and 80 mg/kg) of both sexes shows splenic tissue with preserved architecture comprising the red pulp (splenic cords) and white pulp (perivascular lymphoid follicles) (Figure 9). In the control and treatment groups (n-hex 40 and 80 mg/kg) of both sexes, the section shows the usual brain tissue consisting of nerve cell clusters surrounded by fibrillary glial matrix. The molecular and granular layers within the hippocampus region were intact and well preserved (Figure 10).

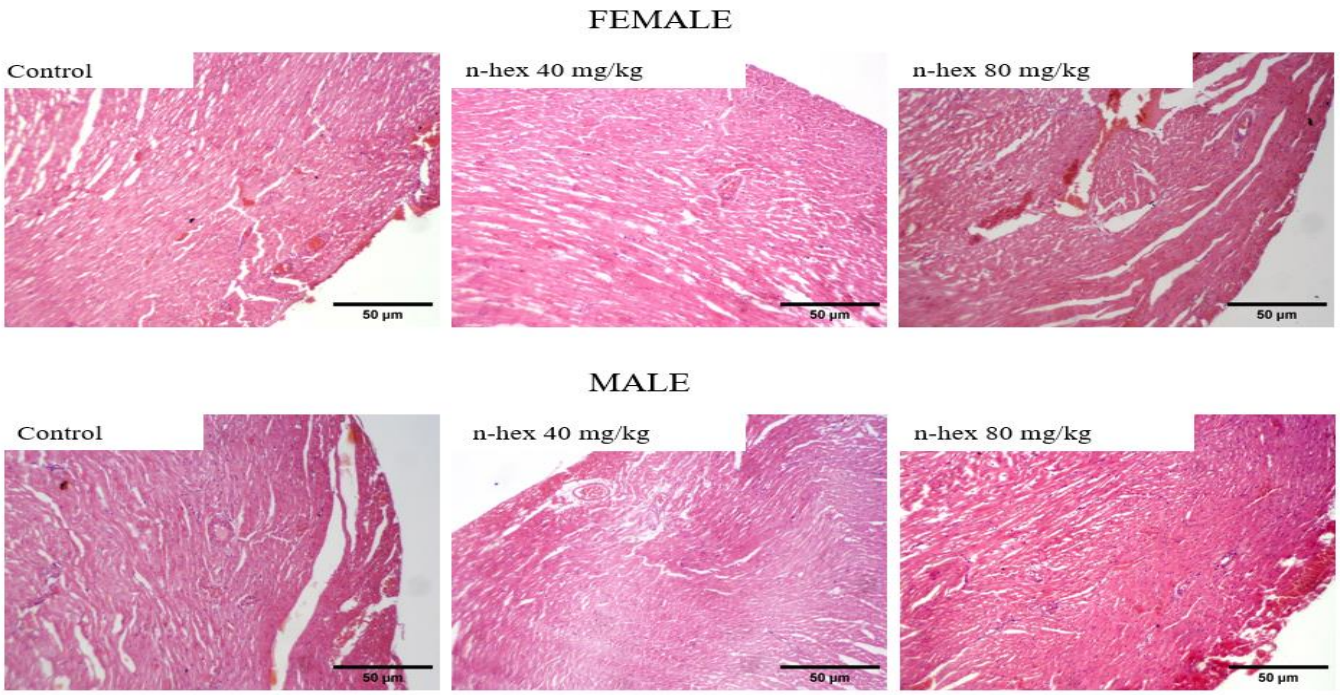

Figure 8: Photomicrographs of the heart in female and male rats given repeated oral treatment with the *n*-hexane fraction of *A. leiocarpus* (40 and 80 mg/kg bw) for 28 days. n-hex: *n*-hexane; magnification-40x; scale bar-50 µm**.**

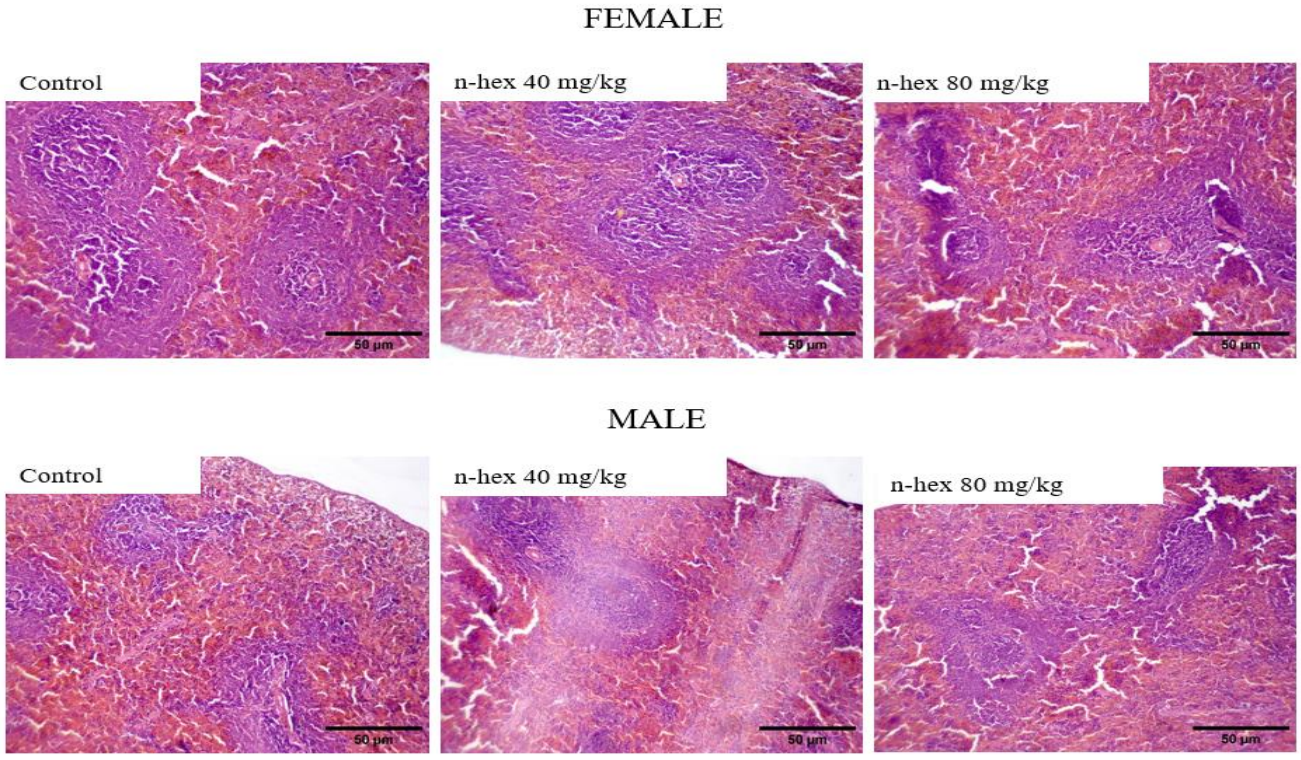

Figure 9: Photomicrographs of the spleens of female and male rats given repeated oral treatment with the *n*-hexane fraction of *A. leiocarpus* (40 and 80 mg/kg bw) for 28 days. n-hex: *n*-hexane; magnification-40x; scale bar-50 µm.

Sections from the control and treatment groups (n-hex 40 and 80 mg/kg) of female rats presented normal myometrial (composed of fascicles of smooth muscle) and endometrial tissue (composed of preserved glandular epithelium). No abnormalities were observed. Sections in the control and treatment groups (n-hex 40 and 80 mg/kg) of male rats show testicular tissue composed of late-stage seminiferous tubules comprising pachytene spermatocytes and elongating spermatids. There is characteristically no observable reduction or disruption of spermatogenesis (Figure 11). Histological analysis of the liver, kidney and other vital organs from the control and treatment groups revealed a normal cellular structure (histoarchitecture) in both the female and male rats. The findings corroborate the haematological and biochemical indices following treatment with the *n*-hexane fraction of *A. leoicarpus*, and confirm that oral administration of the extract might not have caused any injury to the liver, kidney or other organs at the tested doses and within the treatment duration

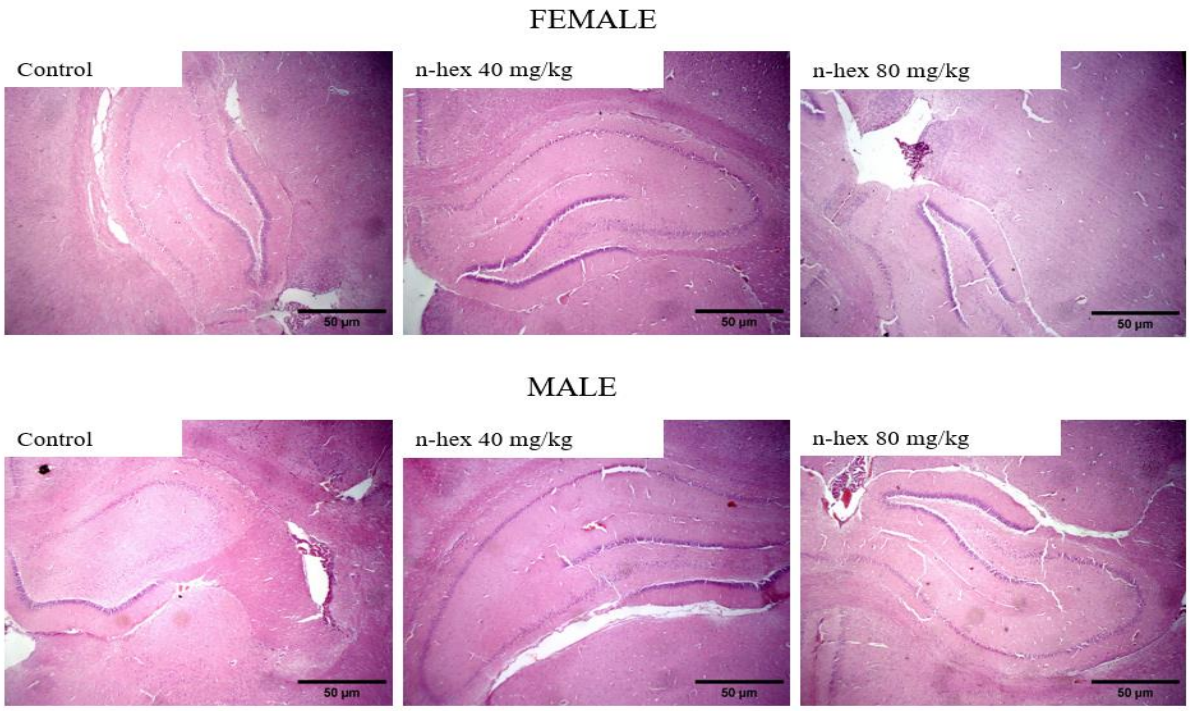

Figure 10: Photomicrographs of brain (hippocampus) of female and male rats given repeated oral treatment with the *n*-hexane fraction of *A. leiocarpus* (40 and 80 mg/kg bw) for 28 days. n-hex: *n*-hexane; magnification-40x; scale bar-50 µm.

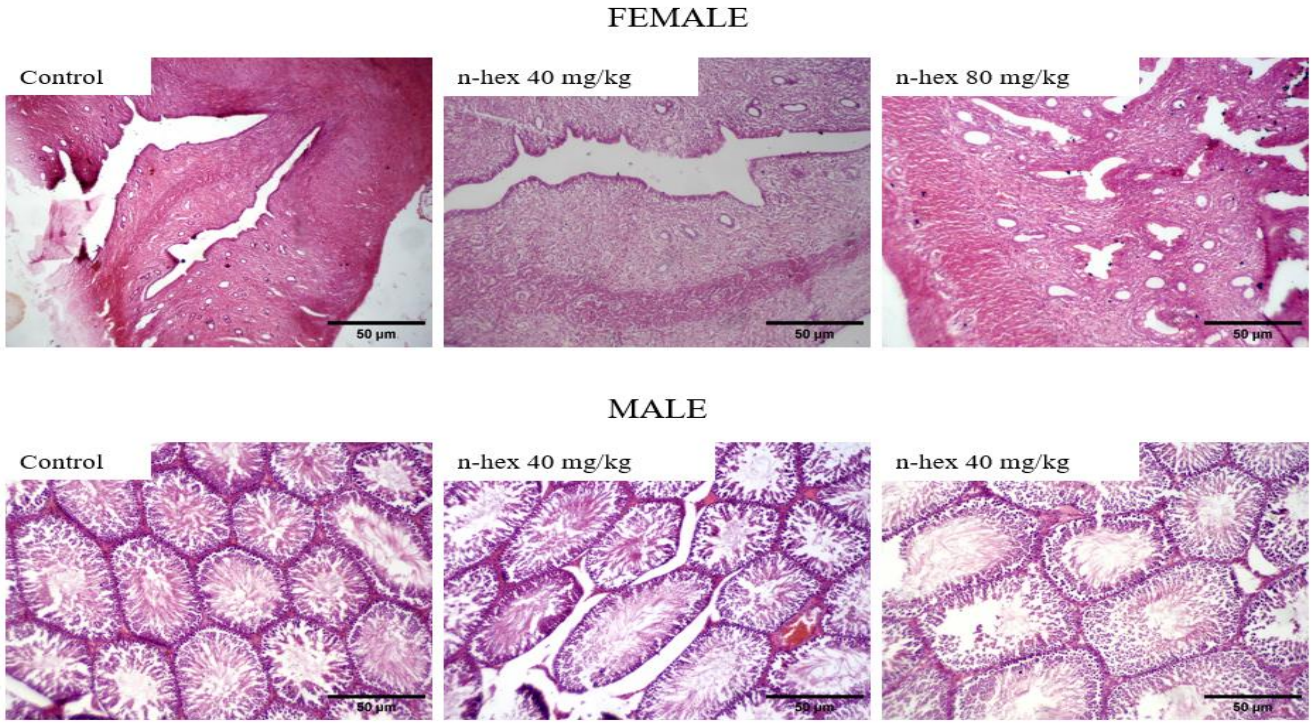

Figure 11: Photomicrographs of the uterus and testes of rats given repeated oral treatment with the *n*-hexane fraction of *A. leiocarpus* (40 and 80 mg/kg bw) for 28 days. n-hex: *n*-hexane; magnification-40x; scale bar-50 µm.

Conclusion

The study demonstrated that the mean lethal dose (LD50) for the hydomethanolic extract of the stem bark of *A. leiocarpus* exceeded 5000 mg/kg bw. Furthermore, the *n*-hexane fraction of *A. leiocarpus* did not cause any detectable adverse effects on female or male rats. The extract did not cause death in rats during the 28 days' sub-acute toxicity studies. The haematological, biochemical, and histological parameters were not substantially altered relative to those of the control group. Future pre-clinical studies should consider a chronic toxicity to further accentuate the safety profile of the *n*-hexane fraction of *A. leoicarpus*.

Conflict of interest

The author reports no conflicts of interest in this work.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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