

HPLC ANALYSIS, SAFETY PROFILE AND SUBACUTE EFFECTS OF *Aspilia africana* (PERS.) C. D ADAMS (ASTERACEAE) LEAVES ON STEROIDAL HORMONES ASSOCIATED WITH FIBROID

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ABSTRACT

Uterine fibroids present a significant threat to women's reproductive health worldwide and estrogen and progesterone play a vital role in its growth. This study investigated the safety profile and effects of ethanol extract of *Aspilia africana* leaves on serum estradiol and progesterone levels in female Wistar rats. The rats were administered the plant extract in graded doses (100 and 200 mg/kg) for 28 days after which blood samples and organs were collected for toxicity studies and hormonal assay. Additionally, High-Performance Liquid Chromatography (HPLC) was employed to identify bioactive compounds in the extract. The extract did not cause any significant adverse effect on most of the tested biochemical and hematological parameters except the creatinine levels and platelet distribution width. There was no toxic damage to most of the selected organs of the rats except the liver which revealed portal hepatitis. A boost in the local immune system was observed in the organs. Serum estradiol and progesterone levels were significantly lowered by 55.05 ($p < 0.01$) and 51.85 % respectively, at 100 mg/kg dose. HPLC analysis of the ethanol leaf extract of *A. africana* revealed the presence of majorly limonene, alpha-pinene, phytol, alpha-copaene, beta-elemene, and cis-ocimene. This result shows that *A. africana* leaves have some toxic effect on the liver, but is able to lower estradiol and progesterone levels.

Keywords: *Aspilia africana*, Estradiol, Fibroid, HPLC, Progesterone, Safety profile.

INTRODUCTION

Uterine leiomyomas, commonly referred to as uterine fibroids, present a significant threat to women's reproductive health worldwide (Uimari *et al.*, 2021). It occurs in more than 70% of women but only around 30% experience symptoms (Navarro *et al.*, 2021). Although the precise cause of uterine leiomyoma formation remains unclear, epidemiological research strongly suggests that reproductive hormones such as estrogen and progesterone, as well as increased protein and cholesterol levels promote tumor growth. This is evident from the fact that fibroids seldom appear before menarche and tend to regress after menopause (Navarro *et al.*, 2021).

Treatment and prevention options are limited, with medical professionals frequently recommending myomectomy or hysterectomy (Ezejiofor and Okoroafor, 2022). Although uterine artery embolization and high-intensity focused ultrasound ablation are effective treatment options, their high costs and associated risks discourage

patients from seeking these options (Arip *et al.*, 2022). Conventional medications are both expensive and generally provide only temporary relief, underscoring the importance of exploring alternative treatment options (Imade *et al.*, 2024) such as herbal remedies which are accessible, affordable, and of cultural importance (Imade *et al.*, 2024).

Aspilia africana (Pers.) C. D. Adams (Asteraceae) is reported to be used ethnomedicinally to treat various diseases including fibroid. Its antibacterial, antifungal, anticoagulant, antiulcer, and antimalarial effects have been documented. Obideje *et al.* (2024) reported the effect of *Aspilia africana* leaf extract on total serum protein levels of monosodium glutamate (MSG) treated female Wistar rats. Other studies indicate that aqueous extract of *Aspilia africana* leaves protect against MSG-induced fibroids in rat models (Eze-Stephen *et al.*, 2019). Further research is needed to ascertain the mechanisms through which this medicinal plant works against fibroid growth, as well

determine its effect on prolonged usage since fibroid is a chronic condition.

The aim of this study therefore was to evaluate the safety of the ethanol leaf extract of *Aspilia africana* and investigate its effect on hormones implicated with the etiology of fibroid such as estradiol and progesterone.

MATERIALS AND METHODS

Chemicals and reagents

Absolute ethanol (99.5%), distilled water, chloroform, Accu-bind estradiol Enzyme Linked Immunosorbent Assay (ELISA) kit, Accubind Progesterone ELISA kit, Picric acid.

Plant collection, authentication and extraction

In October 2024, fresh leaves of *A. africana* were gathered from within University of Benin, located in Benin City, and was confirmed at the Department of Plant Biology and Biotechnology at the same university, where a voucher specimen (UBH-A192) was kept. The leaves were sun-dried for two days and then pulverized with a grinding machine. The powdered plant was subjected to 99.5% absolute ethanol extraction for 48 hours using a Soxhlet apparatus. The extract was reduced to dryness with a water bath at 50°C, weighed and stored in the fridge until required.

Grouping of experimental animals

Non-pregnant Wistar rats (15), weighing between 110 – 200 g were obtained from and acclimatized at the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin. The animals were placed in separate plastic cages and allowed unrestricted access to dry grower rodent pellet (Chikun feed) and water. They were grouped into three with each group (A, B and C) having five rats each. Proper ventilation was ensured and wood shavings provided as bedding material to collect urine and excreta. Ethical permission was sought from the Research Ethics Committee of the Faculty of

Pharmacy, University of Benin (EC/FP/025/08).

Dosing of experimental animals

Group A was the control given feed and water freely. Doses of 100 and 200 mg/kg of *A. africana* extract were administered to groups B and C. They were chosen based on the plants LD₅₀, which exceeds 5000 mg/kg (Arunsi *et al.*, 2020). The rats were given daily doses for twenty-eight days via orogastric tube, their weights were taken on day 0, 7, 14, 21 and 28 and the dose administered adjusted, based on their recorded body weight each week (Rachmaini *et al.*, 2024).

Biochemical assays

On the 29th day, the animals were euthanized with chloroform, and blood samples collected via cardiac puncture. The blood was transferred into both plain and EDTA bottles for biochemical analysis of subacute toxicity, plasma progesterone levels and plasma estradiol levels. Organs excised for histological examination were the kidney, liver, heart, spleen, lungs and uterus (Ezejiyor and Okoroafor, 2022).

Serum obtained from blood in plain bottles was assayed for lipids, renal and liver function tests with an automated chemistry analyzer (Selectra Pro S, Germany). Ion selective electrode (SFRI 4000, France) was utilized for electrolyte assay. Lipid profile tests carried out were total cholesterol, serum triglycerides, low-density lipoproteins, and high-density lipoprotein. The kidney function values assayed were creatinine, urea, and serum electrolytes (K⁺, Cl⁻, Na⁺, HCO₃⁻). Liver function parameters assayed were aspartate aminotransferase (AST), alkaline phosphatase (ALP), alanine aminotransferase (ALT), conjugated bilirubin (Cb), total bilirubin (Tb), albumin (ALB), total protein (Tp) and globulin (GLO) (Arunsi *et al.*, 2020).

Hematological analyses

Additionally, hematological analyses were obtained by a blood count with the aid of an automated hematology analyzer with blood

placed in EDTA bottles (Dymind 2000, China). The parameters analyzed include red blood cells (RBC), white blood cell (WBC), red blood cell distribution width (SD), red blood cell distribution width (CV), mean corpuscular volume (MCV), hemoglobin (Hb), neutrophil (NEU), basophil (BAS), monocyte (MON), platelet (PLT), mean corpuscular hemoglobin (MCH), hemoglobin (HGB), granulocytes (GRAN), red blood cell distribution width SD (RDW-SD), red blood cell distribution width-coefficient of variation (RDW-CV), mean platelet volume (MPV), lymphocytes (LYM), platelet larger cell ratio (P-LCR), platelet distribution width (PDW), eosinophil (EOS), mean corpuscular hemoglobin concentration (MCHC), platelets count (PCT), and hematocrit (HCT) (Ezejirofor and Okoroafor, 2022).

Histopathology analysis of organs

The liver, heart, kidney, spleen, lungs, and uterus of the rats were preserved in a 10% neutral buffered formalin solution for histological examination. Tissues were dehydrated, cleared, waxed and sectioned to slides. They were subsequently treated with xylene, hydrated and stained with hematoxylin. Sections were counter-stained with eosin after differentiation in 1% acid alcohol. Dehydration was repeated, and the slides treated with xylene and mounted with dibutylphthalate polystyrene before microscopic analysis (Imade *et al.*, 2024).

Assay of serum estradiol and progesterone levels

This was carried out using the Microplate Reader (Mindray MR-96A). E₂ AccuBind ELISA Kit was used for serum estradiol analysis while progesterone ELISA Kit was used for serum progesterone analysis. The instructions in the manufacturers' manuals were followed (Lin *et al.*, 2020).

HPLC procedure

HPLC analysis was conducted using a Shimadzu Nexera MX system. The chromatography was performed on a uBondapak C₁₈ reverse-phase column with dimensions of 100 mm in length, 4.6 mm internal diameter, and 7 µm thickness, following standard methods. Mobile phase employed was acetonitrile/water, 70:30. The HPLC system was attached to a UV-Vis diode array detector set at an analytical wavelength of 254 nm, within the UV-visible region. Pump pressure employed was 15 mpa. Solutions of standards were first injected into the HPLC system to generate a chromatogram with a given peak and peak profile. These were used to create a window in preparation for the test sample analysis. The analysis adopted a 2 ml/min constant flow rate as 5 µl of extract was injected into the HPLC system also to obtain a corresponding peak area and peak profile in a chromatogram (Imade *et al.*, 2024).

Data analysis

Bar graphs were generated with GraphPad Prism for Windows (version 7), and the data were analyzed with one-way analysis of variance (ANOVA) followed by the Tukey-Kramer multiple comparison test, with $P \leq 0.05$ regarded as significant in all analyses.

RESULTS

Effect of *A. africana* leaves extract on body weight

Daily dosing for 28 days did not result in death or any outward display of toxicity in all treated groups. Eating habits were normal and a noticeable rise in body weight was evident (Table I), but significant weight reduction was observed at day 7 and 21 for 200 mg/kg group, and day 28 for 100 mg/kg group compared to the control.

Table 1: Change in mean body weights (g) of the rats at different days of the experiment

Dose (mg/kg)	0	7	14	21	28
Control	161±23.54	13±28.63	14.8±30.28	12.2±19.81	26±17.97
100	197.8±13.3	0.6±14.93	9.65±12.24	4.4±9.26	-4.4±8.80
200	162.2±6.95	-7.8±8.30	7.2±11.82	-9.8±10.58	1±7.705

Data are expressed as mean ± Standard Error of Mean, n=5

Effect of *A. africana* leaves extract on serum biochemical parameters

Effect on lipid profile

The lipid profile data obtained from the test groups were not substantially different ($p \geq 0.05$) from the control group (Table 2).

Table 2: Result of lipid profile

Test (mg/dL)	Control	100 mg/kg	200 mg/kg
Total cholesterol	82.20 ± 4.03	81.20 ± 8.89	92.00 ± 2.45
High-density lipoprotein	25.40 ± 0.98	24.40 ± 2.60	27.60 ± 0.75
Low density lipoprotein	36.60 ± 2.94	39.40 ± 6.38	45.00 ± 2.03
Triglyceride	100.60 ± 2.89	86.20 ± 2.62	97.00 ± 3.54

Data are expressed as mean ± SEM, n=5.

Effect on renal function parameters

Renal function values such as the sodium, bicarbonate ion, and chloride ($P \geq 0.05$) levels were not considerably altered at both doses.

Urea content was significantly reduced at 200 mg/kg ($P \leq 0.01$) while creatinine and potassium levels were significantly raised at 100 mg/kg ($P \leq 0.05$) (Table 3).

Table 3: Result of renal function tests

Tests	Dose (mg/kg)		
	Control	100	200
Urea (mg/dL)	45.40 ± 2.25	41.80 ± 1.24	32.20 ± 1.88 ^b
Creatinine (mg/dL)	0.68 ± 0.06	0.92 ± 0.04 ^a	0.72 ± 0.04
Na ⁺ (mmol/L)	140.60 ± 1.47	137.80 ± 0.92	141.00 ± 1.27
K ⁺ (mmol/L)	4.12 ± 0.10	5.06 ± 0.11 ^a	4.58 ± 0.27
HCO ₃ ⁻ (mmol/L)	21.40 ± 0.28	19.00 ± 0.89	21.40 ± 1.08
Cl ⁻ (mg/dL)	104.80 ± 0.73	104.20 ± 0.73	106.6 ± 0.81

Data are expressed as mean ± SEM, n=5. a and b have some level of significance with $p < 0.05$ and $p < 0.01$, respectively compared to the control.

Effect on liver function parameters

Administration of the extract at both doses did not significantly change the liver function parameters (Table 4).

Table 4: Result of liver function tests

Parameters	Dose (mg/kg)		
	Control	100	200
AST (μ /L)	77.40 $\pm$ 4.27	70.80 $\pm$ 6.63	63.00 $\pm$ 5.93
ALT (μ /L)	45.80 $\pm$ 2.42	38.00 $\pm$ 3.11	41.00 $\pm$ 1.34
ALP (μ /L)	59.60 $\pm$ 3.54	67.40 $\pm$ 6.52	50.60 $\pm$ 5.55
Tb (mg/dL)	0.28 $\pm$ 0.02	0.26 $\pm$ 0.02	0.28 $\pm$ 0.02
Cb (mg/dL)	0.10 $\pm$ 0.00	0.10 $\pm$ 0.00	0.10 $\pm$ 0.00
Tp (g/dL)	6.34 $\pm$ 0.12	6.14 $\pm$ 0.33	6.48 $\pm$ 0.34
ALB (g/dL)	2.82 $\pm$ 0.02	2.66 $\pm$ 0.05	2.90 $\pm$ 0.19
GLO (g/dL)	3.52 $\pm$ 0.14	3.54 $\pm$ 0.29	3.58 $\pm$ 0.36

Data are expressed as mean $\pm$ SEM, n=5.

Effect of *A. africana* leaves extract on hematological parameters

The results revealed that there was no significant alteration ($p \geq 0.05$) in the white blood cell and lymphocyte count as well as most

of the other hematological parameters tested (Table 5). Only the platelet distribution width (PDW) was significantly reduced in relation to the control ($p, 0.01$).

Table 5: Result of hematological assay

Parameters	Dose (mg/kg)		
	Control	100	200
WBC ($10^3 / \mu$ L)	14.64 $\pm$ 1.50	13.76 $\pm$ 1.50	11.66 $\pm$ 1.31
LYM (%)	83.00 $\pm$ 1.66	75.92 $\pm$ 3.11	80.86 $\pm$ 0.91
MON (%)	2.06 $\pm$ 0.33	2.10 $\pm$ 0.41	1.62 $\pm$ 0.20
NEU (%)	10.56 $\pm$ 1.65	17.36 $\pm$ 2.37	13.54 $\pm$ 0.82
EOS (%)	0.84 $\pm$ 0.37	0.64 $\pm$ 0.21	0.62 $\pm$ 0.16
BAS (%)	3.50 $\pm$ 0.42	3.98 $\pm$ 0.96	3.36 $\pm$ 0.25
RBC ($10^6 / \mu$ L)	6.38 $\pm$ 0.24	6.19 $\pm$ 0.21	6.36 $\pm$ 0.17
HGB (g/dL)	13.50 $\pm$ 0.51	13.52 $\pm$ 0.36	14.00 $\pm$ 0.27
HCT (%)	40.20 $\pm$ 1.56	38.16 $\pm$ 1.46	41.80 $\pm$ 0.80
MCV (μ m ³)	50.28 $\pm$ 0.42	53.06 $\pm$ 0.86	53.30 $\pm$ 0.80
MCH (pg)	21.16 $\pm$ 0.10	21.88 $\pm$ 0.26	22.04 $\pm$ 0.43
MCHC (g/dL)	42.12 $\pm$ 0.37	41.22 $\pm$ 0.26	41.32 $\pm$ 0.39
RDW-CW (%)	16.28 $\pm$ 0.88	14.80 $\pm$ 0.27	15.16 $\pm$ 0.38
RDWS (μ m ³)	26.96 $\pm$ 1.65	25.16 $\pm$ 0.83	28.20 $\pm$ 2.40
PLT ($10^3 / \mu$ L)	741.20 $\pm$ 98.38	643.60 $\pm$ 29.72	708.40 $\pm$ 81.79
MPV (μ m ³)	6.54 $\pm$ 0.27	6.92 $\pm$ 0.22	6.32 $\pm$ 0.07
PCT (%)	0.49 $\pm$ 0.08	0.46 $\pm$ 0.02	0.56 $\pm$ 0.03
PDW (%)	24.98 $\pm$ 1.43	14.88 $\pm$ 0.24 ^d	16.76 $\pm$ 1.11 ^b
P-LCR (%)	5.96 $\pm$ 2.29	6.56 $\pm$ 1.25	3.60 $\pm$ 0.08

Data are expressed as mean $\pm$ SEM, n = 5. Letters b and d have some level of significance with $p < 0.01$ and $p < 0.0001$ respectively compared to the control.

Histopathology Result

Panel A shows normal spleen architecture with well-defined white pulp (WP), sinuses (SI), splenic arterioles (SA), and red pulp (RP). Sections taken from rats treated with 100 and 200 mg/kg body weight of *A. africana* extract revealed vasodilatation, and active congestion (DC) as well as follicular activation (FA) and increase in the sequestration of red blood cells (RS).

In the control group, the liver showed normal structure (Panel B), with normal hepatocytes (NH), sinusoids (SI) and portal triad (hepatic portal vein (PV), artery (HA) and bile duct (BD)). In addition, sections taken from rats treated with graded doses of *A. africana*, also showed portal vein dilatation (VD), Kupffer cell activation (KA), and periportal infiltrates of inflammatory cells (PI).

For the kidney, the control and treated groups revealed normal histological architecture (Panel C), with well-defined tubules (TU), glomeruli (GL), interstitial space (IS) and arcuate blood vessels.

Lungs from the control group in Panel D revealed well-defined alveolar sacs (AL), interstitial space (IS), bronchioles (TB), bronchial blood vessels (BA) and active vascular congestion (AC). Sections from rats given the extract revealed normal alveolar sacs (NA) and added beneficial changes of bronchiolar dilation (BD) and activation of bronchiolo-alveolar lymphoid aggregates (AB). Panel E shows normal heart architecture with well-defined bundles of cardiomyocytes (CM), interstitial space (IS), coronary artery (CA) and cardiac veins. Sections of the heart taken from rats treated with the plant extract also showed normal architecture as well as active vascular congestion and vasodilatation (DC). The uterus taken from the control and treated groups (Panel F) revealed normal histological architecture with well-defined uterine cavity, surrounded by the endometrial membrane (EM) and endometrium containing the glands (EG) embedded in the stroma (ES). The endometrial membrane ulceration (MU) observed was not taken to be a toxic effect since it was also observed in the control group. It was thus considered to be artefactual (error introduced during tissue processing) and should be discountenanced.

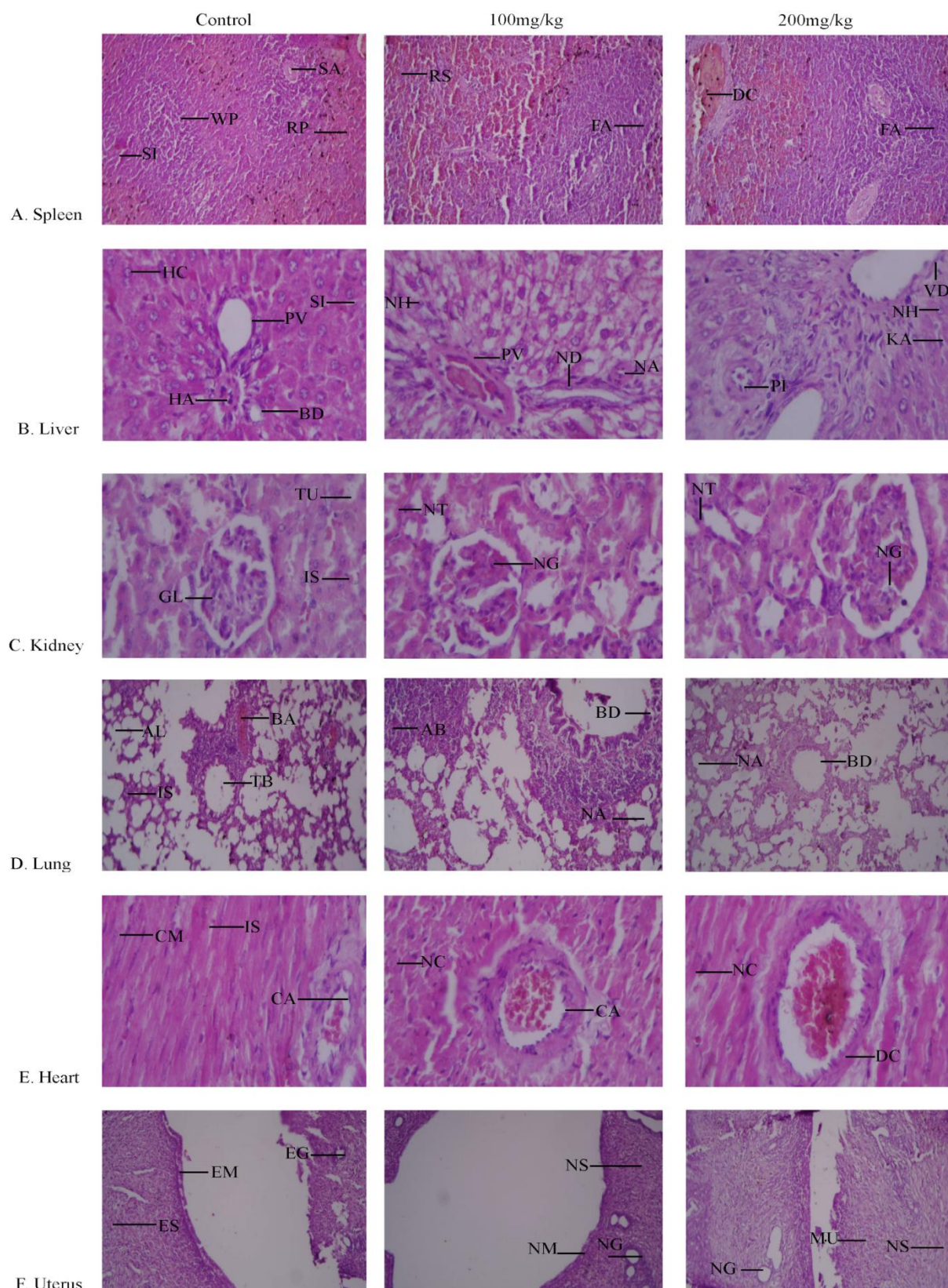


Figure 1: Histological Sections of Wistar Rat Organs Treated with *A. Africana* extract.

Total serum estradiol

Treatment with 100 mg/kg *A. africana* leaf extract significantly reduced serum estradiol level by 55.05% compared to the control

($p < 0.01$). A decrease of 16.25% in serum estradiol levels relative to the control ($p > 0.05$) was observed with 200 mg/kg extract (Figure 2).

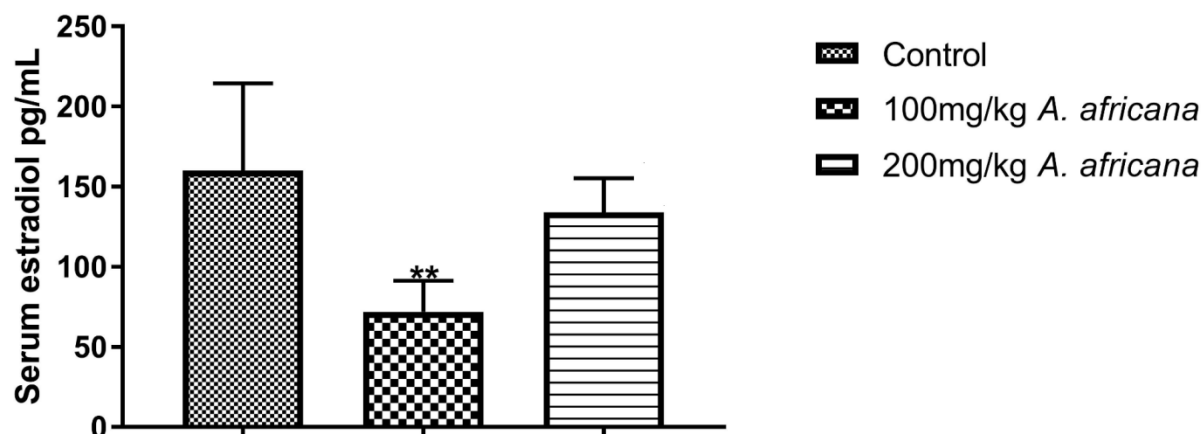


Figure 2: Effect of the ethanol extract of *A. africana* leaves on total estradiol in female Wistar rats compared with control group

Total serum progesterone

Treatment with 100 and 200 mg/kg of the extract exerted 51.85 and 32.44% decrease, respectively, in progesterone level (Figure 3) compared to the control ($p > 0.05$)

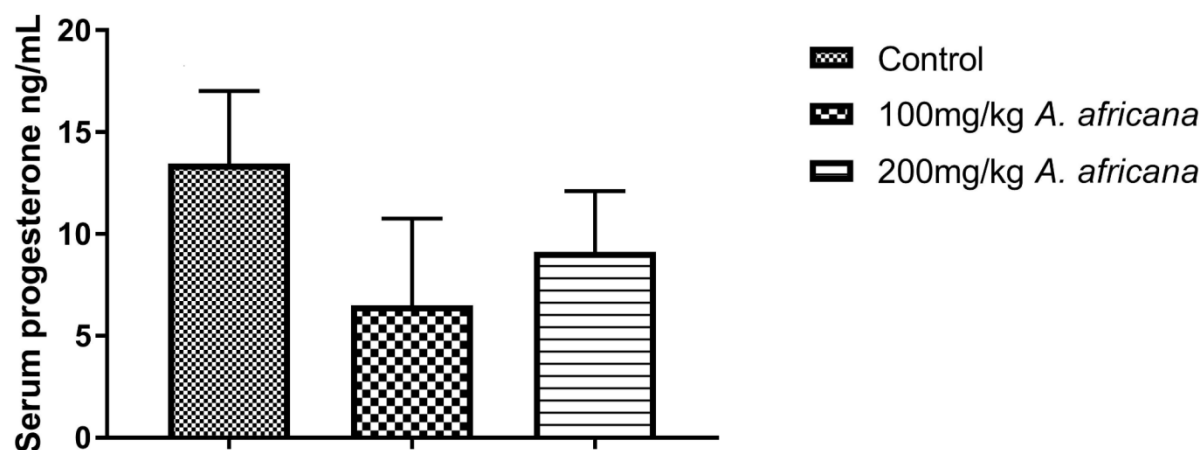


Figure 3: Effect of the ethanol extract of *A. africana* leaves on total progesterone level in female Wistar rats compared with control group

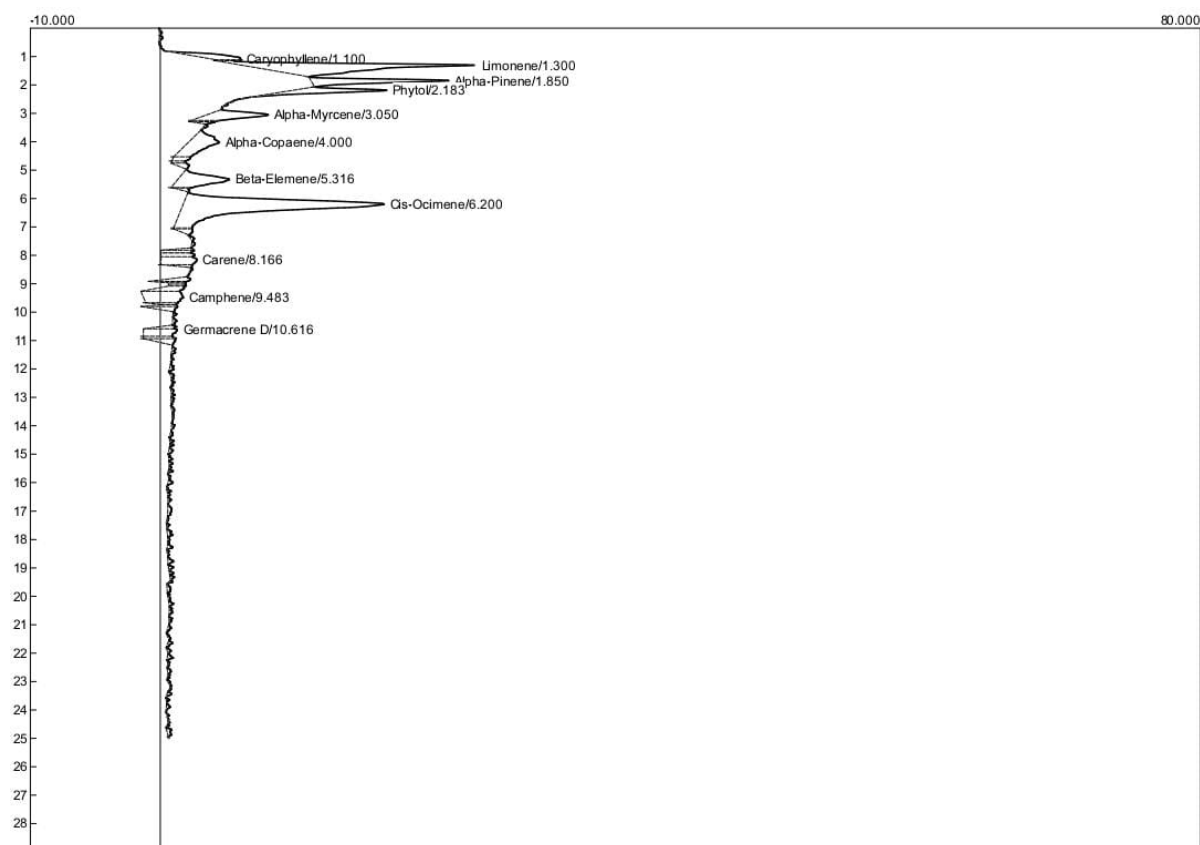
HPLC analysis result

HPLC analysis of the ethanol leaf extract of *A. africana* uncovered different constituents (Table 6). The main components were limonene, alpha-pinene, phytol, alpha-copaene, beta-

elemene, and cis-ocimene. Other constituents present but in smaller amounts were caryophyllene, alpha-myrcene and germacrene. Figure 3 depicts the HPLC chromatogram with the distinct peaks of the detected components.

Table 6: HPLC result of *A. africana* leaf ethanol extract showing the various individual concentrations of the constituents.

Components	Retention time (min)	Area	Height	Concentration (mg/g)
Caryophyllene	1.100	36.9560	2.077	0.0248
Limonene	1.300	247.0630	18.081	0.1656
Alpha -pinene	1.850	95.5140	10.666	0.0640
Phytol	2.183	73.1910	7.1770	0.0491
Alpha-myrcene	3.050	70.6485	4.7260	0.0474
Alpha-copaene	4.000	84.7555	2.3260	0.0568
Beta-elemene	5.316	86.7300	3.9500	0.0581
Cis-Ocimene	6.200	421.2310	15.4590	0.2824
Carcene	8.166	44.5470	2.7800	0.0299
Camphene	9.483	69.5660	3.0640	0.0466
Germacrene	10.616	38.7180	2.6230	0.0256

**Figure 4:** HPLC chromatogram of phytochemicals in ethanol extract of *A. africana* leaves

DISCUSSION

Adult female Wistar rats were treated with *A. africana* ethanol extract. The control group showed a constant weight gain from the beginning to the completion of the investigation however, the group receiving the extract showed significant reduction in average weekly weight gained on some of the days (Table I). This could be associated with a lot of factors. Natural products have been known to stimulate metabolic processes and enhance thermogenesis, which increases energy expenditure (Dekan *et al.*, 2022). They may also promote the browning of white adipose tissue, which is more metabolically active. Certain phytochemicals can enhance lipolysis and inhibit adipogenesis, thereby contributing to weight loss (Dekan *et al.*, 2022). *A. africana* has been reported to possess anti-obesity potential due to its effects on glucose and fat metabolism (Gang *et al.*, 2025).

LDL, total cholesterol and triglycerides are three primary lipids that are linked to cardiovascular disease (Zakaria *et al.*, 2020). The levels of these parameters were not significantly different ($p > 0.05$) from the control group, which is converse to other published studies (Arunsi *et al.*, 2020). This could have been as a result of the differences in doses administered to the rats. The kidneys' principal role is to eliminate toxic waste produced by regular bodily functions and delivered by the blood. Urea and creatinine are formed from proteins and through the process of lipid peroxidation. In fact, previous studies amply supports the capacity of plant extracts to function as potent free radical scavengers in the kidney, preventing the harmful effects of lipid peroxidation, which raises biochemical markers like creatinine and urea by rupturing membranes (Shaik Mohamed Sayed *et al.*, 2023). In this study, urea content was significantly reduced ($P \leq 0.01$) but creatinine and potassium levels were considerably raised ($P \leq 0.05$) (Table 3). Elevation in serum creatinine as obtained in this study could be attributed to altered renal functions associated with loss of normal excretory function of creatinine and muscular cell damage (Arunsi *et al.*, 2020). Significant elevation in potassium content was observed in

the result. Potassium is known to help in the regulation of the heart rate and muscle function.

Increased serum levels of biochemical indicators such as alanine aminotransferase and aspartate aminotransferase are linked to liver toxicity (Ogunro and Yakubu, 2021). These parameters were reduced, though not significantly, revealing the hepatoprotective capacity of the extract, which is contrary to what was reported by Arunsi *et al.* in 2020 with 250 and 500 mg/kg dose of the extract, which elevated these parameters. Blood indices are used to monitor the physiological and pathological state of the body (Ogunro and Yakubu, 2021), and the extract did not significantly alter these parameters.

The plant extract showed beneficial changes of bronchiolar dilation in the rats. The extract boosted the local immune system of the lungs (activation of the bronchiolo-alveolar lymphoid aggregates and mobilization of cells of the mononuclear phagocyte system). Sections of the heart taken from rats treated with graded doses of the extract also showed beneficial haemodynamic and vasogenic changes of increased blood circulation and vasodilatation. Arunsi *et al.*, in 2020, reported toxic changes in the kidney and liver, as well as alterations in serum biochemical markers, when 250 and 500 mg/kg doses of *A. africana* extract was given sub-acutely. The only sign of organ toxicity observed in this study was mild inflammation in the portal region of the liver. Thus, the extract at 100 and 200 mg/kg appeared to mostly preserve the normal organs architecture and have added beneficial effects.

This study examined the anti-fibroid potential of the leaves of *A. africana*, by examining its ability to reduce serum progesterone and estradiol levels in female Wistar rats. The progression and stability of uterine fibroids are regulated by a complex hormonal network involving estrogen and progesterone, as well as other mechanisms (Koyejo *et al.*, 2021). Gaining insight into the roles of these hormones is crucial for advancing effective therapeutic strategies for this prevalent

gynecological condition (Farris *et al.*, 2019). According to the result obtained from the experiment, significant reduction in estradiol level was observed at 100 mg/kg dose. Estradiol exerts its effects through estrogen receptors (ER α and ER β), particularly ER α , which enhances fibroid cell proliferation (Ahmed *et al.*, 2020). It also stimulates the production of collagen and growth factors that contribute to fibroid expansion (Ali *et al.*, 2019). Fibroids not only respond to circulating estrogen but also produce their own estrogen via aromatase-mediated conversion of androgens such as testosterone. A cholesterol-rich environment further enhances estrogenic activity within fibroid tissues (Ahmed *et al.*, 2020) therefore, weight reducing/lipid-lowering effects may indirectly impact estrogen metabolism by reducing cholesterol availability for steroidogenesis. Eze-Steven *et al.*, in 2019 reported the ameliorating effect of *A. africana* aqueous extract on monosodium glutamate-induced hyperplasia in the uterus of rats. Our study shows the likely mechanism through which the extract functions against fibroid. There was 51.85% reduction of progesterone content at 100 mg/kg. Drugs such as mifepristone are known for their antiprogesterone activity and are associated with beneficial effects such as decreased volume and symptoms of uterine fibroids (Koyejo *et al.*, 2021). Progesterone signaling is essential for the development and growth of fibroids, as it stimulates its cell proliferation by triggering various genetic and epigenetic signaling pathways (Ali *et al.*, 2023). The lowering of serum progesterone levels will impact on the effect of progesterone on fibroid cells as its lowering will consequently result in decrease in the expression of proliferating cell nuclear antigen (PCNA), which will in turn lead to a fall in both the number of fibroid cells and the overall tumor size. And same goes for other signaling pathways through which progesterone plays its role in uterine fibroid growth (Koyejo *et al.*, 2021). The extracellular matrix (ECM) in fibroids are aberrant and may help tumors develop and maintain themselves. Collagen is a prominent part of the extracellular matrix of fibroids and is necessary for their development and stability. Progesterone has been shown to

increase collagen synthesis in fibroid cells (Koyejo *et al.*, 2021).

A. africana leaves have been reported to possess tannins, phenols, alkaloids, flavonoids, terpenoids and saponins as dominant secondary metabolites (Obideje *et al.*, 2024). The phytoconstituents with the highest concentration in the extract were cis-ocimene, limonene, alpha-pinene, beta-elemene, alpha-copaene and phytol. These constituents have various pharmacological actions which include antioxidant and anti-inflammatory activities (Carvalho-Freitas and Costa, 2002), which are relevant mechanisms in fibroid treatment (Koyejo *et al.*, 2021).

CONCLUSION

The plant extract in the doses deployed in this study induced portal hepatitis in the liver and increased creatinine content, but no damage was observed in other selected tissues of the rats. It also lowered serum progesterone and estradiol in female Wistar rats to significant levels. The plant contains many bioactive components which may be responsible for its activity.

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CONFLICT OF INTEREST

Authors declare no conflict of interest.

AUTHORS' CONTRIBUTION

Imade, R.O. designed the study, Imade R. O. and Osayamwen, N. I. administered the extracts, Osayamwen, N. I. performed the statistical analysis and data interpretation, Imade R. O. wrote the paper, and the final manuscript was proofread and approved by both authors.

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