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SUBACUTE TOXICITY, HORMONAL EFFECTS AND HPLC PHYTOCHEMICAL ANALYSIS OF *SPONDIAS MOMBIN* LEAF EXTRACT IN FEMALE WISTAR RATS

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ABSTRACT

Uterine fibroids are benign growths in the uterus that commonly develop during a woman's reproductive years. Since estrogen and progesterone play a key role in fibroid development, researchers have explored herbal treatments that may help regulate these hormones. This study investigated the safety profile and effects of ethanol extract of *Spondias mombin* Linn (Anacardiaceae) leaves on serum estradiol and progesterone levels in female Wistar rats. Wistar 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 significantly alter adversely, any of the tested biochemical and hematological parameters. It induced minimal toxic damage to the selected tissues of the rats. The biochemical assay showed that *Spondias mombin* extract did not significantly ($P>0.05$) reduce serum estradiol levels. The doses of 100 and 200 mg/kg decreased serum progesterone by 39.52 % and 39.26 % respectively. HPLC analysis identified several bioactive compounds such as caryophyllene, quercetin, kaempferol, gallic acid, and β-sitosterol. This result shows that *Spondias mombin* leaves are relatively safe and suggests that they have potential antiprogestogenic activity that warrants further study.

Keywords: Estradiol, Fibroid, HPLC, Progesterone, Safety profile, *Spondias mombin*

INTRODUCTION

Uterine fibroids are non-cancerous tumors that develop in the uterine wall and are influenced by sex steroid hormones. Composed mainly of smooth muscle cells and extracellular matrix, they are among the most common tumors in women of reproductive age (Navarro, 2021). The estimated lifetime prevalence in premenopausal women varies between 40% and 89%, depending on factors such as detection methods, study population, and age group. Fibroid sizes can range from under 1 cm to over 20 cm (Marsh *et al.,* 2024). They can grow within the uterine wall, inside the uterine cavity, or on the outer surface of the uterus, leading to their classification as intramural, submucosal, or subserosal fibroids. Their size varies widely, ranging from as small as a pea to as large as a watermelon (Uimari *et al.*, 2021). The exact cause of uterine leiomyoma development is still uncertain. However, epidemiological studies provide strong evidence that estrogen and progesterone promote tumor growth, likely due to repeated menstrual cycles. This is supported by the fact that fibroids rarely appear before menarche and typically shrink after menopause (Navarro, 2021).

Several epidemiological factors contribute to the risk of developing uterine fibroids (UF), including age, race, early menstruation

onset, low or no pregnancy history, genetic factors, obesity, diet, physical activity levels, smoking, contraceptive use, hormone replacement therapy, and exposure to high levels of estrogen and progesterone in the environment (Navarro, 2021). Among these, age remains a consistent risk factor, with women aged 40–60 being 4 to 11 times more likely to develop UF than younger women. Furthermore, early menarche is associated with an increased risk, whereas having multiple pregnancies reduces the likelihood of UF by 20%–50% compared to women who have never given birth (Morhason-Bello and Adebamowo, 2022). Since many women with uterine fibroids are asymptomatic, they often receive minimal medical attention, and the condition may go undetected. However, those who do experience symptoms commonly report heavy menstrual bleeding, painful periods, pelvic pressure, an enlarged lower abdomen, frequent urination, discomfort during intercourse, lower back pain, pregnancy and labor complications, and fertility issues (Badawy *et al.,* 2022). The severity of these symptoms typically varies based on the size and location of the fibroids (Badawy *et al.,* 2022).

Treatment and prevention options for uterine fibroids are quite limited; conventional medications are expensive and often provide only short-term relief, with myomectomy or hysterectomy being the most commonly recommended procedures. This emphasizes the need for alternative treatment approaches (Arip *et al.,* 2022). The shortcomings of conventional medicine, combined with the accessibility, affordability, and cultural significance of herbal remedies, have sparked renewed interest in these natural alternatives (Arip *et al*., 2022). A study found that green tea extract (*Camellia sinensis* (L.) Kuntze, Theaceae) significantly reduced the total fibroid volume in patients who consumed the extract (Badawy *et al.*, 2022). Additionally, *Scutellaria barbata*, *Curcuma longa*, and bioactive compounds like resveratrol, curcumin, and anthocyanins have been shown to inhibit or reduce fibroid growth. They achieve this through mechanisms such as inducing cell-cycle arrest, triggering apoptosis by increasing reactive oxygen species (ROS) levels beyond cell survival thresholds, and decreasing extracellular matrix (ECM) proteins by restricting growth factor activity (Koyejo *et al.*, 2021). Additionally, morphological analysis showed that the entire *Labisia pumila* plant triggered apoptosis in SK-UT-1 uterine leiomyoma cells in a dose-dependent manner and inhibited the growth of uterine fibroid tumors (Zakaria *et al.,* 2020).

Similar to other well-known medicinal plants with multiple economic and ethnomedicinal benefits, *Spondias mombin* plays a vital role in herbal medicine, possessing various ethnopharmacological properties for treating numerous ailments in traditional practices across cultures. Different parts of the plant, including its leaves, flowers, fruits, seeds, stem bark, pulp, and roots, are used in folk medicine to manage conditions such as leprosy, severe cough, diarrhea, dysentery, indigestion, stomach pain, colic, constipation, hemorrhoids, gonorrhea, difficult childbirth, postpartum hemorrhage, inflammation and leiomyoma (Ogunro and Yakubu, 2021). Eze-Steven *et al* in 2019, reported that aqueous extract of the leaves of *S. mombin* provided protection to the uterine cells of female Wistar rats with monosodium glutamate-induced fibroid. Given that its leaves are used in traditional treatments for managing leiomyoma, a chronic condition, it is crucial to evaluate its safety, particularly with prolonged and repeated use.

HPLC is a chromatographic technique that can separate a mixture of compounds and is used to identify, quantify and purify the individual components of a mixture. It's used in characterization and quantification of secondary metabolites in plant extracts, mainly phenol compounds, steroids, flavonoids and alkaloids (Boligon and Athayde, 2014). This study therefore aimed to evaluate the safety profile of *Spondias mombin* Linn (Anacardiaceae), examine its subacute impact on steroid hormones associated with fibroid development and identify its components with High-Performance Liquid Chromatography (HPLC).

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, Acacia gum. All reagents utilized in this study were of analytical grade and sourced from reputable local suppliers.

Plant Collection, Identification and Preparation

Fresh leaves of *Spondias mombin* were harvested from the University of Benin campus, Benin City, Nigeria, on 30th August, 2024. The plant was identified by Prof. Henry Akinnibosun from the Department of Plant biology and biotechnology, Faculty of Life Sciences, University of Benin, and assigned the voucher number UBH-S345. The leaves were air-dried at room temperature, then ground into a fine powder using a milling machine. The powdered material underwent exhaustive extraction with 99.5% absolute ethanol at 65°C using the Soxhlet extraction method. The resulting extract was concentrated to dryness with a water bath maintained at 60°C.

Experimental Animals

Fifteen non-pregnant Wistar rats, each weighing between 100 and 200 grams were obtained from and acclimated at the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmacy, University of Benin, Benin City, Nigeria. The rats were housed individually in plastic cages under controlled room temperature and humidity, with free access to dry grower rodent pellets (Chikun feed) and water. They were divided into three groups (A, B, and C), with each group consisting of five rats. A wire screen was placed on top of the cages for ventilation, and wood shavings were used as bedding to collect urine and waste. In the handling of the animals, we adhered to the National Institute of Health Guidelines for the Care and Use of Laboratory Animals. All experimental procedures were approved by 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 *S. mombin* extract were administered to groups B and C. The dosage was determined based on a report by Porwal *et al.* (2020), where no toxicity was observed for up to 2000 mg/kg. The animals received the extract through an orogastric tube. Throughout the experiment, the animals were given a daily dose for twenty-eight days, with the volume adjusted weekly according to their recorded body weight. The animals were observed daily for any adverse reaction.

Biochemical Assays

On the 29th day, the animals were euthanized in a chloroform-saturated chamber, and blood samples collected via cardiac puncture. The blood was transferred

into both plain and EDTA bottles for 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. The blood samples collected into plain tubes were allowed to sit at room temperature for 45 minutes before being centrifuged at 3400 rpm for 10 minutes and serum stored at -25°C (Ezejiofor and Okoroafor, 2022).

Sub-acute Toxicity Test

The collected serum stored at −25°C was used for the lipid profile, renal and liver function tests. These parameters were assessed with an automated chemistry analyzer (Selectra Pro S, Germany). Electrolyte assay was by ion selective electrode (SFRI 4000, France). The parameters assayed include creatinine (Cr), urea (Ur), aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP), total bilirubin (Tb), conjugated bilirubin, triglycerides (TG), total cholesterol (T-CH), low-density lipoproteins (LDL), high-density lipoprotein (HDL) and serum electrolytes (Na^+, K^+, Cl^-, $HCO3^-$) (Ogunro and Yakubu, 2021).

Hematological Analysis

Additionally, hematological parameters were evaluated by utilizing an automated hematology analyzer on blood collected into EDTA bottles (Dymind 2000, China). The parameters analyzed include white blood cells (WBC), red blood cells (RBC), red blood cell distribution width (CV), red blood cell distribution width (SD), hemoglobin (Hb), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), granulocytes (GRAN), platelets count (PCT) and hematocrit (HCT) (Ezejiofor and Okoroafor, 2022).

Histopathology Analysis of Organs

For histological analysis, the liver, heart, kidney, spleen, lungs and uterus from the sacrificed animals were fixed in 10% neutral buffered formalin solution. These tissues were subsequently dehydrated in ascending grades of alcohol (70%, 90%, 96% and 100%), cleared in xylene, impregnated with molten paraffin wax and sectioned to slides. These sections (4–5 µm thick) were stained with hematoxylin after dewaxing with xylene and hydrating in descending grades of alcohol (100%, 96%, 70%) and water. Differentiation was done in 1% acid alcohol and the sections counter stained with eosin. Dehydration in ascending grades of alcohol was carried out again, and the sections cleared in xylene and mounted with dibutylphthalate polystyrene using cover slips prior to microscopic examination (Eze-Steven *et al*., 2019).

Assay of Serum Estradiol and Progesterone Levels

This was carried out using the Microplate Reader (Mindray MR-96A). E_2 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. The absorbance was read at 450 nm within 15 min of adding the stop solutions. The concentration of estradiol and progesterone in the samples were extrapolated from dose response curves (Lin *et al*., 2020).

HPLC Procedure

The HPLC analysis was carried out at Bato Chemical Laboratory in Lagos, Nigeria, using a Shimadzu Nexera MX system. Chromatography was performed on a uBondapak C_{18} reverse-phase column with dimensions of 100 mm in length, 4.6 mm internal diameter, and a particle size of 7 µm. The mobile phase consisted of

acetonitrile and water, with flow rates of 0.08 mL/min for water and 5 mL/min for acetonitrile. The system was equipped with a UV-Vis diode array detector set at 254 nm within the UV-visible spectrum, and the pump operated at a pressure of 15 MPa.

Initially, standard analytes were injected into the HPLC, generating a chromatogram with defined peak areas and profiles, which were used as a reference for analysis. Subsequently, an aliquot of the extracted test sample was injected to produce a corresponding chromatogram. The peak area of the test sample was then compared to that of the standard, using the standard's known concentration to determine the concentration of the sample (Zakaria *et al*., 2020).

Data Analysis

Results were presented as graphs and tables. Data analysis was performed by one-way analysis of variance (ANOVA), followed by the Tukey–Kramer post hoc test for multiple comparison using GraphPad Prism for Windows (version 7). Statistical significance was set at a P-value of ≤ 0.05.

RESULTS

Sub-acute Toxicity Results

Effect of *Spondias mombin* leaves extract on food consumption and body weight

In test animals, repeated dosing over a 28-day period did not result in mortality or any outward display of toxicity. Normal eating habits were observed and a gradual increase in weight was seen in both the control and test groups (Table I), though 200 mg/kg group was significantly lowered at day 21 and 28 ($P \leq 0.01$) compared to the test groups.

Table I: Change In Mean Body Weights of The Rats At Weekly Intervals of Extract Administration

	Change in mean body weights of rats (g)				
Dose (mg/kg)	**0**	**7**	**14**	**21**	**28**
Control	111.0 ± 9.54	13.0 ± 8.65	27.8 ± 9.81	25.6 ± 9.81	41.6 ± 7.97
100	144.4 ± 9.70	24.8 ± 9.69	35.4 ± 10.06	30.2 ± 10.08	28.6 ± 10.41[a]
200	176.8 ± 11.48	15.6 ± 5.76	14.6 ± 11.29	2.8 ± 10.02[b]	7.6 ± 8.47[b]

Data are expressed as mean ±SEM, n=5. a= $p < 0.05$, b=$p < 0.01$

Effect of *Spondias Mombin* Leaves Extract on Serum Biochemical Parameters

Effect on Lipid Profile Tests

All the lipid profile parameters obtained from the serum of the control and test groups at different doses were not significantly different ($p \geq 0.05$) (Table II).

Table II: **Lipid profile parameters after 28 days of *S. mombin* extract administration**

	Dose (mg/kg)		
Parameters	**Control**	**100**	**200**
T-CH (mg/dL)	82.20 ± 4.03	89.60 ± 4.48	89.00 ± 4.14
HDL (mg/dL)	25.40 ± 0.98	26.40 ± 0.98	25.60 ± 1.12
LDL (mg/dL)	36.60 ± 2.94	44.40 ± 3.31	45.80 ± 3.18
TG (mg/dL)	100.60 ± 2.89	94.00 ± 8.59	87.80 ± 3.68

Data are expressed as mean ± SEM, n=5. Total cholesterol (T-CH), high-density lipoprotein (HDL), low density lipoprotein (LDL), triglyceride (TG).

Effect on kidney function test parameters

For the kidney function tests, extract administration at all doses did not significantly alter the concentration of sodium, potassium, chloride and creatinine ($P \geq 0.05$) but urea content at 200 mg/kg was lowered ($P \leq 0.001$) significantly (Table III).

Table III: Kidney Function Test Parameters after 28 Days of *S. Mombin* Extract Administration

	Dose (mg/kg)		
Parameters	**Control**	**100**	**200**
Urea (mg/dL)	45.40 ± 2.25	49.00 ± 2.61	29.00 ± 1.00[c]
Creatinine (mg/dL)	0.68 ± 0.06	0.70 ± 0.03	0.72 ± 0.05
Na^+ (mmol/L)	140.60 ± 1.47	139.20 ± 1.39	141.5 ± 0.92
K^+ (mmol/L)	4.12 ± 0.10	4.30 ± 0.22	4.40 ± 0.17
HCO_3^- (mmol/L)	21.40 ± 0.28	21.60 ± 0.81	21.00 ± 0.84
Cl^- (mg/dL)	104.80 ± 0.73	105.00 ± 1.05	105.60 ± 0.75

Data are expressed as mean ± SEM, n=5. Letter c level of significance is $p<0.001$ compared to the control.

Effect on Liver Function Test Parameters

The liver function test parameters were not majorly altered by administration of the extract (Table IV) except for the alanine aminotransferase and aspartate aminotransferase levels which were seen to be significantly reduced ($P \leq 0.01$) at 100 mg/kg dose.

Table IV: Liver Function Test Parameters After 28 Days of *S. Mombin* Extract Administration

	Dose (mg/kg)		
Parameters	**Control**	**100**	**200**
AST (µ/L)	77.40 ± 4.27	57.00 ± 4.53[b]	75.00 ± 3.78
ALT (µ/L)	45.80 ± 2.42	33.40 ± 2.62[b]	44.60 ± 2.29
ALP (µ/L)	59.60 ± 3.54	49.80 ± 4.13	51.80 ± 3.50
Tb (mg/dL)	0.28 ± 0.02	0.28 ± 0.02	0.26 ± 0.02
Cb (mg/dL)	0.10 ± 0.00	0.10 ± 0.00	0.10 ± 0.00
Tp (g/dL)	6.34 ± 0.12	6.92 ± 0.11	6.26 ± 0.18
ALB (g/dL)	2.82 ± 0.02	3.00 ± 0.05	2.82 ± 0.02
GLo (g/dL)	3.52 ± 0.14	3.92 ± 0.10	3.42 ± 0.17

Data are expressed as mean ± SEM, n=5. Letter b level of significance is $p<0.01$ compared to the control. Alkaline phosphatase (ALP), alanine aminotransferase (ALT), Aspartate aminotransferase (AST), total bilirubin (Tb), conjugated bilirubin (Cb), total protein (Tp), albumin (ALB), globulin (GLo).

Effect of *Spondias mombin* leaves extract on hematological parameters

The results of the hematological parameters (Table V) revealed no significant effect ($P \geq 0.05$) on most of the hematological parameters tested. Only the red blood cell distribution width-coefficient of variation was observed to be elevated above normal range ($P \leq 0.01$).

Table V: **Results of Hematological Assay After 28 Days of *S. Mombin* Extract Administration**

	Dose (mg/kg)		
Parameters	**Control**	**100**	**200**
WBC (10^3/μL)	14.64 ± 1.50	15.68 ± 3.05	18.90 ± 1.14
LYM (%)	83.00 ± 1.66	80.48 ± 3.53	80.73 ± 3.53
MON (%)	2.06 ± 0.33	1.98 ± 0.30	1.80 ± 0.19
NEU (%)	10.56 ± 1.65	12.56 ± 2.31	12.15 ± 2.80
EOS (%)	0.84 ± 0.37	0.42 ± 0.12	0.92 ± 0.46
BAS (%)	3.50 ± 0.42	4.56 ± 1.29	4.20 ± 0.73
RBC (10^6/μL)	6.38 ± 0.24	6.69 ± 0.24	6.56 ± 0.06
HGB (g/dL)	13.50 ± 0.51	14.40 ± 0.35	14.00 ± 0.35
HCT (%)	40.20 ± 1.56	42.60 ± 0.68	41.00 ± 1.05
MCV (μm^3)	50.28 ± 0.42	50.42 ± 0.45	49.90 ± 1.05
MCH (ρg)	21.16 ± 0.10	21.56 ± 0.27	21.32 ± 0.39
MCHC (g/dL)	42.12 ± 0.37	42.80 ± 0.28	42.78 ± 0.37
RDW-CV (%)	16.28 ± 0.88	15.64 ± 0.42	13.83 ± 0.36^b
RDWSD (μm^3)	26.96 ± 1.65	25.86 ± 0.72	24.32 ± 0.37
PLT (10^3/μL)	741.20 ± 98.38	618.80 ± 18.56	658.00 ± 38.70
MPV (μm^3)	6.54 ± 0.27	6.58 ± 0.09	6.50 ± 0.11
PCT (%)	0.49 ± 0.08	0.41 ± 0.01	0.43 ± 0.02
PDW (%)	24.98 ± 1.43	26.28 ± 1.91	26.02 ± 2.84
P-LCR (%)	5.96 ± 2.29	5.40 ± 0.61	4.68 ± 0.54

Data are expressed as mean ± SEM, n = 5. Letter b level of significance is $p<0.01$ compared to the control.

Histopathology Result

Panel A in Figure 1 shows normal heart sections which revealed well-defined bundles of myocardiac fibres (CM), interstitial space (IS), coronary arteries (CA) and cardiac veins. Sections taken from rats administered the extract showed observable toxic effect of vascular ulceration (AU).

Panel B shows the liver with well-defined hepatocytes (HC), sinusoids (SI) and portal triad (hepatic portal vein (PV), artery (HA) and bile duct (BD)). The same architecture was observed in sections taken from rats treated with 100 mg dose of *S. mombin.* In addition, 200 mg/kg activated the sinusoidal Kupffer cells (KA) and showed normal hepatocytes (HN) with conspicuous nucleoli (HN) as well as vasodilatation and active congestion (DC).

For the kidney, the control as well as sections taken from rats treated with the extract showed normal histological architecture, with well-defined tubules (TU), glomeruli (GL), interstitial space (IS) and arcuate blood vessels (Panel C).

Panel D shows normal spleen architecture with well-defined splenic arterioles (SA), white pulp (WP) (comprising the lymphoid follicles), red pulp (RP), and the splenic sinuses (SI), which constitute the lymphatic channels. Sections treated with graded doses of the plant extract showed varying degrees of activation of the lymphoid follicles (FA) and increase in the sequestration of red blood cells in the splenic red pulp (RS).

Panel E shows normal lung architecture with well-defined alveolar sacs (AL), interstitial space (IS), bronchioles (TB) and bronchial blood vessels (BA). Sections taken from rats given graded doses of the extract showed normal alveolar sacs. An added beneficial change of bronchiolar dilation (BD) in the rats was also observed. Activation of the bronchiolo-alveolar lymphoid aggregates (AB) and mobilization of cells of the mononuclear phagocyte system were also

observed (AM). In addition, the extract group revealed bronchiolar ulceration (BU). Panel F shows normal uterus architecture with well-defined uterine cavity, surrounded by the endometrial membrane (EM) and endometrium containing the glands (EG) embedded in the stroma (ES). The extract group revealed normal cell structures (SI).

Result of hormonal assay

Total serum estradiol

There was no significant decrease in estradiol levels of rats administered *Spondias mombin* extract in comparison with the control ($p \geq 0.05$). The control group's estradiol level was 160±24.38 pg/mL, while 100 and 200 mg/kg groups produced 107±6.86 and 158.1±22.20 pg/mL, resulting in 33.13 and 1.19% reductions respectively (Figure 2).

Total serum progesterone

There was a reduction in progesterone levels (Figure 3) in rats administered *S. mombin* extract. The control group's progesterone level was 13.46±1.59 ng/mL, while 100 and 200 mg/kg groups gave 8.14±2.00 and 8.18±1.54 ng/mL, resulting in 39.52 and 39.26% reduction in progesterone respectively ($p \geq 0.05$).

HPLC analysis result

HPLC analysis of *S. mombin* leaves ethanol extract identified caryophyllene, chlorogenic acid, quercetin, kaempferol, rutin, rhamnetin, and isorhamnetin as the predominant compounds. Additionally, smaller quantities of gallic acid, ellagic acid, catechin, allicin, kolatin, β-sitosterol, isoquercitrin, lupeol, humulene, cardinine, geranin, and astragalin were detected (Table VI). The HPLC chromatogram displaying the peaks corresponding to these compounds is shown in Figure 4.

DISCUSSION

Adult female Wistar rats were treated with graded doses (100mg and 200mg/kg body weight) of *S. mombin* extract. The reduced body weight of test animals in comparison with the control could be associated with a lot of factors. Research has it that some natural products can stimulate metabolic processes and enhance thermogenesis, which increases energy expenditure. 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 (Shaik Mohammed Sayed *et al,* 2023). *S. mombin* have been reported to possess anti-obesity potential through some of these mechanisms (Wang *et al.*, 2019).

LDL, total cholesterol and triglycerides are three primary components of the lipid profile that are linked to cardiovascular diseases (Zakaria *et al*., 2020). The levels of these parameters were not significantly different ($p > 0.05$) from the control group, which is in tandem with other published studies. The primary function of the kidneys is to remove harmful waste produced by the normal functioning of the body and transported by the blood (Taychaworaditsakul *et al*., 2024). In fact, additional research amply supports the capacity of plant extracts to function as potent free radical scavengers in the kidney and prevent 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, all the kidney function test parameters were within the normal range. Urea content was further lowered pointing to the protective ability of the plant.

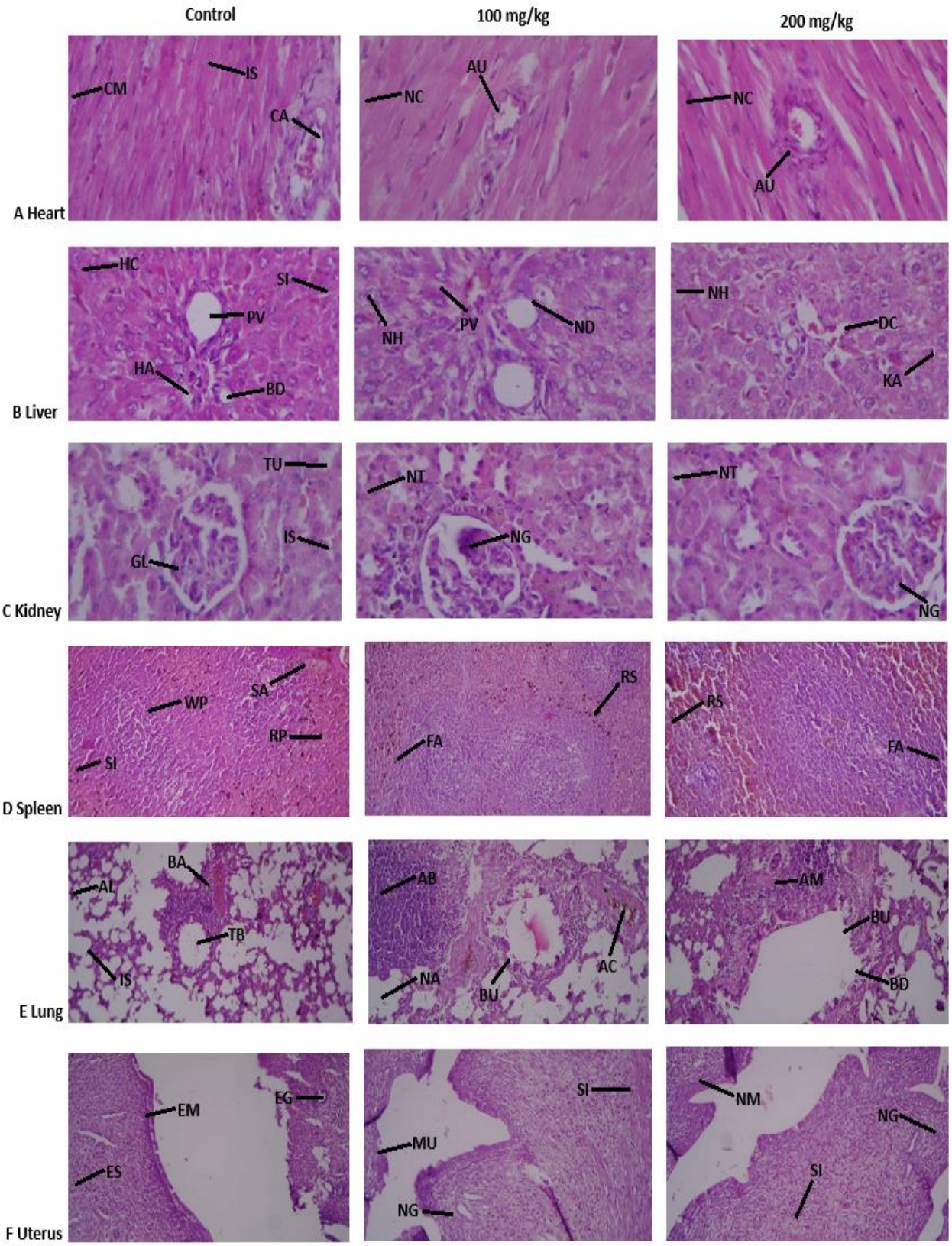

Plate 1: H&E 400 X, Wistar Rat organ sections

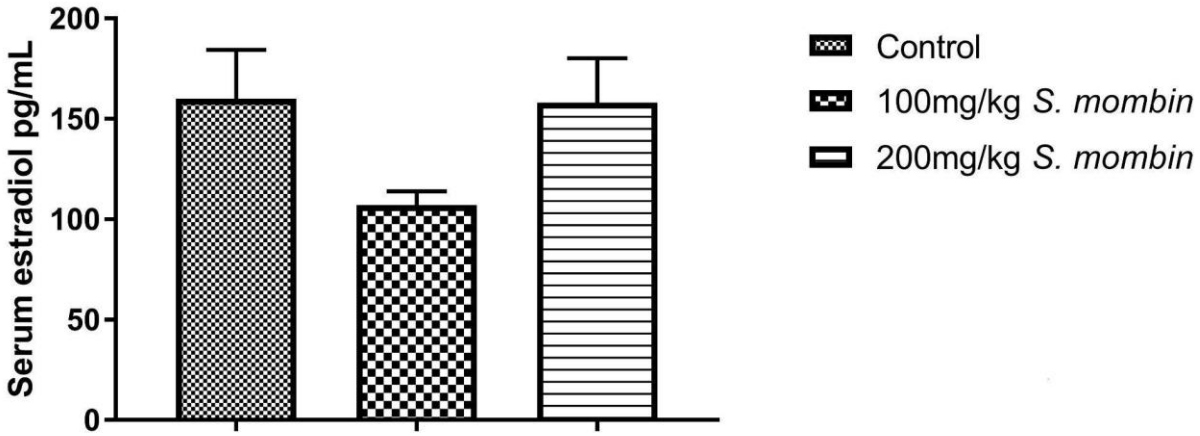

Figure 2: Effect of *S. mombin* leaves ethanol extract on serum estradiol in female Wistar rats.

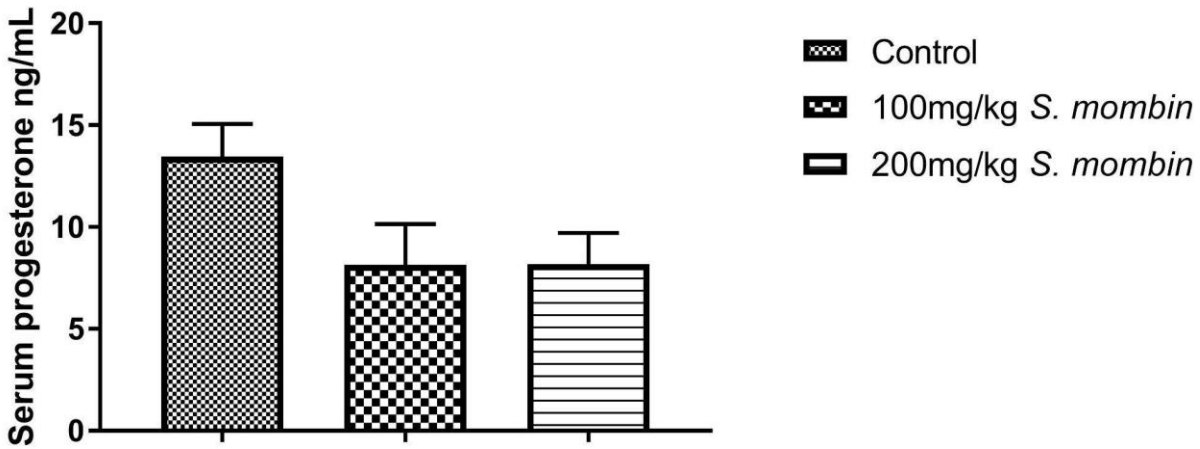

Figure 3: Effect of *S. mombin* leaves ethanol extract on serum progesterone in female Wistar rats.

Table VI: HPLC Analysis of *S. mombin* Leaves Ethanol Extract

Component	Retention	Area	Height	Concentration (mg/g)
Caryophyllene	1.266	610.2830	23.413	0.42
Chlorogenic Acid	2.750	1422.0485	16.960	0.97
Gallic Acid	4.450	282.2360	5.778	0.19
Ellagic Acid	5.466	92.2670	2.116	0.06
Catechin	6.500	81.1300	8.408	0.06
Allicin	7.333	81.9950	5.385	0.06
Kolatin	7.950	102.3055	5.087	0.07
B-Sitosterol	8.416	82.3060	5.048	0.06
Isoquercetin	9.350	69.3030	6.499	0.05
Quercetin	11.050	8228.2380	150.738	5.61
Kaempferol	12.166	2592.5880	52.347	1.77
Rutin	13.700	985.4310	23.542	0.67
Rhamnetin	15.716	152.6880	6.464	0.10
Isorhamnetin	17.616	435.2880	7.511	0.30
Lupeol	18.900	65.2410	5.403	0.04
Humulene	20.233	136.4740	12.774	0.10
Cadinene	21.300	81.9340	6.247	0.06
Geranin	22.466	109.0890	9.204	0.07
Astragalin	24.133	138.0130	6.256	0.09
Total		15748.8580		10.73

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 significantly reduced in relation to the control at 100 mg/kg, thereby revealing the hepatoprotective capacity of the extract at this dose.

S. mombin leaves has been reported to possess tannins, steroids, terpenoids, alkaloids, flavonoids, and saponins as dominant secondary metabolites (Ogunro *et al*., 2023). The protective effect of the extract could be explained by the antioxidant potential of its flavonoid and phenolic constituents.

Blood indices are used to monitor the physiological and pathological state of the body. The extract did not significantly alter these parameters except to lower the red blood cell distribution width-coefficient of variation.

The plant extract boosted the activity of the local immune system of the spleen and caused increase in trapping and storage of erythrocytes, as well as destruction and excretion of affected red blood cells. In the liver, the extract showed additional beneficial haemodynamic and vasoactive effects of increased blood circulation and vasodilatation. It also boosted the local immune system of the liver, by activating the sinusoidal Kupffer cells. Thus, the extract appeared to preserve the normal liver architecture and have some added beneficial effects.

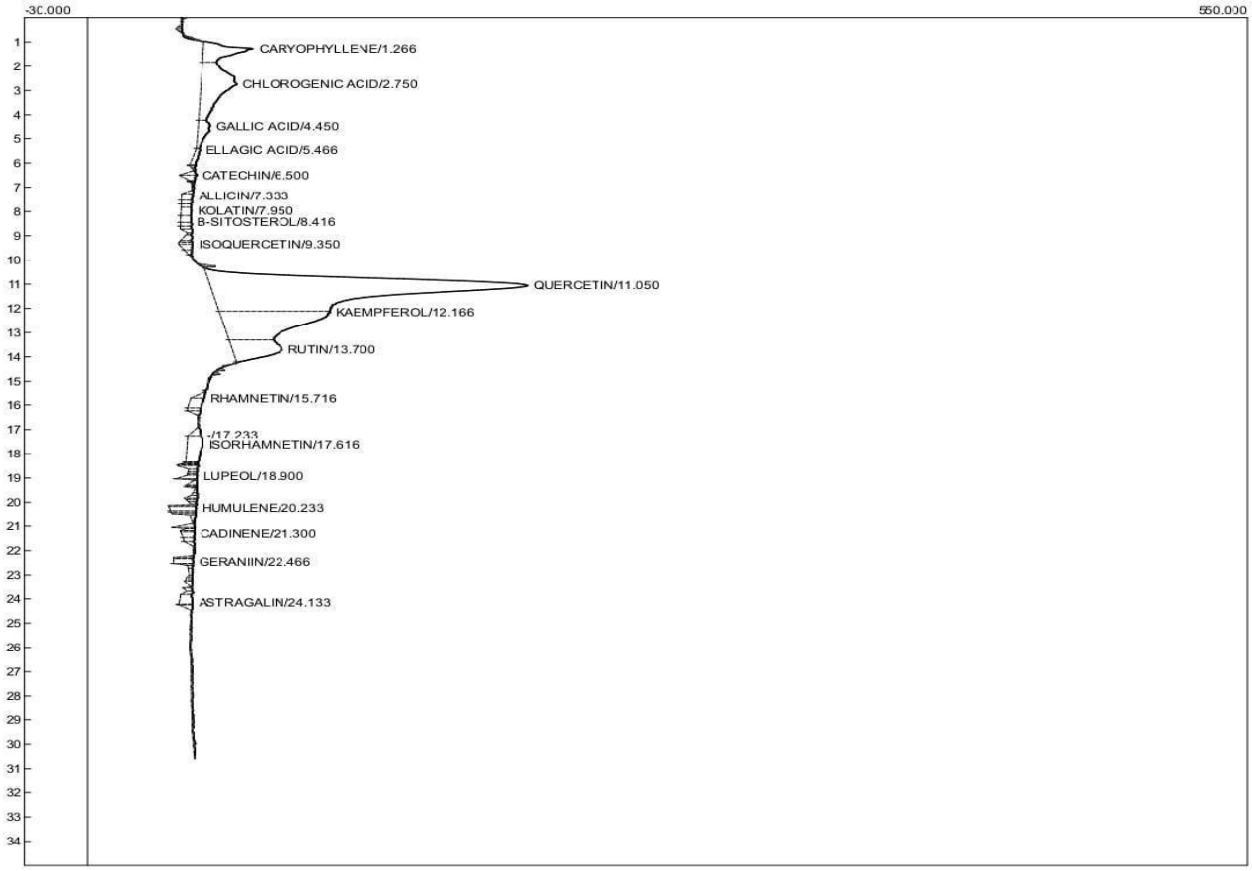

Figure 4: HPLC chromatogram of compounds in ethanol extract of *Spondias mombin* leaves

It further boosted the local immune system of the lungs through activation of the bronchiolo-alveolar lymphoid aggregates and mobilization of cells of the mononuclear phagocyte system. Toxic damage induced manifested as vascular ulceration in the heart and bronchiolar ulceration in the lungs. Conversely, the methanol extract of this plant, according to Porwal *et al*., (2020) did not reveal any alteration in the histopathology of the organs. This may be explained by the different chemicals that the extracting solvents produce.

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).

The ethanol extract of *Spondias mombin* leaves at the concentrations used did not result in significant lowering of the steroidal hormones. Eze-Steven *et al* in 2019, reported that aqueous extract of the leaves of *S. mombin* provided protection to the uterine cells of female Wistar rats with monosodium glutamate-induced fibroid. Mechanisms through which plant phytochemicals carry out their anti-fibroid actions include hormonal modulation, antioxidant activity, reduced growth factors, induced apoptosis etc (Koyejo *et al*., 2021).

The inability of the plant extract in this study to cause a significant decrease in levels of both serum estradiol and progesterone could be as a result of the doses used. The HPLC analysis of *Spondias*

mombin revealed several bioactive constituents including chlorogenic acid, quercetin, kaempferol, and ellagic acid.

Chlorogenic acid, a polyphenol has been documented to have antioxidant, anticancer, anti-tumor, anti-inflammatory, hepatoprotective and anti-obesity properties (Nguyen *et al,* 2024; Huang *et al*, 2023). Ellagic acid has also been reported to possess anti-obesity activity as it enhances the conversion of white adipose tissues to brown and inhibits genes responsible for white adipocyte maintenance in rats with high-fat diet-induced obesity (Wang *et al,* 2019). The statistically significant reduction in body weight of the test groups compared to the control could be attributed to the activities of these constituents. Quercetin and kaempferol are flavonoids also reported to have anti-inflammatory, anticancer, and antioxidant activities (Wang *et al,* 2022; Bangar *et al,* 2023). Additionally, quercetin has been reported to reduce serum estradiol. It has also been suggested that kaempferol might regulate a suitable level of estrogenic activity in the body and as such, is expected to have potential beneficial effects in preventing estrogen imbalance diseases (Oh *et al*., 2006; Bergsten *et al*., 2023). While specific phytochemicals were identified in this study, they constitute just a fraction of the total extract, and the biological activity observed could be due to the synergy between these constituents and other unidentified constituents.

CONCLUSION

Spondias mombin leaves extract in the doses deployed in this study induced minimal toxic damage to the selected tissues of the rats. There were some beneficial effects such as boosting of the local immune system, increase in the blood circulation and vasodilatation. The extract lowered serum estradiol and progesterone levels in female Wistar rats though not to a statistically significant level. The plant contains many bioactive components, chiefly quercetin and kaempferol, which may be responsible for its activity.

Conflict of interest

The authors have no competing interests.

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