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Eluehike N

Department of Medical Biochemistry,
School of Basic Medical Sciences,
University of Benin, Benin City, Edo
State, Nigeria

Ikponmwosa Eweka

Department of Medical Biochemistry,
School of Basic Medical Sciences,
University of Benin, Benin City, Edo
State, Nigeria

Onoagbe IO

Department of Medical Biochemistry,
School of Basic Medical Sciences,
University of Benin, Benin City, Edo
State, Nigeria

Correspondence:

Eluehike N

Department of Medical Biochemistry,
School of Basic Medical Sciences,
University of Benin, Benin City, Edo
State, Nigeria
Email: nkeiruka.ezeugwu@uniben.edu

Safety assessment of aqueous and ethanol extract of *Spondias mombin* leaf: Biochemical and histopathological study

Eluehike N, Ikponmwosa Eweka, Onoagbe IO

ABSTRACT

Background: *Spondias mombin* leaf is a medicinal plant used in the treatment and management of various disease conditions. However, there are limited reports on its toxicity effects. **Objectives:** The present study evaluated the acute and sub chronic toxicity effects of aqueous and ethanol extract of *Spondias mombin* leaves. **Materials and Methods:** For the first phase of the acute toxicity study, 18 male wistar rats grouped into three were administered single doses of 10, 100 and 1000 mg/kg body weights of the extracts respectively, they were observed for 24 h for signs of toxicity. In the second phase, six rats were grouped into three consisting of one rat each and they received extracts of doses 1600, 2900 and 5000 mg/kg body weight orally then observed for 24 h for behavioral changes as well as mortality. For the sub chronic studies 60 male wistar rats arranged into six groups each for both extracts consisting of the control group and the varying doses (200, 500, 1000, 3000 and 5000 mg/kg body weight) groups. They were administered extracts for 28 days. Blood samples collected were used to determine the hepatic, renal, cardiac and hematological changes. Also, histopathological analysis was determined on the liver, heart and kidneys of the animals. **Results:** The result of acute toxicity study revealed no mortality neither was any physiological or behavioral changes observed. Sub chronic studies showed non-significant changes in the renal and cardiac parameters, significant ($p \leq 0.05$) increase in the red blood cell, hematocrit and hemoglobin concentration at the higher doses treated with both extracts and significant alterations of some hepatic parameters at the lower doses. Mild to normal histological changes were observed in the liver, heart and kidney tissues of the extracts treated rats. **Conclusion:** The study has therefore shown that aqueous and ethanol extracts of *Spondias mombin* leaf orally did not cause abnormal alterations in the blood parameters but resulted in slight histological alterations to the tissues; as a result, care must be used while using this plant's extracts.

Keywords: Acute toxicity, Biochemical parameters, Sub-chronic toxicity, *Spondias mombin*, Histology.

INTRODUCTION

Since time immemorial, man has relied heavily on the use of plants and natural products for the treatment of a variety of disease condition. Approximately 75% of people worldwide, according to WHO estimates, depend on herbs to meet their medical needs.^[1] According to estimates, between 70 and 80 percent of patients in Africa receive treatment through traditional medicine.^[2] The perception that natural substances don't have major negative effects is one of the primary causes of people's increasing interest in herbal products. Nevertheless, toxicological and pharmacological data indicate that this is a false belief. However, unless otherwise demonstrated in a lab, all medicinal plants and natural products have the potential to be harmful regardless of their source. Furthermore, to ensure that the usage of these plants is acceptable and safe, systematic toxicity studies—particularly pharmacological activity studies—must be carried out^[3]. Studies have revealed the toxicity of medicinal herbs in the biological system,^[4-6] which may be acute or chronic in character, despite their therapeutic approach. This may explain why the WHO urged an assessment of the quality, safety, and effectiveness of herbal medicines in 2002.^[7] The initial action in determining a substance's adverse effects within 14 days following a single administration is to conduct an acute toxicity assessment.^[8, 9] In order to determine the median lethal dose (LD₅₀) of a particular hazardous substance in laboratory animals, such as rats or mice, it is usually administered via oral ingestion.^[10] Sub-chronic toxicity testing involves exposing animals to a substance for a period ranging from 28 to 90 days, with the aim of identifying any adverse reverse that could result from prolong exposure.

Spondias mombin is referred to as hog plum in English, akika in Yoruba, ijikara in Igbo, tsardarmaser in Hausa, chabbuh in Fulani and nsukakara in Efik.^[11] Aqueous extracts of *S. mombin* have been shown to have a number of pharmacological actions, including antiviral properties,^[12] leishmanicidal.^[13]

anti-inflammatory.^[14]

Some scientific reports are available to show the toxicological evaluation of various parts of the plant *Spondias mombin*. Okonkwo et al.^[15], Asuquo et al.^[16], Nwaogwugwu et al.^[17], Abiodun et al.^[18] and Mayur et al.^[19] have all reported on toxicological effects of this plant. Although none of this study gave a detailed report of the toxicological effects using varying doses.

We have reported on several beneficial effects of the leaves of *Spondias mombin*^[20-23]. Our interest in this plant has necessitated a proper documentation of the safety profile of the leaves of *Spondias mombin*. Hence the present study was done to investigate the acute and sub chronic toxicological effects of aqueous and ethanol extracts of the leaves of *Spondias mombin* using varying doses.

MATERIALS AND METHODS

Collection, Identification and Authentication of Plant materials

The leaves of *Spondias mombin* were obtained from fields in the University of Benin campus, Ugbowo, Benin City, Edo State. The fresh leaves were identified and authenticated in the Department of Plant Biology and Biotechnology of the University of Benin, Benin City, Nigeria by Prof. H.A Akinnibosun. Specimen was deposited in the University of Benin Herbarium with voucher no UBHs 345.

Preparation of plant extract

Precisely 6 kg shade dried leaves were pulverized and then 3kg of the ground leaves was soaked in distilled water (aqueous extract) and the other 3 kg in ethanol (ethanol extract) for 72 h (Onoagbe *et al.*,^[24]). The contents were stirred several times a day and at the end of the third day, was filtered using filter paper and the filtrate concentrated using a rotary evaporator. The concentrated extract was weighed and stored in an air-tight container and kept in the refrigerator maintained at 4°C until use.

Experimental animals

A total of one hundred and two (102) male albino rats weighing 130-180 g were used for this study. The rats were obtained from the animal house, Department of Biochemistry, University of Benin. The animals were housed in galvanized rat cages and acclimatized for two weeks on guinea growers mash (Bendel Feed and Flour Mill, Ltd, Ewu, Nigeria) and allowed free access to drinking water. Treatment of the animals was in accordance with the principle of Laboratory Animal Care.^[25] Ethical approval was obtained from the ethical board of the College of Medical Sciences, University of Benin with reference number (CMS/2023/490).

Acute toxicity studies

An acute toxicity study was carried out using the method of Lorke^[26], which involves two phases. In the first phase, nine rats were divided into three groups, each receiving a single oral dose of 10, 100, or 1000 mg/kg body weight of the extract. The animals were observed for 24 hours for signs of toxicity. In the second phase, twelve rats were divided into four groups, each consisting of a single rat. They were administered oral doses of 1600, 2900, and 5000 mg/kg body weight of the extract and observed for 24 hours for behavioral changes and mortality. The median lethal dose (LD₅₀) was calculated based on the results from the second phase.

Then the LD₅₀ is calculated by the formula:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

D₀ = Highest dose that gave no mortality,

D₁₀₀ = Lowest dose that produced mortality.

Sub-chronic toxicity study

A total of 60 (sixty) male albino Wistar Rats were divided into twelve groups of five rats each. Six groups (for the ethanol extract) while the other six groups (for the aqueous extract). The extracts (aqueous and ethanol) were administered orally. Doses of 200, 500, 1000, 3000 and 5000 mg/kg body weight of the aqueous and ethanol extracts were administered daily by oral means using the oro-gastric tube (gavage) to the rats for 28 days. The rats were grouped as follows:

Group A was the normal control

Group B received 200 mg/kg body weight of either extract

Group C received 500 mg/kg body weight of either extract

Group D received 1000 mg/kg body weight of either extract

Group E received 3000 mg/kg body weight of either extract

Group F received 5000 mg/kg body weight of either extract

After 28 days, all surviving animals was fasted overnight and sacrificed under chloroform anesthesia. Blood samples were collected through cardiac puncture into lithium heparinized containers and EDTA containers for biochemical analysis and hematological parameters determination respectively (using automated hematology analyzer -Sysmex America Inc, USA). The heart, liver and kidneys were excised out. The tissues were fixed in 10 % buffered formalin for histopathological examinations

Weekly body weight and Absolute organ weight

The body weight of each rat was assessed using a sensitive balance during acclimatization period, then on day 28th. The different organs viz: liver, heart and kidney were dissected out and weighed to get the absolute organ weight.

Biochemical assays

The Method of Roy^[27], and Reitman and Frankel,^[28] was used to determine alkaline phosphatase, alanine transferase and aspartate transferase activities. Total protein, albumin, creatinine, and urea were measured according to the procedure of Gornall *et al.*^[29], Doumas and Biggs.^[30], Henry^[31] and the Urease-Berthelot.^[32] respectively. Sodium, chloride, bicarbonate and potassium ions were determined using the electrolyte auto-analyser (Sysmex America Inc., USA). Plasma LDH activities was determined using the procedure of Amador *et al.*,^[33] while creatine kinase activity was determined by the IFCC^[34]

Statistical Analysis

Data were expressed as mean ± SEM. The results were computed statistically using Statistical Package for Social Sciences (SPSS), version 17. Analysis of variance and Tukey's multiple comparison tests was used to compare all treatment groups. Values of p < 0.05 was taken as significant.

RESULT

Acute Toxicity Study

The acute toxicity study showed no deaths in rats at any of the tested doses (10 to 5000 mg/kg) for both aqueous and ethanol extracts of *Spondias mombin* leaves (Table 1). The mortality ratio was 0 for all groups, indicating the extracts were non-toxic at these levels. Therefore, the oral LD₅₀ (median lethal dose) is greater than 5000 mg/kg body weight, suggesting a high safety margin.

Sub chronic Toxicity Study

Organ and Body weight measurement of rats treated with aqueous and ethanol extracts of leaves of *Spondias mombin*

A significant increase ($p \leq 0.05$) was observed in the body weight of the rats administered doses of 200-5000 mg/kg body weight of aqueous and ethanol leaves extract *Spondias mombin* (Table 2). However, there were no significant differences ($p \geq 0.05$) in the absolute organ weight in both extract group (Table 3).

Indices of liver function in rats administered with extracts of *Spondias mombin* leaves

Aside the significant reduction in ALP concentration for the ethanol extract groups administered 200, 500 and 1000 mg/kg body weight and the significant reduction in the AST activity for the aqueous extract groups administered 200, 500 mg/kg body weight, no significant differences ($p \geq 0.05$) were observed in the liver function parameters (ALT, AST, ALP, TP, and ALB) particularly in the high doses administered groups for both extract (Tables 4a and 4b).

Concentration of hematological parameters in rats administered with extracts of *Spondias mombin* leaves

Significant increase ($p \leq 0.05$) in red blood cell (RBC), hematocrit (HCT) and hemoglobin (HB) concentration was observed in the groups exposed to high doses (1000-5000 mg/kg body weight) of ethanol and aqueous extract. (Table 5a and 5b).

Indices of Kidney Function

No significant differences were observed in the plasma concentration of creatinine, urea, and electrolytes in rats administered aqueous and ethanol extract of *Spondias mombin* (table 6, 7a and 7b).

Indices of Cardiac Function

Administration of aqueous and ethanol extracts of *Spondias mombin* leaves did not cause any significant change in the cardiac function enzymes lactate dehydrogenase (LDH) and creatine kinase (CK) (Tables 8).

Histological results

Figures 1a–f shows photomicrographs of liver sections from rats administered ethanol extract of *Spondias mombin* at doses of 200, 500, 1000, 3000, and 5000 mg/kg body weight, along with the normal control. Similarly, Figures 2a–f present liver sections from rats treated with the aqueous extract at the same dose levels and the control group. Figures 3a–f depicts photomicrographs of heart sections from rats administered ethanol extract, while Figures 4a–f shows heart sections from those treated with aqueous extract, both at the same dose range and including controls. Figures 5a–f illustrates kidney sections from rats given ethanol extract, and Figures 6a–f represents kidney sections from those treated with the aqueous extract, all at doses of 200, 500, 1000, 3000, and 5000 mg/kg body weight, including the normal control.

Table 1: Acute toxicity study of aqueous and ethanol extracts of leaves of *Spondias mombin*

Dose (mg/kg body weight)	No of rats	No of deaths	Ratio of mortality
10	3	0	0/3
100	3	0	0/3
1000	3	0	0/3
1600	1	0	0/1
2900	1	0	0/1
5000	3	0	0/1

Data are number of death and ratio of mortality of rats. No of death recorded = Nil
Mortality ratio= No of death/ Initial no of animals. The oral LD₅₀ of each extract was greater than 5000 mg/kg body weight.

Table 2: Body weight of rats treated with aqueous and ethanol extracts of leaves of *Spondias mombin*

Groups	Time	Body weight of rats (g)	
		Aqueous	Ethanol
Control	Day 0	140.56±2.47	152.4±1.98
	Day 28	142.45±1.60	152.5±2.05
200 mg/kg bwt	Day 0	140.05±0.70	150.8±2.80
	Day 28	150.49±0.90*	162.5±2.81*
500 mg/kg bwt	Day 0	136.02±2.00	148.5±2.05
	Day 28	169.35±0.30*	160.0±2.0*
1000 mg/kg bwt	Day 0	137.50±2.40	150.5±0.96
	Day 28	179.42±1.28*	157.7±1.80*
3000 mg/kg bwt	Day 0	140.02±0.70	151.5±0.05
	Day 28	151.09±2.00*	162.5±1.80*
5000 mg/kg bwt	Day 0	137.60±2.50	148.8±1.65
	Day 28	162.10±2.30*	156.5±1.20*

Values represent the body weight of rats administered extracts for 28 days and are the means ± SEM (n=5). * $p \leq 0.05$ when compared with values at day 0. bwt: Body weight.

Table 3: Organ weight of rats treated with aqueous and ethanol extracts of leaves of *Spondias mombin*

Treatment (aqueous and ethanol extract)	Organ weight of rats (g)					
	Liver		Heart		Kidney	
	Aqueous	Ethanol	Aqueous	Ethanol	Aqueous	Ethanol
Control	5.16±0.15	3.85±0.22	0.61±0.15	0.59±0.22	0.99±0.15	1.09±0.02
200 mg/kg bwt	4.95±0.19	4.45±0.29	0.71±0.83	0.65±0.05	1.03±0.12	1.18±0.07
500 mg/kg bwt	4.90±0.12	4.49±0.14	0.65±0.06	0.61±0.03	0.95±0.04	1.18±0.05
1000 mg/kg bwt	4.94±0.15	4.07±0.15	0.63±0.05	0.59±0.04	1.02±0.05	1.15±0.03
3000 mg/kg bwt	5.11±0.27	3.59±0.10	0.64±0.05	0.57±0.04	1.05±0.05	1.18±0.07
5000 mg/kg bwt	5.24±0.03	4.25±0.15	0.66±0.01	0.59±0.02	1.06±0.03	1.16±0.04

Values represent the organ weight measurement (g) of rats administered extracts for 28 days and are the means ± SEM (n=5). bwt: Body weight.

Table 4a: Activities of Alanine amino transferase (ALT), Aspartate amino transferase (ALT), alkaline phosphatase (ALP) and concentration of Albumin and total protein in Rats treated with ethanol extracts of *Spondias mombin* leaves

Treatment (Ethanol extracts)	Time	Indices of liver function				
		ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Albumin (g/dl)	Total protein (g/dl)
Control	Day 28	17.45±0.98	71.57±0.92	66.50±1.43	4.30±0.34	7.60±1.75
200 mg/kg bwt	Day 28	15.91±0.63	70.50±1.32	61.30±4.10*	4.80±0.25	5.75±0.23*
500 mg/kg bwt	Day 28	18.23±2.75	70.10±1.13	59.00±1.40*	4.84±0.15	6.80±0.96
1000 mg/kg bwt	Day 28	17.53±1.93	68.15±1.07	59.20±0.20*	4.80±0.15	7.40±1.15
3000 mg/kg bwt	Day 28	17.30±2.04	69.0±1.02	65.10±1.97	4.40±0.35	7.50±0.60
5000 mg/kg bwt	Day 28	17.94±0.54	68.0±1.83	65.15±1.60	5.35±0.03	7.10±1.01

Data are indices of liver function of rats administered extracts for 28 days and are expressed as means ± SEM, (n=5). *Indicates statistically significant (p≤0.05) when compared with the control. bwt: Body weight.

Table 4b: Activities of Alanine amino transferase (ALT), Aspartate amino transferase (ALT), alkaline phosphatase (ALP) and concentration of Albumin and total protein in rats treated with aqueous extracts of *Spondias mombin* leaves

Treatment (Aqueous extract)	Time	Indices of liver function				
		ALT (IU/L)	AST (IU/L)	ALP (IU/L)	Albumin (g/dl)	Total protein (g/dl)
Control	Day 28	16.52±1.44	54.02±1.25	60.50±2.20	4.25±0.60	7.48±0.05
200 mg/kg bwt	Day 28	16.02±1.25	47.25±1.26*	58.25±1.10	4.40±0.10	7.35±2.50
500 mg/kg bwt	Day 28	16.22±0.88	47.30±0.75*	58.50±0.01	4.55±0.40	7.35±2.00
1000 mg/kg bwt	Day 28	17.01±1.45	53.20±0.20	59.05±2.20	4.00±0.50	7.50±1.50
3000 mg/kg bwt	Day 28	17.12±1.48	54.25±0.50	57.00±2.55	4.50±0.20	7.40±1.40
5000 mg/kg bwt	Day 28	17.11±0.02	54.00±0.50	61.10±0.22	4.50±0.54	7.50±0.80

Data are indices of liver function of rats administered extracts for 28 days and are means ±SEM, (n=5). indicates statistically significant (p≤0.05) when compared with the control. bwt: Body weight.

Table 5a: Concentration of hematological parameters in rats treated with ethanol extracts of *Spondias mombin* leaves

Treatment (Ethanol extract)	Time	Hematological parameters				
		Red blood cells (×10 ³ μL)	White blood cells (×10 ³ μL)	Hemoglobin (g/dl)	Hematocrit (%)	Platelets (×10 ³ μL)
Control	Day 28	7.01±0.12	18.45±2.50	15.0±0.43	38.30±0.01	396.00±2.65
200 mg/kg bwt	Day 28	7.79±0.20	16.45±1.15	15.00±0.15	38.20±0.01	400.0±4.05
500 mg/kg bwt	Day 28	7.80±0.21	16.20±0.50	17.30±0.71*	40.00±2.20*	395.30±4.50
1000 mg/kg bwt	Day 28	10.90±0.20*	17.75±0.30	18.20±0.35*	42.22±0.01*	398.54±3.40
3000 mg/kg bwt	Day 28	9.12±0.13*	17.70±0.05	18.20±0.38*	45.50±1.25*	400.70±4.20

5000 mg/kg bwt	Day 28	9.91±0.05*	17.46±1.15	18.50±0.27*	40.20±1.15*	398.30±1.54
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Data are concentrations of hematological indices of rats administered ethanol extract for 28 days and are expressed as means ±SEM, (n=5). *p<0.05 when compared with the control. bwt: Body weight.

Table 5b: Concentration of Hematological parameters in rats treated with aqueous extracts of *Spondias mombin* leaves

Treatment (Aqueous extract)	Time	Hematological parameters				
		Red blood cells (×10 ⁶ μL)	White blood cells (×10 ³ μL)	Hemoglobin (g/d)	Hematocrit (%)	Platelets (×10 ³ μL)
Control	Day 28	7.25±0.05	12.21±0.05	13.60±2.40	42.30±0.06	456.00±2.34
200 mg/kg bwt	Day 28	7.50±0.20	13.35±0.80	14.50±0.01	41.00±0.52	452.50±2.00
500 mg/kg bwt	Day 28	7.40±0.02	13.50±2.20	16.50±2.20*	42.90±0.02	455.05±3.80
1000 mg/kg bwt	Day 28	12.80±0.40*	14.01±2.01	16.50±2.20*	44.00±0.45*	456.30±1.80
3000 mg/kg bwt	Day 28	10.82±0.05*	14.25±0.05	16.50±0.01*	44.80±0.05*	455.30±1.55
5000 mg/kg bwt	Day 28	12.01±0.20*	14.80±2.45	14.00±1.55	44.50±0.20*	456.54±2.82

Data are concentrations of hematological indices of rats administered aqueous extract for 28 days and are expressed as means ±SEM, (n=5). bwt: Body weight.

Table 6: Concentrations of creatinine and urea in rats treated with ethanol extract of *Spondias mombin* leaves

Treatment (Ethanol/ Aqueous extract)	Time	Indices of kidney function			
		ETHANOL EXTRACT		AQUEOUS EXTRACT	
		Creatinine (mg/dl)	Urea (mg/dl)	Creatinine (mg/dl)	Urea (mg/dl)
Control	Day 28	26.50±0.62	63.95±3.90	32.70±2.35	60.15±2.55
200 mg/kg bwt	Day 28	26.00±0.30	63.15±0.90*	33.35±1.05	60.25±2.35
500 mg/kg bwt	Day 28	26.80±0.75*	63.60±0.50	30.26±3.80	60.40±2.55
1000 mg/kg bwt	Day 28	26.60±2.15	62.35±0.70	32.56±2.65	60.28±1.90
3000 mg/kg bwt	Day 28	27.10±4.80	62.95±0.90	32.55±2.35	61.85±3.40
5000 mg/kg bwt	Day 28	27.70±0.30	63.50±2.30	30.00±1.58	61.70±2.60

Data are indices of kidney function of rats administered ethanol and aqueous extracts of *Spondias mombin* for 28 days and are expressed as means ±SEM (n=5). *p<0.05 when compared with control. bwt: Body weight.

Table 7a: Concentrations of plasma electrolytes in rats treated with ethanol extracts of *Spondias mombin* leaves

Treatment (Ethanol extract)	Time	Indices of kidney function			
		Sodium (Na ⁺) (mmol/L)	Potassium (K ⁺) (mmol/L)	Bicarbonate (HCO ₃ ⁻) (mmol/L)	Chloride (Cl) (mmol/L)
Control	Day 28	99.67±6.36	5.15±0.37	26.70±1.27	101.5±1.60
200 mg/kg bwt	Day 28	100.45±2.58	4.13±0.31	28.00±2.30	100.50±1.25
500 mg/kg bwt	Day 28	100.30±2.63	4.86±0.29	26.05±3.50	100.35±2.10
1000 mg/kg bwt	Day 28	100.30±2.63	5.15±0.22	25.35±0.80	99.75±1.10
3000 mg/kg bwt	Day 28	98.32±2.00	5.05±0.15	24.40±0.01	99.80±1.15
5000 mg/kg bwt	Day 28	99.66±2.60	4.35±0.35	27.00±1.68	100.85±2.50

Data are indices of kidney function of rats administered ethanol extract of *Spondias mombin* for 28 days and are expressed as means ±SEM (n=5). bwt: Body weight.

Table 7b: Concentrations of plasma electrolytes in rats treated with aqueous extracts of *Spondias mombin* leaves

Treatment (Aqueous extract)	Time	Concentrations of plasma electrolytes			
		Sodium (Na ⁺) (mmol/L)	Potassium (K ⁺) (mmol/L)	Bicarbonate (HCO ₃ ⁻) (mmol/L)	Chloride (Cl) (mmol/L)
Control	Day 28	102.20±2.00	4.01±1.20	30.15±0.85	100.50±0.30
200 mg/kg bwt	Day 28	100.25±1.25	3.80±0.52	28.10±2.55	98.40±0.80
500 mg/kg bwt	Day 28	101.30±2.25	3.66±1.20	25.65±1.25*	102.60±0.85
1000 mg/kg bwt	Day 28	102.75±2.50	4.11±0.98	28.65±1.50	103.25±1.25
3000 mg/kg bwt	Day 28	103.35±2.25	3.82±0.80	30.23±0.57	100.25±1.08
5000 mg/kg bwt	Day 28	101.65±0.05	4.00±0.50	28.75±0.01	101.60±1.22

Data are indices of kidney function of rats administered aqueous extract of *Spondias mombin* for 28 days and are means ± SEM (n=5). bwt: Body weight.

Table 8: Indices of cardiac function in rats treated with ethanol extracts of *Spondias mombin* leaves

Treatment (Ethanol/Aqueous extract)	Time	Indices of cardiac function			
		ETHANOL EXTRACT		AQUEOUS EXTRACT	
		Creatine kinase (U/L)	Lactate dehydrogenase (U/L)	Creatine kinase (U/L)	Lactate dehydrogenase (U/L)
Control	Day 28	40.90±1.00	20.38±0.50	42.95±0.25	20.45±2.45
200 mg/kg bwt	Day 28	42.00±0.55	20.25±0.40	39.52±3.45	21.80±2.55
500 mg/kg bwt	Day 28	42.65±0.80	19.30±0.45	40.35±2.12	20.67±0.45
1000 mg/kg bwt	Day 28	42.10±1.15	20.40±0.65	40.18±1.37	19.40±0.15
3000 mg/kg bwt	Day 28	40.20±0.50	19.62±1.10	42.45±2.70	19.05±1.15
5000 mg/kg bwt	Day 28	43.00±0.90	19.42±0.56	42.65±1.58	19.20±2.15

Data are indices of Cardiac function of rats administered ethanol extract of *Spondias mombin* for 28 days and are expressed as means ± SEM (n=5). bwt: Body weight.

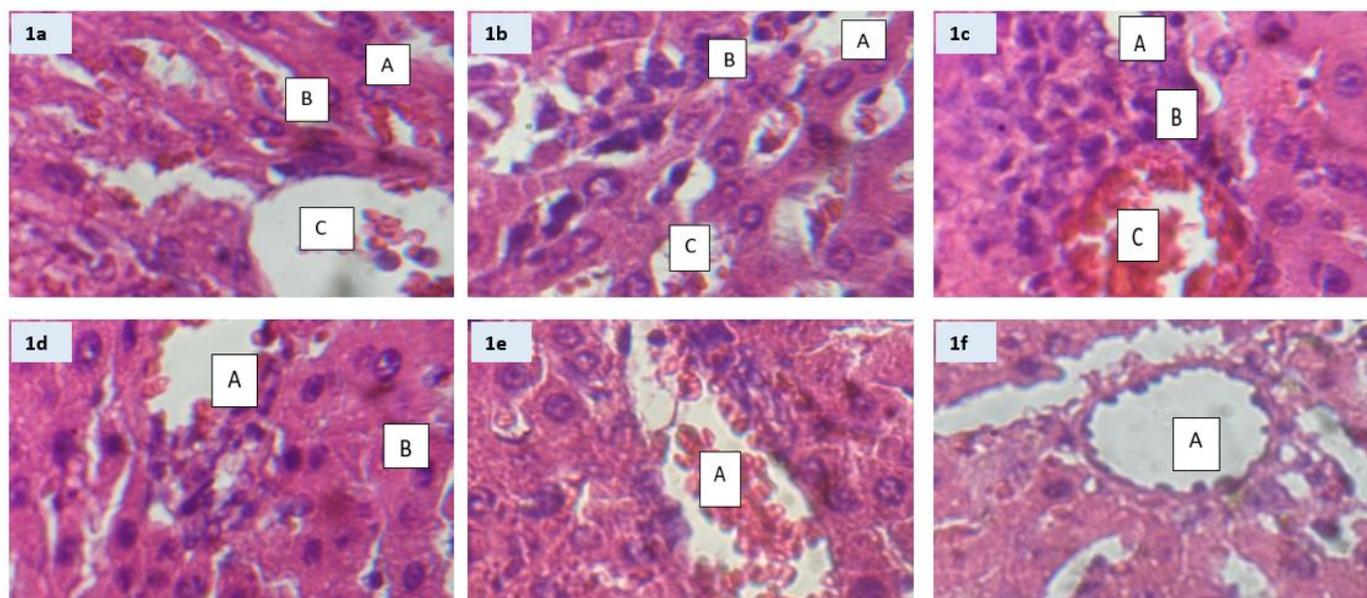


Figure 1: Histopathology examination of the liver tissue administered with ethanol extract

Figure 1a: (Normal control): Rat liver composed of A (central vein), B (hepatocytes); and C (Sinusoids) (H&E x 400). Figure 1b: Rat liver treated with 200 mg/kg body weight ethanol leaf extract of *Spondias mombin* showing A (mild vasodilatation), B (mild periportal infiltrates of lymphocytes) and C (mild kupffer cell activation) (H&E x 400). Figure 1c: Rat liver treated with 500 mg/kg body weight of ethanol extract of *Spondias mombin* leaves showing mild vascular congestion A, mild periportal infiltrates of lymphocytes B and mild kupffer cell activation C (H&E x 400). Figure 1d: Rat liver given 1000 mg/kg body weight of ethanol extract of *Spondias mombin* leaves showing mild congestion A and kupffer cell activation B (H&E x 400). Figure 1e: Rat liver exposed to 3000 mg/kg body weight of ethanol extract showing A (mild vascular dilatation) (H&E x400). Figure 1f: Rat liver exposed to 5000 mg/kg body weight of ethanol extract showing A (mild vascular dilatation) (H&E x400).

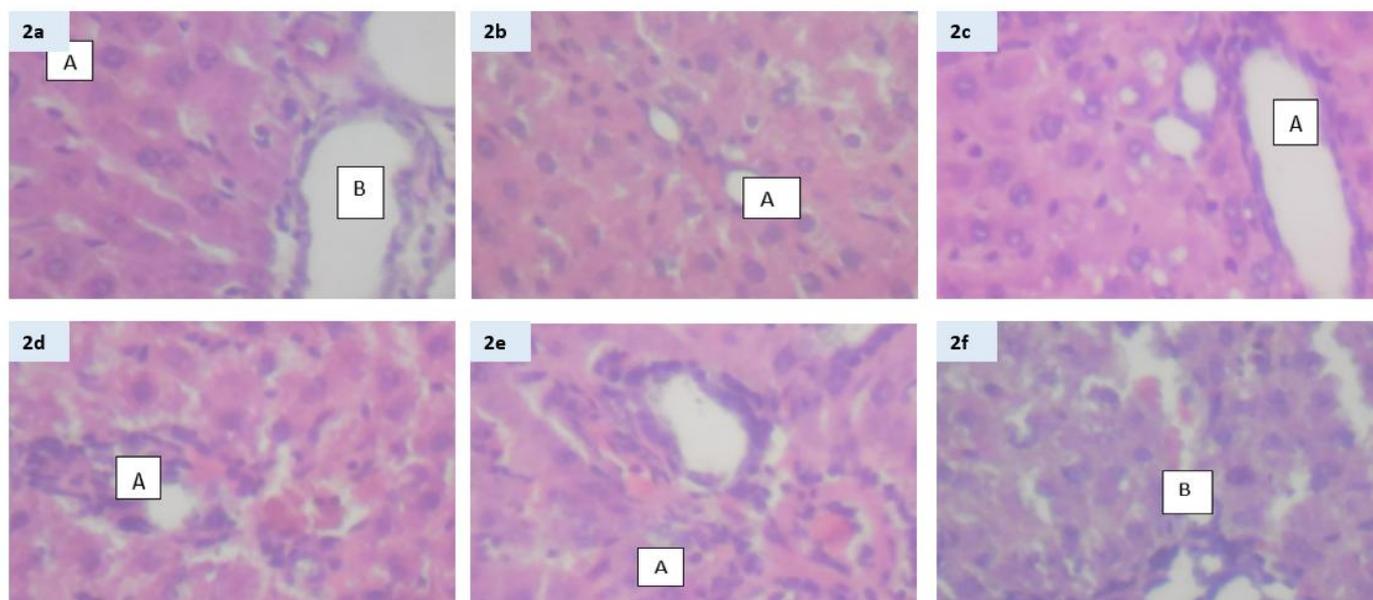


Figure 2: Histopathological examination of the liver of rats administered aqueous extract

Figure 2a: Control: Rat liver composed of A, hepatocytes, B, bile duct and C, sinusoids (H&E x 400). Figure 2b: Rat liver given 200 mg/kg body weight aqueous extract of *Spondias mombin* leaves showing A, mild kupffer cell activation (H&E x 400). Figure 2c: Rat liver given 500 mg/kg body weight aqueous extract of *Spondias mombin* leaves showing A (mild kupffer cell activation) (H&E x 400). Figure 2d: Rat liver given 1000 mg/kg body weight aqueous extract of *Spondias mombin* leaves showing A (mild periportal lymphocytosis) (H&E x 400). Figure 2e: Rat liver given 3000 mg/kg body weight extract of *Spondias mombin* leaves showing A (mild periportal lymphocytosis) and B (kupffer cell activation) (H&E x 400). Figure 2f: Rat liver given 5000 mg/kg body weight extract of *Spondias mombin* leaves showing A (mild portal activation) (H&E x 400).

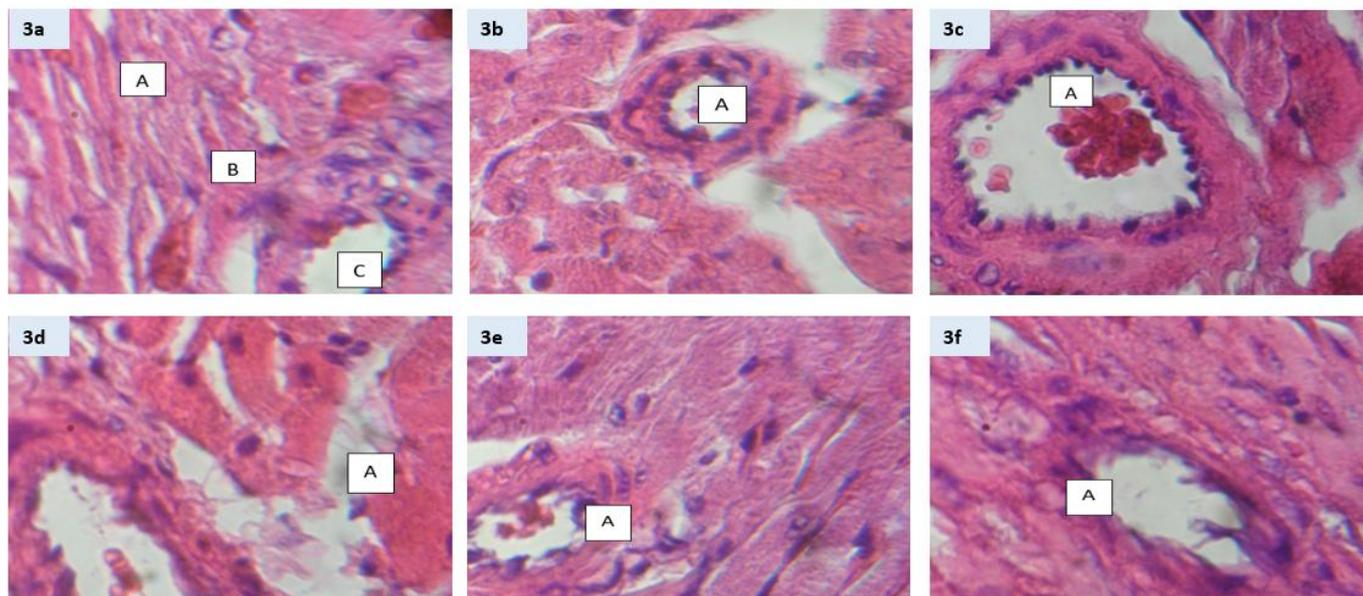


Figure 3: Histopathological Examination of the Heart of rats administered ethanol extract

Figure 3a: (Normal control): rat heart composed of A (Myocardial fiber); B (Interstitial vessels); C (Coronary fiber) (H&E x400). Figure 3b: Rat heart treated with 200 mg/kg body weight of ethanol leaf extract of *Spondias mombin* showing mild coronary vascular dilatation A (H&E X 400). Figure 3c: Rat heart given 500 mg/kg body weight ethanol leaf extract of *Spondias mombin* showing A (dilatation) (H&E x 400). Figure 3d: Rat heart given 1000 mg/kg body weight ethanol leaf extract of *Spondias mombin* showing A (dilatation) (H&E x 400). Figure 3e: Rat heart given 3000 mg/kg body weight ethanol leaf extract of *Spondias mombin* showing mild coronary vascular dilatation A (H&E x 400). Figure 3f: Rat heart given 5000 mg/kg body weight of ethanol leaf extract of *Spondias mombin* showing normal rat architecture A (H&E x 400).

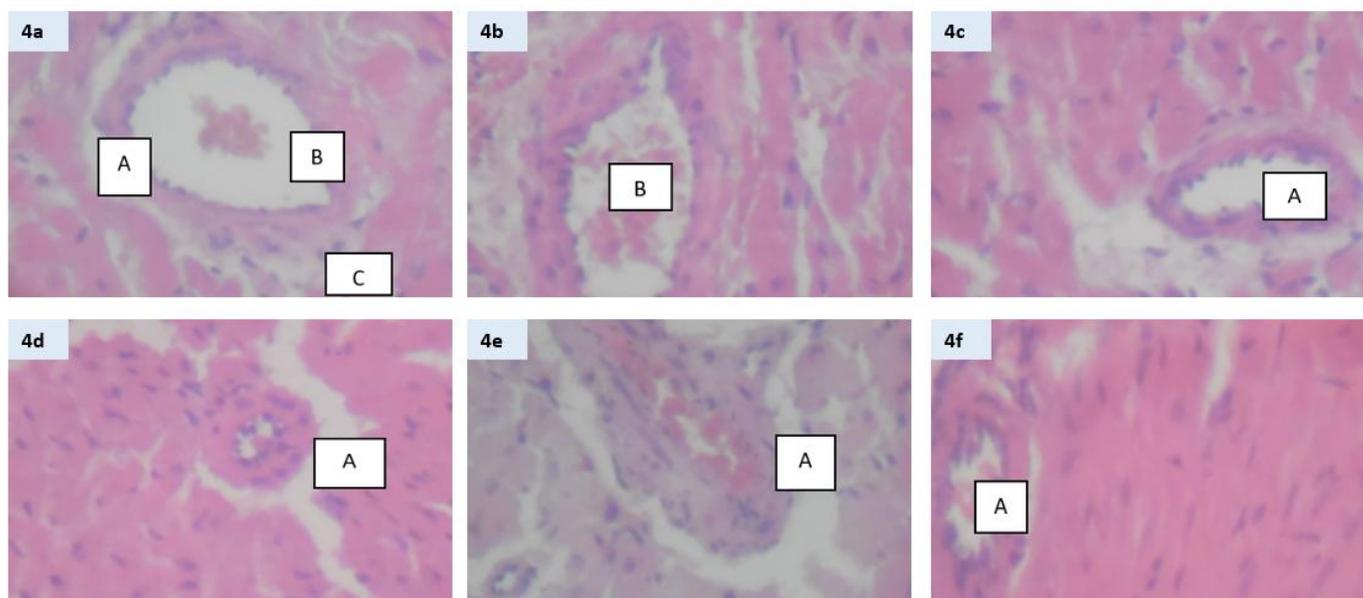


Figure 4: Histopathological Examination of the Heart of rats administered aqueous extract

Figure 4a: Control: Rat heart composed of A, bundles of myocardial fibres, B, coronary artery and C, interstitial space (H&E x 4 00). Figure 4b: Rat heart given 200 mg/kg body weight aqueous extract of *Spondias mombin* leaves showing B (mild vascular dilatation) (H&E x 400). Figure 4c: Rat heart given 500 mg/kg body weight aqueous extract of *Spondias mombin* leaves showing A, normal myocardiac architecture (H&E x 400). Figure 4d: Rat heart given 1000 mg/kg body weight aqueous extract showing A, coronary artery (H&E x 400). Figure 4e: Rat heart given 3000 mg/kg body weight aqueous extract of *Spondias mombin* leaves showing A, mild vascular congestion (H&E x 400). Figure 4f: Rat heart given 5000 mg/kg body weight aqueous extract of *Spondias mombin* leaves showing A, mild vascular dilatation (H&E x 400).

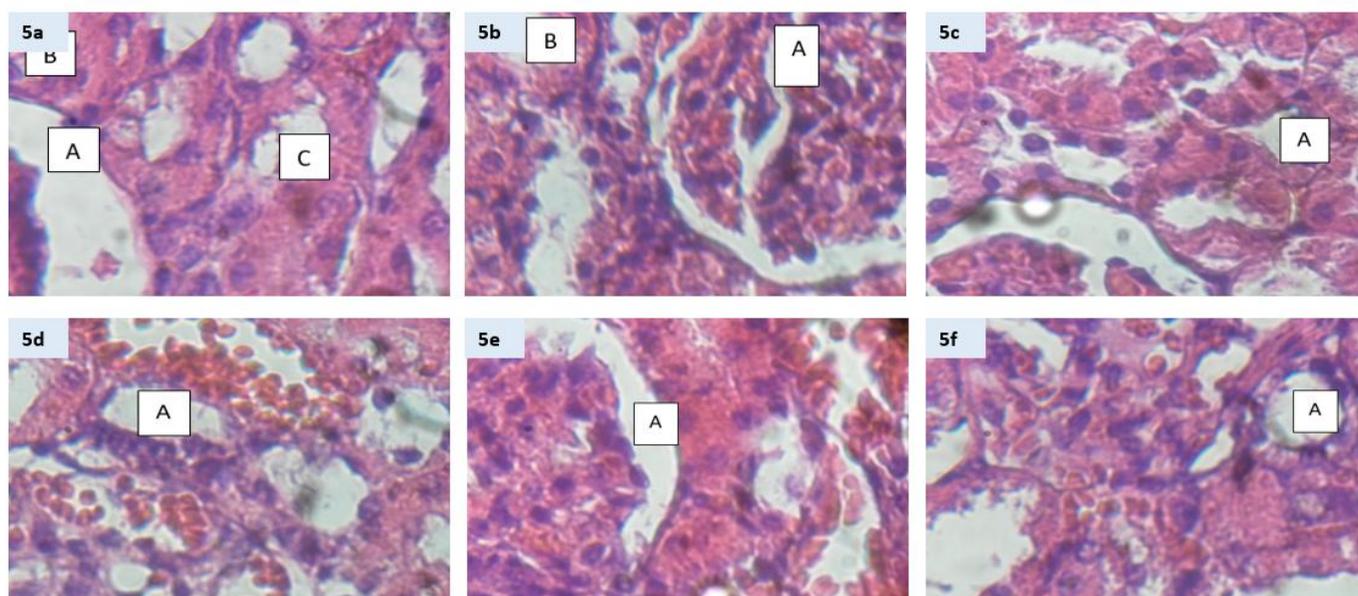


Figure 5: Histopathological examination of kidney tissue administered ethanol extract

Figure 5a: (Normal Control): Rat kidney composed of A (glomerulus); B (tubules); and C (interstitial space) (H&E \times 400). Figure 5b: Rat kidney given 200 mg/kg body weight of ethanol extract of *Spondias mombin* leaf showing normal glomerulus A and tubules B (H&E \times 400). Figure 5c: Rat kidney given 500 mg/kg body weight of ethanol extract of *Spondias mombin* leaf showing normal architecture (H&E \times 400). Figure 5d: Rat kidney given 1000 mg/kg body weight of ethanol extract of *Spondias mombin* leaf showing A (mild interstitial congestion) (H&E \times 400). Figure 5e: Rat kidney given 3000 mg/kg body weight ethanol extract of *Spondias mombin* leaves showing mild interstitial dilatation A (H&E \times 400). Figure 5f: Rat kidney given 5000 mg/kg body weight of ethanol extract of *Spondias mombin* leaves showing mild interstitial dilatation A (H&E \times 400).

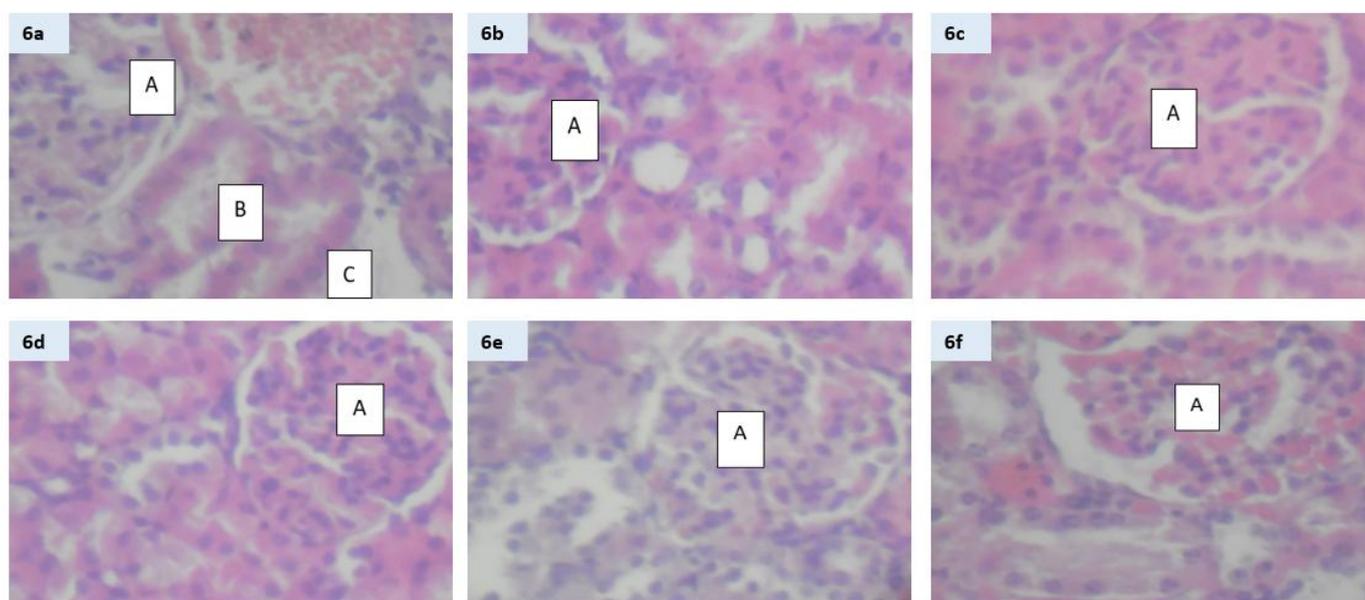


Figure 6: Histopathological examination of the kidney of rats administered aqueous extract

Figure 6a: Control: Rat kidney composed of A, glomerulus, B, tubules and C, arcuate artery (H&E \times 400). Figure 6b: Rat kidney given 200 mg/kg body weight aqueous extract of *Spondias mombin* leaves showing A, normal renal architecture (H&E \times 400). Figure 6c: Rat kidney given 500 mg/kg body weight aqueous extract of *Spondias mombin* leaves showing A, normal renal architecture (H&E \times 400). Figure 6d: Rat kidney given 1000 mg/kg body weight extract of *Spondias mombin* leaves showing A, normal renal architecture (H&E \times 400). Figure 6e: Rat kidney given 3000 mg/kg body weight of aqueous extract of *Spondias mombin* leaves showing A, normal renal architecture (H&E \times 400). Figure 6f: Rat kidney given 5000 mg/kg body weight aqueous extract of *Spondias mombin* leaves showing A, normal renal architecture (H&E \times 400).

DISCUSSION

A plant or plant product can only be considered safe after undergoing toxicological research utilizing the most recent clinical and scientific methodologies.^[35]

According to the findings of the acute toxicity research, there were no discernible signs of morbidity or mortality during the treatment period. No death was noted even at the highest doses of 5000 mg/kg body weight (Table 1). This could imply that the extract was well

tolerated by the experimental animals. Also, the rats presented no signs of behavior changes as shown by the normal display of respiration pattern, color of body surfaces, frequency and nature of movement. The LD₅₀ can be said to be \geq 5000 mg/kg body weight as no death was observed at this highest dose.

When comparing the groups treated with aqueous and ethanol extract to the control in the sub-chronic toxicity research, a notable rise in body weight measurement was observed (Table 2). Weight gain is typically the result of eating when there is no illness. This could be

associated with the normal growth of the rats, or it could be because they are consuming more food. Body weight changes have been utilized as a sign of negative pharmacological and chemical effects.^[36]

Another crucial indicator of an animal's physiological and pathological state is organ weight. The main organs impacted by toxicant-induced metabolic reactions include the heart, liver, kidney, spleen, and lungs.^[37] Organ weight measurements for the two extract-administered groups revealed no decline in the liver, kidney, or heart.

Due to its exposure to foreign toxins that are absorbed in the intestine before entering the bloodstream, the liver is the primary target for hazardous compounds ^[38,39].

Alanine amino transferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) are markers of liver function. These enzymes are released into the bloodstream in large quantities from the cytosol and subcellular organelles when the liver cells are injured.^[40] The non-significant differences in the liver enzymes (AST, ALT and ALP) (Tables 4a and 4b) for the ethanol extract and for the aqueous extract treated groups observed in this study suggest that both extracts were non-toxic to the liver.

For all hazardous substances, urea and creatinine are regarded as suitable biomarkers of renal failure and dysfunction.^[41] This study showed a non-significant difference in urea, creatinine, and electrolyte levels for the varying doses of extracts administered (Table 6, 7a and 7b). This would suggest that renal function is comparatively in good working condition. This result is not in agreement with the findings of Olaitan et al.^[42]

The results of hematological assay showed a significant increase in the blood counts in the ethanol extract treated groups and a non-significant increase in these hematological indices in the aqueous extract treated groups. Defective hematopoiesis causes a decrease in erythrocytes and hemoglobin count, but we did not find any evidence of anemia.^[43] The significant increase in hematocrit, red blood cell and hemoglobin concentration (Tables 5a, 5b) observed in the groups administered ethanol extract could indicate that the bone marrow is operating normally during the erythropoiesis process. Asuquo et al.^[18] also reported a significant increase in these parameters.

Assessing treatment-related pathological alterations in tissues and organs is done by histopathological analysis.^[44] According to studies, increased liver enzymes are invariably accompanied by abnormalities in the liver's microstructure, such as steatosis, necrosis, or centrilobular degenerative alterations.^[45] These changes were not observed in the liver of the rats from our study. The mild Kupffer cell activation observed in some liver tissues of the treated animals is essential for the liver as it limits cellular and organ damage. Mild dilation and congestion of tissues are two histological findings observed in some of the extract treated groups. Although Mild vascular dilatation can increase blood flow and improve oxygen delivery to tissues. It can also be a sign of inflammation or other underlying health condition. mild vascular congestion may not immediately cause severe harm, but it is crucial to address it to prevent further complications and ensure proper tissue function.

CONCLUSION

In summary the aqueous and ethanol extract of *Spondias mombin* leaves administered orally did not cause abnormal alterations in the blood parameters but caused slight histological alterations to the tissues; as a result, care must be used while using this plant's extracts.

Conflict of interest

The authors declared no conflict of interest.

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None declared.

REFERENCES

1. World Health Organization. Traditional medicine and modern health care. Progress report by the Director General, Document A. Geneva: WHO; 1991. p. 1-11.
2. Diallo D, Paulsen BS, Hvem B. Production of traditional medicine: preparations accepted as medicines in Mali. In: Hostettmann K, Chinyanganya F, Maillard M, Wolfender JL, editors. Chemistry, biological and pharmacological properties of African medicinal plants. Proc 1st Int IOCD Symp, Victoria Falls, Zimbabwe; 1996.
3. Andrade F, Albuquerque CAA, Maraschin Silva EL. Safety assessment of yerba mate (*Ilex paraguariensis*) dried extract: results of acute and 90 days subchronic toxicity studies in rats and rabbits. Food Chem Toxicol. 2012;50(2):328-34.
4. Amadi BA, Agomuo EN, Duru MKC. Toxicological studies of *Asmina triloba* leaves on haematology, liver, and kidney using rat model. Int Sci Res J. 2013;4:11-17.
5. Duru MKC, Amadi BA, Amadi CT, Lele KC, Anudike JC, Chima-Ezika OR, et al. Toxic effect of *Carica papaya* bark on body weight, haematology, and some biochemical parameters. Biokemistri. 2012;24:67-71.
6. Ugbogu AE, Okezie E, Uche-Ikonne C, Duru M, Atasi OC. Toxicity evaluation of the aqueous stem extracts of *Senna alata* in Wistar rats. Am J Biomed Res. 2016;4:80-86.
7. Gbogbo M, Kone M, Bleyere N, Mathieu Y, Kouadio E, Yapo AP. Effect of total aqueous stem bark extract of *Spondias mombin* L. on some biochemical and anthropometric parameters in Wistar albino rats. Int J Biosci. 2014;4:1-8.
8. Rhiouani H, El-Hilaly J, Israili ZH, Lyoussi B. Acute and sub-chronic toxicity of an aqueous extract of the leaves of *Herniaria glabra* in rodents. J Ethnopharmacol. 2008;118:378-86.
9. Bhardwaj S, Gupta D. Study of acute, subacute and chronic toxicity test. Int J Adv Res Pharm Biol Sci. 2012;2:103-29.
10. Gadanya A, Sule M, Atiku M. Acute toxicity study of "GADAGI" Tea on rats. Bayero J Pure Appl Sci. 2011;4:147-49.
11. Gill LS. Ethnomedical uses of plants in Nigeria. Benin City: UNIBEN Press; 1992. p. 222-3.
12. Irvine JR. Woody plants of Ghana. 2nd ed. London: Oxford Univ. Press; 1961. p. 51.
13. Accioly MP, Bevilaqua CM, Rondon FCM, Morais SM, Machado LKA, Almeida CA, et al. Vet Parasitol. 2012;187(1-2):79-84.
14. Cabral B, Siqueira EMS, Bitencourt AO, Lima MCS, Lima AK, Ortmann CF, et al. Phytochemical study and anti-inflammatory and antioxidant potential of *Spondias mombin* leaves. J Appl Pharm Sci. 2016;26(3):304-11.
15. Okonkwo TJN, Okorie O, Nzekwe IT. Subchronic hepatotoxicity evaluation of aqueous ethanol extract of *Spondias mombin* leaves in rats. Niger J Pharm Res. 2010;8(1):183-7.
16. Asuquo RO, Ekanem BT, Udoh BP, Mesembe EO, Ebong EP. Haematinic potential of *Spondias mombin* leaf extract in Wistar rats. Adv Biores. 2013;4(2):53-6.
17. Nwaogwugwu J, Friday U, Okereke S, Egege A, Atasi O. Toxicological evaluation of aqueous leaf extract of *Spondias mombin* using albino rats. J Med Herbs Ethnomed. 2018;4:23-30.
18. Abiodun OO, Nnoruka ME, Tijani RO. Phytochemical constituents, antioxidant activity, and toxicity assessment of the seed of *Spondias mombin* L. Turk J Pharm Sci. 2020;17(3):343-8.

19. Mayur P, Sandeep KG, Najam AK, Kamal KM. Evaluation of toxicity and antihyperlipidemic activity of *Spondias mombin* L. leaves methanolic extract in laboratory rats. *Cardiovasc Hematol Disord Drug Targets*. 2020;20(4):289–96.
20. Eluehike N, Onoagbe I. Changes in organ weight and body weight, serum amylase and antidiabetic effects of tannins from *Spondias mombin* on streptozotocin-induced diabetic rats. *J Insulin Resist*. 2018;3(1):1–5.
21. Eluehike N, Onoagbe IO. Preliminary nutrient determination and regeneration of pancreatic islet cells by extracts of *Spondias mombin* leaves in streptozotocin-induced diabetic rats. *Avicenna J Med Biochem*. 2020;8(1):1–14.
22. Eluehike N, Onoagbe I. Amino acid contents of *Spondias mombin* leaves and in-vitro antioxidant capacity of aqueous and ethanol extracts of *Spondias mombin* leaves. *J Phytomed Ther*. 2018;17(2):221–36.
23. Eluehike N, Erema GV, Onoagbe IO. Atherogenic indices, cardiovascular risk index of serum and alteration in lipid profile in organs of experimental diabetic rats treated with extracts of *Spondias mombin* leaves. *Biokemistri*. 2020;32(3):185–95.
24. Onoagbe IO, Lau HU, Esekheigbe A, Dawha IM, Salami CO. Effects of *Irvingia grandifolia* and *Spondias mombin* on blood glucose and triglyceride concentrations in streptozotocin-induced diabetic rabbits. *Biochemistry*. 1999;9(1):79–84.
25. Alade PI, Irobi ON. Antimicrobial activities of crude leaf extracts of *Acalypha wilkesiana*. *J Ethnopharmacol*. 1993;39(3):171–4.
26. Harborne JB. *Phytochemical methods: a guide to modern techniques of plant analysis*. 3rd ed. London: Chapman & Hall; 1998. p. 302.
27. Gossell-Williams M, Simon OR, West ME. The past and present use of the castor oil plant as a medicinal plant. *West Indian Med J*. 2006;55(4):346–9.
28. OECD. *OECD Guidelines for the testing of chemicals: Acute oral toxicity – acute toxic class method*. OECD Guidelines. Paris: OECD Publishing; 2001. No. 423.
29. Teo S, Stirling D, Thomas S, Hoberman A, Kiorpes A, Khetani V. A 90-day oral gavage toxicity study of d-methylphenidate and d,l-methylphenidate in Sprague–Dawley rats. *Toxicology*. 2002;179(3):183–96.
30. Obidike IC, Salawu OA. Screening of herbal medicines for potential toxicities. In: Kuatsienu A, editor. *New insights into toxicity and drug testing*. London: IntechOpen; 2013. p. 63–88.
31. Mukinda JT, Syce JA. Acute and chronic toxicity of the aqueous extract of *Artemisia afra* in rodents. *J Ethnopharmacol*. 2007;112(1):138–44.
32. Akinmoladun FO, Akinrinlola BL, Komolafe OA, Farombi EO. Sub-acute toxicity study of hydroethanol stem bark extract of *Ficus exasperata* (Vahl) in Wistar rats. *Toxicol Rep*. 2020;7:1048–56.
33. OECD. Test No. 407: Repeated Dose 28-Day Oral Toxicity Study in Rodents. *OECD Guidelines for the Testing of Chemicals*. Paris: OECD Publishing; 2008.
34. OECD. Test No. 408: Repeated Dose 90-Day Oral Toxicity Study in Rodents. *OECD Guidelines for the Testing of Chemicals*. Paris: OECD Publishing; 2018.
35. OECD. Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure. *OECD Guidelines for the Testing of Chemicals*. Paris: OECD Publishing; 2008.
36. Gleason MN, Gosselin RE, Hodge HC, Smith RP. *Clinical toxicology of commercial products*. 5th ed. Baltimore: Williams & Wilkins; 1986.
37. Turner RA. *Screening methods in pharmacology*. Vol. 1. New York: Academic Press; 1965. p. 299.
38. Vogel HG. *Drug discovery and evaluation: pharmacological assays*. 2nd ed. Berlin: Springer; 2002. p. 67–9.
39. Awodele O, Popoola TD, Amadi KC, Coker HA, Akintonwa A. Traditional herbal medicines: adverse effects and drug interactions. *Afr J Biotechnol*. 2015;14(31):2470–83.
40. Rajeh MN, Zuraini A, Sasiidharan S, Latha LY, Amutha S. Acute toxicity and genotoxicity studies of *Nelumbo nucifera* leaves extract. *Int J Pharmacol*. 2010;6(3):215–9.
41. Mukherjee PK. *Quality control of herbal drugs: an approach to evaluation of botanicals*. New Delhi: Business Horizons; 2002.
42. Ghannoum MA, Perfect JR. *Antifungal therapy*. Boca Raton: CRC Press; 2009. p. 258.
43. Hemmings HC, Egan TD. *Pharmacology and physiology for anesthesia: foundations and clinical application*. Philadelphia: Elsevier Saunders; 2013.
44. Gujarat Rajya Bheshaj Samiti. *Bheshaj Samhita*. Ahmedabad: Swasthya Mantralaya Gujarat; 1966. Chapter 13. p. 745.
45. Government of India. *The Ayurvedic Pharmacopoeia of India*. Part I, Vol. I. New Delhi: Ministry of Health and Family Welfare, Department of AYUSH; 2001.

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