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Research Article

Antioxidant, Anti-Inflammatory, and Liver-Protective Properties of n-Hexane and Chloroform Fractions of *Spondias mombin* Leaves in Albino Rats

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ABSTRACT

Body weight serves as a key marker for assessing drug effects and potential toxicity. In this study, extract-treated rats exhibited no significant changes in body weight, indicating the absence of phytochemicals that influence weight. Additionally, the antioxidant properties of Spondias mombin extracts were analyzed, revealing a significant increase in superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH) activity, along with a reduction in malondialdehyde (MDA) levels, demonstrating potent antioxidant effects. The anti-inflammatory potential was evaluated using the egg albumin-induced paw edema model, where the 400 mg/kg extract dose exhibited greater inhibition compared to diclofenac. This suggests additional anti-inflammatory mechanisms, possibly involving antioxidant and membrane-stabilizing effects. Liver function tests showed that extract administration reduced ALT, AST, and ALP levels, demonstrating hepatoprotective potential. Similarly, renal function markers improved, with reduced urea and uric acid levels at higher extract concentrations, likely due to antioxidative and nephroprotective properties. Electrolyte balance was also positively influenced by extract treatment. These findings highlight the therapeutic potential of Spondias mombin extracts in modulating oxidative stress, inflammation, and organ function, supporting its traditional medicinal applications.

INTRODUCTION

Medicinal plants have long been utilized for their therapeutic benefits, particularly in traditional

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medicine, where they serve as rich sources of bioactive compounds for disease treatment. *Spondias mombin*, commonly known as hog plum, has been traditionally used across various cultures to manage inflammatory conditions, gastrointestinal disorders, and microbial infections [1]. Its pharmacological effects are primarily linked to its diverse phytochemical composition, including flavonoids, tannins, alkaloids, and terpenoids, which exhibit strong antioxidant and anti-inflammatory properties [2]. Inflammation is a natural biological response to injury and infection; however, chronic or excessive inflammation contributes to the development of various diseases, such as arthritis, cardiovascular conditions, and liver dysfunction [3]. While non-steroidal anti-inflammatory drugs (NSAIDs) like diclofenac are commonly used to treat inflammation, prolonged use is associated with adverse effects, including gastrointestinal distress and liver toxicity [4]. As a result, there is growing interest in plant-based anti-inflammatory alternatives that offer therapeutic efficacy with fewer side effects. Oxidative stress is a major contributor to inflammatory and degenerative diseases, arising from an imbalance between reactive oxygen species (ROS) production and the body's antioxidant defense mechanisms [5]. Key enzymatic antioxidants, including catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH), play a crucial role in mitigating oxidative damage by neutralizing free radicals [6]. Lipid peroxidation, marked by elevated malondialdehyde (MDA) levels, is a key indicator of oxidative stress-induced cellular damage. Plant-derived antioxidants have been reported to mitigate oxidative stress and enhance antioxidant enzyme activity, thereby protecting against inflammation-related tissue damage [7]. Given the traditional use of *Spondias mombin* in inflammation-related disorders, this study aimed to evaluate its anti-inflammatory, antioxidant, and hepatoprotective effects using an egg white-induced paw edema model in albino rats. Additionally, the impact of *Spondias mombin* extracts on liver and renal function markers was assessed to determine its potential toxicity or protective role in vital organs. The findings from this study contribute to the growing body of evidence supporting the pharmacological benefits of *Spondias mombin* and its potential as a natural alternative for managing inflammatory and oxidative stress-related diseases.

2.0MATERIALS AND METHODS

2.1Collection, Identification and Processing of Plant Fractions

An ethnobotanical survey guided the selection of *Spondias mombin*, a medicinal plant traditionally used by herbal practitioners in Niger State for managing inflammatory diseases. The plant leaves were collected from Gara village along Katcha Road in Katcha Local Government Area.

Dr. Grace E. Ugbabe, a specialist in Plant Taxonomy and Biosystematics at the National Institute for Pharmaceutical Research and Development (NIPRD), Idu, Abuja, identified the plant specimens. The authenticated samples were subsequently stored in the herbarium and assigned the voucher number NIPRD/H/7354. A total of 400 g of air-dried and coarsely ground plant material was subjected to methanol extraction for two hours using a Soxhlet apparatus. The resulting crude extract was obtained by evaporating the methanol to complete dryness under reduced pressure at 40 °C. A 20 g portion of the crude extract was dissolved in 250 mL of water and subsequently partitioned with equal volumes of n-hexane, chloroform, ethyl acetate, and water. The eluents were then evaporated to dryness under reduced pressure at 40 °C.

2.1Determination of Body Weight Changes

The experimental rats' body weights were recorded weekly by weighing each rat daily with a compact

digital weighing scale (3000 g capacity, FEJ-3000B; China).

2.1In-vivo Antioxidant Assay

Forty-eight albino rats of mixed sex (n = 6 per group) were randomly divided into seven groups. Group I served as the control, while Groups II, III, IV, V, VI, and VII received oral doses of *Spondias mombin* chloroform and n-hexane solvent-partitioned extracts at 100, 200, and 400 mg/kg, respectively, for 14 days. On the final day, the animals were euthanized via cervical dislocation, and blood samples were collected into anticoagulant-free test tubes and left to clot at room temperature. Serum was separated by centrifugation at 3,000 rpm for 15 minutes using an electric desktop lab centrifuge (220V EU Plug, Jersey, UK). The obtained serum was transferred into anticoagulant-free test tubes using a micropipette and stored at 4°C for subsequent biochemical analyses.

2.1.1Lipid peroxidation assay

The thiobarbituric acid reactive substances (TBARS) assay was used to evaluate lipid peroxidation during an acid-heating reaction. Sample aliquots were mixed with 15% trichloroacetic acid and 0.38% thiobarbituric acid, then heated in a boiling water bath for one hour. The extent of lipid peroxidation was determined by measuring the absorbance of the pink-colored complex at 532 nm using a spectrophotometer, following the method outlined by Satoh [8].

2.1.2Superoxide dismutase assay

The assessment was conducted using a reaction mixture comprising 0.1 mL of phenazine methosulfate (186 µM) and 1.2 mL of sodium pyrophosphate buffer (0.052 M, pH 7.0). To this mixture, 0.3 mL of the supernatant—obtained through sequential centrifugation of the homogenate at 1,500 × g for 10 minutes, followed by 10,000 × g for 15 minutes—was added. The enzyme reaction was initiated by introducing 0.2 mL of NADH (780 µM) and terminated after one minute with the addition of 1 mL of glacial acetic acid. The resulting chromogen was quantified by measuring absorbance at 560 nm, with results expressed in units per milligram of protein, as described by Kakkar *et al*. [9].

2.1.3Catalase assay

The analysis was conducted using a reaction solution containing 2.5 mL of 0.05 M phosphate buffer (pH 8.3), 0.7 mL of 0.2 M hydrogen peroxide (H_2O_2), and 0.1 mL of tissue homogenate. The absorbance change at 570 nm was recorded after one minute. Results were expressed in units per milligram of protein, following the methodology outlined by Maehly and Chance [10].

2.1.4Reduced glutathione assay

The assessment was conducted using dithiobisnitrobenzoate as a substrate, generating a yellow-colored product. The absorbance was measured immediately at 412 nm, and the results were expressed as µM GSH per gram of protein, following the method described by Ellman [11].

2.2Anti-inflammatory Activity

The anti-inflammatory potential of chloroform and n-hexane solvent-partitioned extracts of *Spondias mombin* was assessed using the egg white-induced paw edema model [12,13]. The study utilized mixed-sex Wistar rats weighing between 150–200 g, which were housed in the animal facility of the School of Life Sciences, Federal University of Technology, Minna. The animals were maintained under controlled room temperature conditions with a natural day/night cycle, with access to food and water. The rats were randomly divided into nine groups, each consisting of six animals. They were fasted for one hour before the experiment. Group I was administered distilled water (5 mL/kg) as a blank control, while Group II received distilled water (5 mL/kg) as a negative control. Group III was treated with diclofenac (5 mg/kg) as a positive control. Groups IV, V, VI, VII, VIII, and IX received chloroform and n-hexane solvent-

partitioned extracts of *Spondias mombin* at doses of 100, 200, and 400 mg/kg, respectively. One hour after administering the standard drug and plant extracts, the baseline paw diameter of each rat was measured using a caliper (0 h). Inflammation was induced in all groups except the neutral control by injecting 100 µL of a 1% egg white solution intradermally into the left hind paw using a 1 mL syringe. Paw diameters were recorded at 1 h, 2 h, 4 h, 8 h, and 24 h post-injection. The extent of edema was determined by calculating the percentage increase in paw volume over time.

Increase of Paw Volume (IPV)=

$$\left(\frac{\text{Paw Volume at time T} - \text{Initial Paw Volume}}{\text{Initial Paw Volume}}\right) \times 100 \quad (1)$$

$$\text{Percentage Inhibition} = \left(\frac{(\text{IPV})\ \text{control} - (\text{IPV})\ \text{treated}}{(\text{IPV})\ \text{control}}\right) \times 100 \quad (2)$$

2.3Data Analysis

All experiments were performed in triplicate, and the results were presented as mean ± standard error of the mean (SEM). Statistical analysis was conducted using one-way ANOVA in SPSS, followed by Dunnett's post hoc test for comparisons with the control group. A p-value of less than 0.05 was considered statistically significant.

3.0RESULTS AND DISCUSSION

The effects of n-hexane and chloroform solvent-partitioned fractions of *Spondias mombin* on the body weight of animals are shown in Figure 1. The results indicate that the extracts did not adversely affect the weight gain of Wistar rats throughout the treatment period. Body weight increased progressively from week 1 to week 2.

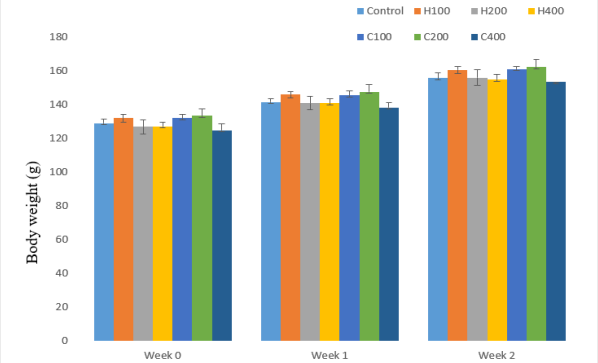


Figure 1: Effect of n-Hexane and Chloroform solvent partitioned extract of *Spondias mombin* on Body Weight of Animals

3.1Effect of n-Hexane and Chloroform Solvent Partition Fractions of *Spondias mombin* on Serum Antioxidant enzymes

The findings of the serum antioxidant enzyme assays for n-hexane and chloroform solvent-partitioned extracts are summarized in Table 1. The activities of key antioxidant enzymes, including catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GSH), were significantly increased ($P < 0.05$) in the treatment groups compared to the control. Notably, administration of 200 mg and 400 mg/kg body weight of the extracts led to a substantial elevation ($P < 0.05$) in CAT, SOD, and GSH enzyme activities in treated rats. Furthermore, lipid peroxidation levels were significantly reduced ($P < 0.05$) in the treatment groups, with the extent of reduction correlating with higher extract concentrations.

Table 1: Effect of n-Hexane and Chloroform Solvent Partition Fractions of *Spondias mombin* on Serum Antioxidant enzymes

Group	CAT (U/mg protein)	SOD (U/mg protein)	GSH (µg/mL)	MDA (µM)
Control	4.75±0.16[a]	9.00±0.18[a]	55.09±0.13[a]	34.10±0.14[f]
H100	4.91±0.00[a]	9.45±0.18[a]	79.87±0.07[d]	30.28±0.25[e]
H200	7.98±0.20[b]	17.14±0.12b	80.18±0.15[d]	27.13±0.11[d]
H400	7.84±0.35[b]	15.79±0.77[b]	95.62±0.16[f]	26.18±0.19[c]
C100	5.56±0.07[a]	10.15±0.07[a]	64.78±0.25[b]	26.21±0.38[c]
C200	9.08±0.26[c]	16.29±0.66[b]	78.89±0.25[c]	24.06±0.17[a]
C400	10.11±0.47[d]	20.35±0.63[c]	86.46±0.24[e]	25.51±0.10[b]

Values are presented as mean ± standard error of mean (SEM) of three replicates. Values with different superscripts along column are significantly different at $p < 0.05$.

3.2Effect of n-Hexane and Chloroform partitioned Fractions of *Spondias mombin* on Egg Albumin-induced Paw Oedema in Rats

The impact of n-hexane and chloroform partitioned fractions of *Spondias mombin* on acute paw edema induced by egg albumin is summarized in Table 2. Injection of egg albumin caused a significant ($P < 0.05$) increase in paw thickness in all animals within the first hour compared to the neutral control group. The peak increase in paw thickness was observed in the negative control group during the second hour post-injection relative to baseline measurements. However, administration of the extracts at doses of 100, 200, and 400 mg/kg led to a significant ($P < 0.05$) reduction in paw thickness compared to the negative control group (which received distilled water) at 2, 4, and 8 hours post-injection, respectively. Additionally, a significant ($P < 0.05$) decrease in paw thickness was observed in all extract-treated groups (100, 200, and 400 mg/kg), as well as in the diclofenac-treated group (5 mg/kg), compared to the negative control group at 24 hours post-injection.

Table 2: Effect of n-Hexane and Chloroform partitioned Fractions of *Spondias mombin* on Egg Albumin-induced Paw Oedema in Rats

	Oedema volume (mm)					
Group	**0hr**	**1hr**	**2hr**	**4hr**	**8hr**	**24hr**
Normal control	0.44±0.02[a]	0.44±0.02[a]	0.44±0.02[a]	0.44±0.02[a]	0.44±0.02[a]	0.44±0.02[a]

Negative control	0.43±0.02^{a}	1.05±0.03^{f}	1.16±0.03^{d}	1.07±0.03^{f}	0.87±0.03^{d}	0.80±0.04^{b}
Positive control	0.42±0.02^{a}	0.91±0.01^{e}	0.72±0.02^{c}	0.56±0.01^{bcd}	0.43±0.02^{a}	0.42±0.02^{a}
H100	0.43±0.01^{a}	0.82±0.02^{bcd}	0.76±0.02^{c}	0.66±0.03^{e}	0.54±0.02^{c}	0.47±0.01^{a}
H200	0.43±0.02^{a}	0.77±0.03^{b}	0.63±0.01^{b}	0.56±0.01^{bcd}	0.46±0.02^{ab}	0.44±0.02^{a}
H400	0.44±0.02^{a}	0.80±0.03^{bc}	0.64±0.01^{b}	0.54±0.01^{bc}	0.47±0.01^{abc}	0.45±0.02^{a}
C100	0.43±0.02^{a}	0.90±0.05^{de}	0.75±0.04^{c}	0.62±0.03^{de}	0.51±0.04^{bc}	0.46±0.01^{a}
C200	0.44±0.02^{a}	0.88±0.04^{cde}	0.73±0.04^{c}	0.60±0.02^{cde}	0.51±0.03bc	0.45±0.01^{a}
C400	0.42±0.02^{a}	0.86±0.02^{bcde}	0.63±0.01^{b}	0.52±0.01^{b}	0.43±0.01a	0.42±0.02^{a}

Values are presented as mean ± standard error of mean (SEM) of three replicates. Values with different superscripts along column are significantly different at $p < 0.05$.

3.3Mean Percentage Inhibition of Egg Albumin-induced Paw Edema in Rats administered n-Hexane and Chloroform partitioned Fractions of *Spondias mombin*

The mean percentage inhibition of egg albumin-induced paw edema in rats treated with n-hexane and chloroform partitioned fractions of *Spondias mombin* is presented in Table 3. The highest inhibition, 42.65%, was observed in the group administered 400 mg/kg of the extract (C400) at 24 hours post-injection, whereas diclofenac exhibited a maximum inhibition of 39.40%.

Table 3: Mean Percentage Inhibition of Egg Albumin-induced Paw Oedema in Rats administered n-Hexane and Chloroform partitioned Fractions of *Spondias mombin*

	% Inhibition at various times (hour)					
Treatment (mg/kg)	1hr	2hr	4hr	8hr	24hr	Mean of %Inhibition
Positive control	13.33	37.93	47.66	50.57	47.5	39.40
H100	21.91	45.69	38.32	37.93	41.25	37.02
H200	26.67	45.69	47.66	47.13	45.00	42.53
H400	23.81	44.83	49.53	45.98	43.75	41.58

C100	14.28	35.34	42.06	41.38	42.50	35.11
C200	16.19	37.07	43.93	41.40	43.75	36.47
C400	18.1	45.69	51.40	50.57	47.5	42.65

Values are presented as mean ± standard error of mean (SEM) of three replicates. Values with different superscripts along column are significantly different at $p < 0.05$.

3.4 Effect of n-Hexane and Chloroform Solvent Partitioned Fractions of *Spondias mombin* on ALT, AST and ALP

The impact of n-hexane and chloroform partitioned fractions of *Spondias mombin* on selected serum biochemical parameters is summarized in Table 4. Egg albumin injection led to a significant increase ($p < 0.05$) in ALT, AST, and ALP levels in the negative control group compared to the neutral control group. However, treatment with *Spondias mombin* n-hexane and chloroform fractions at doses of 100, 200, and 400 mg/kg significantly ($p < 0.05$) reduced the activities of these enzymes relative to the negative control. Notably, at the highest dose of 400 mg/kg, the fractions exhibited a more substantial reduction ($p < 0.05$) in enzyme activities than the reference drug (positive control) when compared to the negative control.

Table 4: Effect of n-Hexane and Chloroform Solvent Partitioned Fraction of *Spondias mombin* on ALT, AST and ALP

Group	ALT (U/L)	AST (U/L)	ALP (U/L)
Normal control	13.31 ± 0.19^{a}	32.95 ± 0.05^{e}	55.98 ± 0.21^{d}
Negative control	35.24 ± 0.58^{h}	45.02 ± 0.53^{h}	66.42 ± 0.53^{f}
Positive control	18.09 ± 0.49^{de}	34.86 ± 0.27^{f}	53.29 ± 0.64^{c}
H100	20.21 ± 0.20^{f}	28.97 ± 0.12^{c}	53.81 ± 0.35^{c}
H200	17.44 ± 0.23^{cd}	27.74 ± 0.17^{b}	49.09 ± 0.21^{b}
H400	14.29 ± 0.18^{b}	23.69 ± 0.15^{a}	44.72 ± 0.36^{a}
C100	23.60 ± 0.21^{g}	35.70 ± 0.14^{g}	59.00 ± 0.38^{e}
C200	18.79 ± 0.19^{e}	29.02 ± 0.41^{c}	56.10 ± 0.14^{d}
C400	16.90 ± 0.24^{c}	30.07 ± 0.21^{d}	49.04 ± 0.23^{b}

Values are presented as mean ± standard error of mean (SEM) of three replicates. Values with different superscripts along column are significantly different at $p < 0.05$.

Key:

H= nHaxane

C= Chloroform

3.5 Effect of n-Hexane and Chloroform solvent partitioned Fraction of *Spondias mombin* on Total protein, Albumin and Bilirubin

Table 5 illustrates the effects of the n-hexane and chloroform solvent-partitioned fractions of *Spondias mombin* on total protein, albumin, and bilirubin levels. The negative control group showed a significant decrease ($P < 0.05$) in total protein and albumin levels, accompanied by a significant increase ($P < 0.05$) in bilirubin levels compared to the neutral control group. Treatment with the fractions resulted in a significant ($P < 0.05$) increase in total protein and albumin levels, with the effect being more pronounced at higher extract concentrations. However, the reference drug-treated group demonstrated a greater improvement in these parameters than the extract-treated groups.

Table 5: Effect of n-Hexane and Chloroform solvent partitioned Fraction of *Spondias mombin* on Total protein, Albumin and Bilirubin

Group	Total protein (g/L)	Albumin (g/L)	Bilirubin (mg/dL)
Normal control	10.61 ± 0.22^{b}	7.72 ± 0.15^{b}	0.85 ± 0.03^{d}
Negative control	8.00 ± 0.29^{a}	5.73 ± 0.23^{a}	0.99 ± 0.01^{f}
Positive control	13.29 ± 0.21^{d}	9.72 ± 0.28^{d}	0.61 ± 0.01^{a}
H100	13.49 ± 0.21^{d}	8.71 ± 0.15^{c}	0.70 ± 0.02^{b}
H200	16.28 ± 0.27^{f}	9.83 ± 0.28^{d}	0.77 ± 0.01^{c}
H400	18.86 ± 0.10^{g}	12.62 ± 0.14^{e}	0.62 ± 0.02^{a}
C100	11.63 ± 0.18^{c}	8.66 ± 0.12^{c}	0.83±0.02
C200	13.40 ± 0.06^{d}	8.29 ± 0.10^{c}	0.81±0.02
C400	15.19 ± 0.17^{e}	9.99 ± 0.08^{d}	0.91±0.02

Values are presented as mean ± standard error of mean (SEM) of three replicates. Values with different superscripts along column are significantly different at $p < 0.05$.

3.6Effect of n-Hexane and Chloroform Solvent Partitioned Fraction of *Spondias mombin* on Creatinine, Urea and Uric acid

Table 6 presents the effects of *Spondias mombin* n-hexane and chloroform solvent-partitioned fractions on creatinine, urea, and uric acid levels. Significant differences ($P < 0.05$) were observed across all treated groups compared to the control. Administration of the n-hexane fraction at 200 mg/kg resulted in a significant ($P < 0.05$) reduction in urea and uric acid levels relative

to the positive control. A similar effect was noted for the chloroform fraction at a dose of 400 mg/kg.

Table 6: Effect of n-Hexane and Chloroform Solvent Partitioned Fraction of *Spondias mombin* on Creatinine, Urea and Uric acid

Group	Creatinine (mg/dL)	Urea (mg/dL)	Uric acid (mg/dL)
Normal control	8.41±0.05^{b}	23.46±0.27^{cd}	5.71±0.10^{a}
Negative control	12.73±0.31^{d}	32.70±0.27^{f}	9.04±0.40^{d}
Positive control	7.49±0.25^{a}	26.02±0.53^{e}	6.53±0.23^{ab}
H100	9.33±0.31^{c}	22.12±0.21^{bc}	5.95±0.46^{a}
H200	8.46±0.18^{b}	24.44±0.40^{d}	6.46±0.22^{ab}
H400	9.33±0.06^{c}	23.19±0.14^{cd}	8.56±0.24^{cd}
C100	9.62±0.20^{c}	21.79±0.48^{b}	7.49±0.23^{bc}
C200	8.91±0.41^{bc}	20.02±0.57^{a}	7.53±0.70^{bc}
C400	8.54±0.13^{b}	21.36±0.76^{b}	5.72±0.24^{a}

Values are presented as mean ± standard error of mean (SEM) of three replicates. Values with different superscripts along column are significantly different at $p < 0.05$.

4.7Effect of n-Hexane and Chloroform Solvent Partitioned Fraction of *Spondias mombin* on Serum Electrolytes

Table 7 presents the effects of *Spondias mombin* n-hexane and chloroform solvent-partitioned fractions on serum electrolytes. The treated groups exhibited a significant ($P < 0.05$) dose-dependent response compared to the negative control. Notably, rats administered 200 and 400 mg/kg of the fractions showed a significant ($P < 0.05$) reduction in electrolyte levels relative to the positive control.

Table 7: Effect of n-Hexane and Chloroform Solvent Partitioned Fraction of *Spondias mombin* on Serum Electrolytes

Group	**Na (mEq/L)**	**K (mEq/L)**	**Cl (mEq/L)**
Normal control	147.80±0.31^{e}	6.59±0.37^{c}	72.48±0.44^{c}
Negative control	155.27±0.56^{g}	4.20±0.41^{a}	79.22±0.57^{e}
Positive control	150.67±0.61^{f}	7.72±0.23^{d}	68.39±0.61^{a}
H100	142.55±0.40^{c}	6.59±0.16^{c}	70.42±0.56^{b}
H200	141.64±0.40^{c}	7.93±0.30^{de}	75.86±0.37^{d}
H400	136.92±0.25^{a}	5.39±0.16^{b}	67.79±0.32^{a}
C100	145.16±0.17^{d}	8.16±0.40^{de}	75.13±0.46^{d}
C200	142.58±0.41^{c}	8.64±0.13^{e}	78.93±0.42^{e}
C400	140.04±0.11^{b}	9.49±0.15^{f}	71.47±0.67^{bc}

Values are presented as mean ± standard error of mean (SEM) of three replicates. Values with different superscripts along column are significantly different at $p < 0.05$.

DISCUSSION

Body weight is a well-established indicator of drug effects and is frequently used to evaluate responses to drug therapy. Additionally, fluctuations in body weight

can serve as early markers of toxicity [14]. In this study, no significant changes in body weight were observed in extract-treated rats. The gradual increase in body weight from week 1 to week 2 (Figure 1) among treated rats, compared to controls, suggests that the leaf extract lacks phytochemicals that influence body weight. This finding is consistent with Gupta *et al*. [15] but contrasts with the results of Odey *et al*. [16]. The overall weight gain observed in both control and treated groups reflects normal growth, aligning with prior research by Aroni *et al*. [17] on the effects of methanol extract of *Albizia lebbeck* leaves on rat body weight. The antioxidant defense system in animals includes key enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH), which neutralize reactive oxygen species (ROS) by converting them into less harmful molecules [5]. This study demonstrated a significant ($P < 0.05$) increase in CAT, SOD, and GSH activity in the treatment groups compared to controls (Table 1). Notably, administration of 200 mg and 400 mg/kg of the extract significantly enhanced these enzyme activities, indicating a strong antioxidant effect. Malondialdehyde (MDA), a crucial biomarker of lipid peroxidation, is produced when free radicals attack polyunsaturated fatty acids in cell membranes [6]. Elevated MDA levels in untreated control rats (Table 1) suggest an overwhelmed antioxidant defense system, leading to oxidative stress and progressive lipid peroxidation. However, MDA levels significantly decreased ($P < 0.05$) in the extract-treated groups, with reductions correlating with increased extract concentration. Table 2 highlights the anti-inflammatory effects of *Spondias mombin* n-hexane and chloroform fractions in the egg albumin-induced paw edema model. At 400 mg/kg (C400), the extract achieved a maximum inhibition of 42.65% after 24 hours, surpassing diclofenac, which showed a 39.40% inhibition rate. Diclofenac, a widely used non-steroidal anti-inflammatory drug (NSAID), alleviates inflammation by inhibiting cyclooxygenase (COX) enzymes, thereby reducing prostaglandin synthesis [4]. The superior efficacy of the extract suggests additional anti-inflammatory mechanisms, possibly involving antioxidant, immunomodulatory, or membrane-stabilizing effects [3]. The egg albumin-induced paw edema model is a widely used method for assessing acute inflammation, involving mediators such as histamine, serotonin, kinins, and prostaglandins [17]. The observed reduction in edema suggests that the extract interferes with these inflammatory mediators. The anti-inflammatory activity of *Spondias mombin* is likely attributed to the presence of flavonoids, tannins, and triterpenoids, which are known for their anti-inflammatory properties [1]. Similar findings were reported by [2], who demonstrated the efficacy of hydroethanol extract (HELSM) of *S. mombin* in an oral mucositis model. Additionally, Silva *et al.* [18] found that chondroitin sulfate derived from *Oreochromis niloticus* waste significantly reduced leukocyte migration in a peritoneal inflammation model. Injection of egg albumin led to a significant ($P < 0.05$) increase in ALT, AST, and ALP levels in the negative control group compared to the neutral control, indicating liver damage [19,20]. However, administration of *Spondias mombin* extracts at 100, 200, and 400 mg/kg significantly reduced ($P < 0.05$) these enzyme activities compared to the negative control, with the highest dose (400 mg/kg) showing a more pronounced effect than the reference drug. This dose-dependent response aligns with findings in phytomedicine, where higher concentrations of bioactive compounds enhance therapeutic effects within non-toxic limits [7]. Similar results were reported by Calderon et al. [21], who demonstrated that *Spondias mombin* extracts did not alter serum ALT, AST, or ALP levels, suggesting the absence of hepatotoxicity. The hepatoprotective effect observed is likely due to the presence of antioxidants that mitigate oxidative stress and support liver function. A significant decrease ($P < 0.05$) in total protein and albumin levels and an increase in bilirubin were observed (Table 5), suggesting liver dysfunction [22]. These markers indicate impaired hepatic synthesis and excretory function. Similar findings by Aboraya *et al*. [23] demonstrated that plant-based polyphenols restored protein and bilirubin levels in liver injury models. However, the reference drug showed superior efficacy, likely due to its targeted pharmacological action [24].

Significant differences ($P < 0.05$) were observed across treated groups in renal function markers compared to controls (Table 6). Elevated urea and uric acid levels indicate kidney dysfunction due to impaired filtration

[25,26]. However, treatment with 200 mg/kg of the n-hexane extract significantly reduced these levels, suggesting improved renal function, possibly through reduced oxidative stress or enhanced clearance. The chloroform extract at 400 mg/kg exhibited a similar effect, likely reflecting differences in the bioavailability of active compounds. The nephroprotective potential of Spondias mombin may be attributed to flavonoids, alkaloids, and tannins, which have antioxidative and anti-inflammatory properties [27]. These findings align with previous studies demonstrating the beneficial effects of polyphenol-rich extracts on renal dysfunction [28]. Electrolytes play essential roles in enzymatic activation, nutrient uptake, and hormonal balance [29]. The study revealed a dose-dependent effect of Spondias mombin extracts on electrolyte levels, with significant reductions at 200 mg/kg and 400 mg/kg doses compared to the positive control (Table 7). This suggests a regulatory effect on electrolyte homeostasis, potentially useful in managing electrolyte imbalances. The impact may result from phytochemicals influencing renal reabsorption or secretion processes [30]. Similar findings in phytotherapy indicate that plant extracts modulate renal and systemic electrolyte balance, as demonstrated by Ondua *et al*.[31], who reported that polyphenol-rich extracts reduced electrolyte imbalances in renal dysfunction models.

CONCLUSION

Overall, this study highlights the therapeutic potential of Spondias mombin extracts, particularly in their antioxidant, anti-inflammatory, hepatoprotective, nephroprotective, and electrolyte-modulating properties. These effects may be attributed to bioactive phytochemicals such as flavonoids, tannins, and saponins, which exert beneficial physiological actions.

Conflict of Interest

The authors declare no conflicts of interest.

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