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Haematological, hepatic and oxidative stress indices of female Wistar rats treated with ethanolic leaf extract of *Spondias mombin*

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Abstract

Local population utilize the medicinal herb *Spondias mombin* to cure a variety of illnesses. In this study, the toxicity of an ethanolic leaf extract of *Spondias mombin* was investigated using haematological, hepatic, and oxidative stress parameters in female wistar rats. Three sets of five rats each were formed from fifteen (15) female rats weighing 117g to 210g. Group A served as control, Group B received 100mg/kg, and Group C received 200mg/kg body weight of ethanolic leaf extract. The extract was administered orally for fourteen (14) days. At the completion of the treatment, blood and tissue samples were collected for haematological, hepatic enzymes and histological examination. The result showed no significant ($p>0.05$) difference in body weight of rats. However, a significant ($p<0.05$) reduction was observed in the organ (liver) weight of rats treated with the extract. Packed cell volume, haemoglobin concentration, Red Blood Cell and white blood cell values were significantly ($p<0.05$) increased after 14days of treatment with ethanolic leaf extract *Spondias mombin*s. Liver enzymes aspartate transaminase and alanine transaminase showed no significantly changes when compared with the control. However, alkaline phosphatase was significantly increased in the extract treated female rats. There was a significant ($p<0.05$) increase in superoxide dismutase and a significant ($p<0.05$) decrease in malondialdehyde levels compared with the control. Rats administered with the plant extract showed no histological alterations in the liver sections. This result confirms the haematinic potential of *Spondias mombin* plant, and prolonged and high dosages may cause liver damage.

Keywords: Haematological Indices; Liver Enzymes; Oxidative stress markers; *Spondias mombin*

1. Introduction

Since the beginning of time, humans have depended on nature to provide for their basic needs, including the production of food, shelter, clothing, and medicines for their survival and well-being [1]. Plants serve as the foundation for complex conventional medicinal procedures that have been employed for thousands of years and continue to give mankind new treatments [2]. Medicinal plants are frequently utilized in traditional settings for treating illnesses in low-income countries all throughout the world, particularly in the tropics [3-4]. Many modern medications are created from plants through studies of indigenous peoples' traditional healing practices [5].

Recent studies on the use of tropical medicinal plants indicate that traditional medicine is extensively utilized to treat and manage a variety of conditions in West African nations [6]. According to Omage *et al*. [7], these medicinal plants

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contain a number of phytochemicals and nutraceuticals that are used in the development and synthesis of modern drugs. They also play key roles in reducing the prevalence of many illnesses by enhancing the activities of various organs in the human body [8].

Spondias mombin, often known as yellow mombin or hog plug, is one of the most well-liked medicinal herbs in Southwest Nigeria. The Anacardiaceae family includes it [9]. *Spondias mombin* has high levels of vitamins, minerals, and bioactive phytochemicals, such as flavonoids (3.00%), tannins (3.82%), cardiac glycosides, saponins (7.60%), and phenolic derivatives (1%), which have anti-herpes and antioxidant properties [10-11]. Traditional medicine has long valued S. mombin's nutritional and medicinal qualities [12]. *S. mombin* fresh leaves in Southern Nigeria are used as a typical midwife's treatment to stimulate labor, reduce bleeding, ease pain during and after childbirth, and encourage the flow of milk, and prevent or treat uterine or vaginal infections after childbirth [13].

Several animal studies have shown the antioxidant, anti-inflammatory, and anti-denaturation [14], antimicrobial [15], hepato-protective [16], anxiolytic effect, sedative, anti-epilepticand antipsychotic effects of the leaves extract [14]. *S. mombin* has been shown to have a significant hematinic [13] and anti-anaemic properties [17], hence helpful in the management of anemia. Igwe and coworker, [18] also reported the lipid lowering effect of aqueous leaf extract of *Spondias mombin.* Given the diverse ethnomedicinal uses of the plant, such as anti-anaemic effects, anti-inflammatory, hepatoprotective and reported anti-fertility effects, this present study was carried out to further investigate the safety of *S. mombin* leaves using haematological, hepatic and oxidative stress indices of Female Rats.

2. Material and methods

2.1. Plant Material

Spondias mombin leaves were collected from the bushes in Amassoma Community of Southern Ijaw Local Government Area of Bayelsa State. The leaves were taken to the Department of Biological Science Niger Delta University, where identification and authentication was done by a botanist.

2.2. Plant Extract Preparation

The ethanolic extract of S. mombin leaf was prepared according to the method described by Igwe et al. [18]. The fresh leaves were cut from the stems' stalks, cleaned under running water to get rid of the dirt, and dried in the air at room temperature. The dried leaves were grinds into a powder using a mechanical grinder (Heman, Japan), weighed (1.5kg of powder), and stored in an airtight container before extraction. A beaker containing 35000 ml of 75% ethanol was filled with around 1.4 kilogram of the powdered leaves. The mixture was stirred in every three hours for proper mixing and allowed to stand for 24 hours. The resultant decoction was filtered, and the filtrate was heated to a controlled temperature of 60°C on a hot plate to slowly but completely evaporate the solvent. The extract was packaged in an air-tight container, labeled and stored below 4°C in a freezer until required [18]

2.3. Experimental Animals

In the experimental study, fifteen apparently healthy adult female Wister rats weighing between 117g and 210g were procured from the animal house of the Department of Pharmacy, Niger Delta University, Bayelsa State. The rats were carried to the Department of Medical Biochemistry while still in cages, where they were given two (2) weeks to acclimatize. For a period of four weeks, they were fed with regular feed (pellet) and purified water. The Institutional Animal Ethical Committee's (IAEC) guidelines for the control and supervision of animal research (CPCSEA) were followed in all operations.

2.4. Experimental Design

The fifteen albino rats were randomly divided into three (3) groups with 5 rats in each group as follows:

- Group I: (Control): Received distilled water and pellet feed for fourteen (14) days.
- Group 2 (Test group): Received 100 ml/kg per body weight of ethanolic leaf extract of *Spondias mombin* by oral gavage for 14 days.
- Group 3 (Test group): Received 200 ml/kg per body weight of ethanolic leaf extract of *Spondias mombin* by oral gavage for 14 days

2.5. Sample Collection

After receiving *S. mombin* therapy for 14 days, the final body weight of the rats was taken and the rats were anaesthetized using chloroform and sacrificed. Blood samples were collected via cardiac punctures and dispensed in Ethylene Diamine Tetra-acetic Acid and plain containers. The blood in the plain blood was allowed to clot properly at room temperature and then centrifuged for 10 minutes at 3000 rpm to separate the serum for Aspartate transaminases (AST) and Alanine transaminase (ALT). The blood in Ethylene Diamine Tetra-acetic Acid container was used for haematological analysis. Thereafter, the rats were dissected and the liver was excised and clean in normal saline, and part of it was placed in a sample bottle containing 10% formalin for histological examination. After being weighed, 10grams of liver tissue were homogenized in 0.1 M Tris buffer (pH 7.4) and centrifuged for 10 minutes at 3000 rpm. Malondialdehyde levels and superoxide dismutase (SOD) activities were all measured using this homogenized liver solution.

2.6. Analysis of Biochemical and Haematological Indices

2.6.1. Measurement of Liver Function Indices

The activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were estimated using spectrophotometric method as described by Frankel and Reitman [19]. Using phenolphthalein monophosphate as a substrate, Plummer's [20] spectrophotometric approach was used to measure the alkaline phosphatase (ALP) activity.

2.6.2. Measurement of Haematological Indices

Packed cell Volume (PCV), Haemoglobin (Hb), Red Blood Cell (RBC) and total white blood cell (WBC) were determined by SYSMEX pocH-100i automated haematological analyzer (Mindray BC 2300, USA) in accordance with Soldin et al. [21]. This device count blood cell using the Direct Current detection method, haemoglobin analysis by non-cyanide haemoglobin analysis method and calculates the red blood cell constants automatically from red blood cell count, Haemoglobin and Packed Cell Volume.

2.6.3. Measurement of Oxidative Stress Markers

The Misra and Fridovich [22] method was used to evaluate the activity of superoxide dismutase (SOD). Its principle is based on the superoxide dismutase's capacity to prevent the photochemical reduction of nitroblue-tetrazolium (NBT). After being set at 420 nm, the spectrophotometer was zeroed with 3.0 cc of distilled water. The reference tube received 0.2 ml of distilled water, and the appropriate enzyme extracts were poured into the appropriate test tube with the appropriate label. After equilibrating at room temperature, 2.5ml of the phosphate buffer was poured into each of these tubes. The reference solution and each of the test solutions were then each given 0.3ml of the 0.3mM adrenaline solution. This was then allowed to mix, and the absorbance at 420 nm was measured. The enzyme activity is expressed as U/mg protein.

Lipid peroxidation activities was measured as an index of malondialdehyde formation by Shah and Walker‟s. [23] method. Malondialdehyde was determined as a conjugate with TBA. TCA was used to precipitate proteins, which were subsequently centrifuged to be eliminated. The MDA-TBA complex was measured at 534 nm. 200 ul of supernatant of liver homogenate was added and briefly mixed with 1 mL of 50% trichloroacetic acid in 0.1 M HCl and 1 mL of 26 mM thiobarbituric acid. It was mixed properly and samples were maintained at 95 °C for 20 min, and centrifuged at 960g for 10 min and measurement were taken spectrophotometrically at 532 nm. The results were expressed as U/mg protein.

2.6.4. Histopathological Analysis

The liver tissues were processed in accordance with the accepted histological processing protocol described by Aviwioro et al [24]. Liver tissues excised from rats were fixed in 10% buffered formalin and cut into pieces of size 4mm using rotary microtome, and stained with Erhlich's hematoxylin and eosin. The Olympus® digital microscope (Olympus BX60MF, Japan) was used to obtain photomicrograph at a magnification of x400.

2.7. Statistical Analysis

Data obtained from the study were expressed as mean ± standard deviation. The statistical significance was evaluated by T-test using SPSS (Statistical Package for Social Sciences). Values with $p<0.05$ were considered statistically significant.

3. Results

Results from (table 1) indicate that there was no change in the body weight of *Spondias mombin*-treated rats compared to the control group (193.19±3.31 and 197.09±3.10 versus 191.23±1.67), which is considered not significant (p>0.05). The weight of the liver in the rats administered with *Spondias mombin* (groups II and III) (9.70±0.55 and 9.68±0.11) was significantly lower (P<0.05) than in the control group (group I) (9.92±0.56). Results from (Table 2) shows that there is no significant (P>0.05) difference in Packed cell volume (PCV), Red blood cell (RBC), Haemoglobin (Hb) and total white blood cell (WBC) count of *Spondias mombin* administered rat (groups II) (37.19±1.71; 9.18±0.15; 8.75± 0.13 and 7.62± 0.14 respectively) when compared with the control group (26.32±1.67; 6.4 ± 0.27; 6.70± 0.2; 6.67± 0.12) respectively. However, there was statistically significant (P<0.05) increase in PCV, RBC, Hb and WBC count in *Spondias mombin* administered rats (Group III) in comparison with the control.

Table 1 Effects of Ethanolic leaf extracts of *Spondiasmombin* on Bodyand Organ (liver) Weight ofFemale albino Rats

Group	Body weight (g) (X±SD)	Liver (organ) weight (g) (X±SD)	Liver weight/ body weight ratio x 100(%)
Group I	191.23±1.67[a]	9.92±0.56[a]	1.93[a]
Group II	193.19±3.31[a]	9.70±0.55[b]	1.99[b]
Group III	194.09±3.10[a]	9.68±0.11[c]	1.21[c]

Significantly different (p<0.05). Group I= Control (Feed and water); Group II = *Spondias mombin* (100mg/kg body weight).Group III= *Spondias mombin* (200mg/kg body weight).

Table 2 Effects of Ethanolic Extracts of *Spondias mombin* on Haematological Indices; Packed Cell Volume, Red Blood Cell, Haemoglobin and White Blood Cell count of Female albino Rats

Group/ Parameters	PCV (%)(X ± SD)	RBC (x 10^6 /L) (X ±S D)	Hb (g/dl) (X ±SD)	WBC (x 10^6 /L) (X ±S D)
Group I	36.32±1.67[a]	6.4 ± 0.27[a]	6.70± 0.2[a]	6.67± 0.12[a]
Group II	37.19±1.71[a]	9.18±0.15[a]	8.75± 0.13[a]	7.62± 0.14[a]
Group III	40.09±1.80[b]	12.32±1.21[c]	9.15± 0.32[b]	13.44 ± 0.57[c]

Key: Data are represented as Mean ± SD; (n=5); mean values with different superscript letter (s) down the column are significantly different (p<0.05). Group I= Control (Feed and water); Group II = *Spondias mombin* (100mg/kg body weight). Group III= *Spondias mombin* (200mg/kg body weight).

Table 3 Effects of Ethanolic Extracts of *Spondiasmombin* on Hepatic Indices; Aspartate transaminase (AST), Alanine transaminase (ALT) and Alkaline phosphatasen of Female albino Rats

Group/ Aspartate Parameters	Transaminase (U/L) (X ± SD)	Alanine Transaminase (U/L)	Alkaline phosphatase (U/L)
Group I	39.21±2.42[a]	42.47± 1.43[a]	27.51 ± 1.50[a]
Group II	38.19±2.14[a]	43.45± 0.71[a]	30.33 ± 1.42[b]
Group III	37.10±2.20[a]	43.12±0.31[a]	35.51 ± 3.57[c]

Key: Data are represented as Mean ± SD; (n=5); mean values with different superscript letter (s) down the column are significantly different (p<0.05). Group I= Control (Feed and water); Group II = *Spondias mombin* (100mg/kg body weight). Group C= *Spondias mombin* (200mg/kg body weight).

Results from (table 3) shows that there is no significant (p<0.05) difference in AST and ALT in the *Spondias mombin* treated rats (group II) (38.19±2.14; and 43.45± 0.71 respectively) when compared with the control group (39.21±2.42 and 42.47± 1.43 respectively). However, ALP showed a significant (p<0.05) increase in group II and III when compared with the control (group I). Table 4 shows a significant (p<0.05) increase in SOD and a significant (p<0.05) decrease in malondialdehyde levels in the *Spondias mombin* treated rats compared to the control. Figure 1 shows the histological sections of the liver of the control group. In the result, the liver section shows normal morphology. The central vein (CV), hepatocytes (H) as well as sinusoidal space (S) appears normal. Figure 2 and 3 shows the histological of liver of

rats treated with 100mg/kg and 200mg/kg body weight of *Spondias mombin* respectively. The section shows normal section of the liver with a central vein and polygonal shape hepatocytes and also normal sinusoid.

Table 4 Effects of Ethanolic Extracts of *Spondiasmombin* on Superoxide dismutase (SOD) and Malondialdehyde (MDA) of Female albino Rats

Group/ Parameters	Superoxide dismutase (U/mg protein) (X ± SD)	Malondialdehyde (nmol/g protein) (X ± SD)
Group I	5.86±0.10a	399.49± 3.47a
Group II	7.07±0.32b	384.89± 3.18b
Group III	10.29 ±0.70c	388.42±3.31c

Key: Data are represented as Mean ± SD; (n=5); mean values with different superscript letter (s) down the column are significantly different ($p<0.05$). Group I= Control (Feed and water); Group II = *Spondias mombin* (100mg/kg body weight). Group C= *Spondias mombin* (200mg/kg body weight).

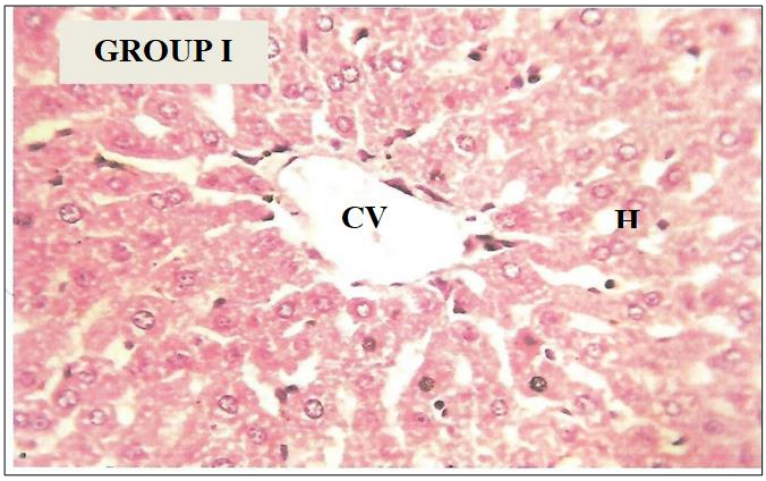

Figure 1 Control (saline water). Microphotograph of liver section showing normal morphology of the liver, central vein (CV), hepatocytes (H) with intact sinusoidal space (S) (Haematoxylin and Eosin X40 Magnification)

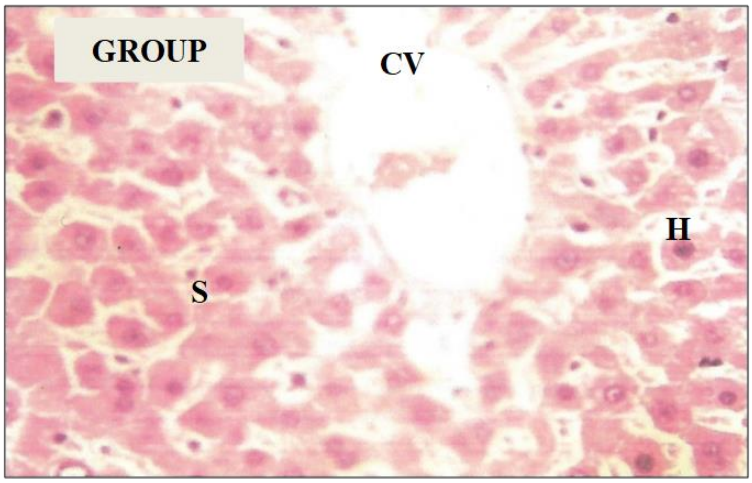

Figure 2 Microphotograph of liver from ratstreated with 100mg/kg body weight of *Spondias mombin*. Slide showing normal section of the liver with a central vein (CV) and polygonal shape hepatocytes (H) and also normal sinusoids. (Haematoxylin and Eosin X40 Magnification)

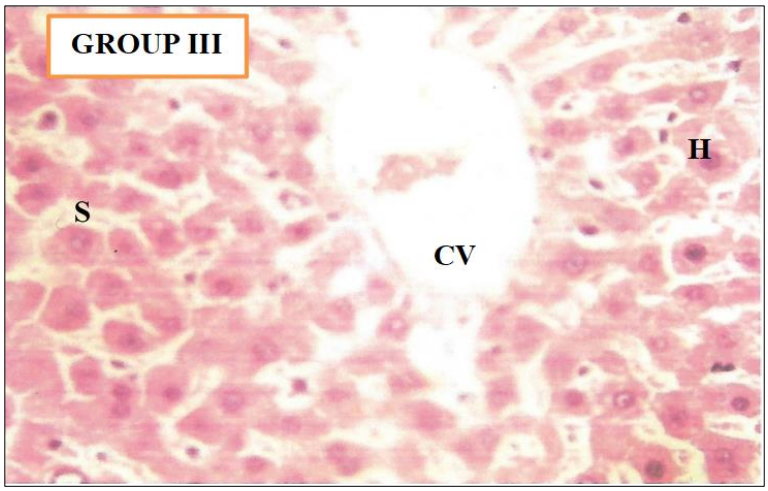

Figure 3 Microphotograph of liver from rats treated with 200mg/kg *Spondias mombin*. Slide shows normal section of the liver with a central vein and polygonal shape hepatocytes and also normal sinusoid. (Haematoxylin and Eosin X40 Magnification)

4. Discussion

Natural remedies are high in demand than ever, particularly in tropical regions. Herbal medicine could be used as a complementary or alternative medicine to the synthetic drugs and this requires more laboratory investigations on their pharmacology activities. Several degenerative diseases are linked with oxidative stress. Due to their excellent therapeutic benefits, plants from the genus *Spondias* were commonly used in traditional medicine. This is attributed to their diverse bioactive phytochemical elements such as phenolic and flavonolds which have strong antioxidant activity and thus prevent degenerative diseases [25].

Treatment of diseases associated with the blood and liver is exceedingly vital and requires extensive care. Despite the fact that there are several herbal treatments for liver issues that have been described, only few of them have had their effectiveness tested pharmacologically. It is believed by most people that since herbal remedies are natural they are nontoxic. However, there have been accounts of natural remedies becoming poisonous. Even scientifically proven haematinic plants have been shown to have toxins [26-27]. Thus, the current study was designed to investigate the safety of *S. mombin* leaves using haematological, hepatic and oxidative stress indices of Female wistar Rats.

The present study revealed that there was no significant ($P>0.05$) difference observed in the body weight of *Spondias mombin* treated rats as compared to the control group. This implies that the extract suppressed body weight, which could result from loss of appetite. This is similar to previous works by Leon et al., [25] and Pakoussi et al. [28]. The finding is inconsistent with the work of Nwaogwugwu and colleagues, [29], who reported a significantly reduced body weight of spondias administered rats. However, the organ (liver) weight was significantly ($P<0.05$) reduced in the *Spondias mombin* treated rats compared to the control group. This is consistent with Olaitan and co-workers, [30], who reported a significant reduction of visceral organs (liver, spleen and kidney) of *Spondias mombin* administered rats.

Haematological indices are of diagnostic importance in the routine clinical assessment of the state of health of humans and animals [31]. The results from table 3 demonstrated an increase in the values of haematological indices with increase in concentration of the plant extract. A statistically significant ($P<0.05$) increase was observed in the values of packed cell volume and haemoglobin levels in the test rats against the control group. It is reported that increase in haemoglobin level usually comes with a corresponding increase in packed cell volume [32]. The increase in Packed Cell Volume and haemoglobin levels may be suggestive of the normal physiological activities of the bone marrow [33]. This implies that the *Spondias mombin* extract may have the capacity to stimulate the production of red blood cells (erythropoiesis). Red blood cell value was significantly ($p<0.05$) increased in the test rats as compared to the control group. This is consistent with the works of Nwaogwugwu et al. [29] and Asuquo *et al.* [33], which reported an increased value of RBC in *Spondias mombin* extract treated rats. This elevation in RBC implies that the extract may have the potential to stimulate the release of erythropoietin from the kidney, which plays a vital role in RBC production [34]. White Blood Cell count value was significantly ($p<0.05$) increased in the extract treated rats when compared with the control group. This is inconsistent with the work of Igwe and colleague [18] who reported non-significant difference in haematological parameters of *Spondias mombin* treated albino rabbits. The increase in white blood cell could be

attributed to presence of antioxidant phytochemicals which has been reported to protect lymphocytes and reduce oxidative DNA damage in WBCs, therefore resulting in the observed increase in white blood cell [35].

Additionally, the observed increase in the haematological indices could be attributed to the reported phytochemicals of the extract like flavonoids and anthraquinones that have been confirmed to possess hematopoietic properties [36]. It could also be associated with the chemical composition of the leaves of *S. mombin* which includes protein, fat, ascorbic acid, calcium, iron, vitamin A, thiamine, riboflavin and nicotinamide, which are directly and indirectly involved in blood cell production [12].

It has been indicated that aspartate aminotransferase (AST) and alanine aminotransferase (ALT) are the specific clinical indicators of liver injury or damage [37]. However, several researchers have confirmed that ALT is more specific than AST in liver damage [38-39]. In the present study, no significant ($p>0.05$) difference was observed in AST and ALT in the *S. mombin* treated rats (group II & III) when compared with the control group. However, ALP showed a significant ($p<0.05$) increase in group II and III when compared with the control. The non-significant increase of these enzymes may imply that extracts of *Spondias mombin* is safe and non-hepatotoxic. This is in agreement with the works of Asuquo et al. [33], who reported a non-significant ($p>0.05$) increase in AST and ALT and a significant ($p<0.05$) increase in ALP of *S. mombin* treated rats. The result disagreed with the works of Nwaogwugwu et al. (2018), who demonstrated significantly elevated AST and ALT activities in *Spondias mombin* treated rats. The elevated ALP could be helpful in diagnosing hepatobiliary or cholestatic obstruction. This could be attributed to the presence of antioxidants in *S. mombin* [40]

Free radical generation can be prevented by turning on redox regulating enzymes like catalase, superoxide dismutase, and glutathione peroxidase as well as radical scavengers and chain terminators like vitamins C and E. S. *mombin* has a multitude of phytochemicals with known antioxidant properties, including the tannins, saponins, alkaloids, flavonoids, phenols, and ascorbic acid [25]. The present study showed a significant ($p<0.05$) increase in superoxide dismutase and a significant ($p<0.05$) reduction in malondialdehyde (MDA) levels in the *S. mombin* treated rats.

The histopathological result revealed that the liver section shows normal hepato-histo-architecture. The central vein, hepatocytes as well as sinusoidal space appears normal in the rats treated with 100 mg/kg and 200 mg/kg body weight of S. *mombin*. The absence of histological changes in the liver alongside liver function test shows that *S. mombin* may not adversely affect the liver. This result confirms the non-hepatotoxic effect of the ethanolic leaf extract of *S. mombin.*

5. Conclusion

The haematinic effect of *Spondias mombin* leaf extract has been confirm by this study. After fourteen days of extract administration, the haematological indices Packed Cell Volume, Red Blood Cell, Haemoglobin, and White Blood Cell levels were significantly increased. The study also revealed a non-significant increase in of AST and ALT levels. ALP activity was significantly elevated in rats treated with the extracts for 14days. SOD activity was significantly increased, while MDA levels was decreased in the rats treated with the plant extract. No histopathological changes were observed in the liver sections of rats treated with the plant extract, thus confirming the biochemical results of liver enzymes.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest.

Statement of ethical approval

The study protocol was approved by the Ethical and Research Committee of Niger Delta University, Bayelsa State, Nigeria. The ethical principles for medical research involving animal subjects as outlined in the Helsinki declaration in 1975 and subsequent revisions were strictly followed in the course of this study.

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