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Research Article

Gastroprotective Effect of Methanol Extract of *Spondias mombin* Stem Bark in Acidified Ethanol-Induced Ulcer in Rats

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ABSTRACT

Ulcer is one of the major diseases faced by the world population. This disease comes with gastrointestinal discomfort and sometimes leads to complications if untreated. Many synthetic drugs are helpful, but they are associated with many side effects. Therefore, there is a need to search for natural herbal medications that are potent and safe for ulcer treatment. This study evaluated the ulcer protective effect of methanol extract of *Spondias mombin* stem bark using acidified ethanol-induced ulcer model. Twenty-four adult rats of 82 – 130g body weight were divided into six groups of four rats per group (n=4). Animals were treated with varying doses (100, 200 and 500 mg) per kilogram body weight of methanol extract of *S. mombin* stem bark for two weeks. Groups treated with 100, 200 and 500 mg/kg b.w of methanol extract of *S. mombin* stem bark extract showed dose-dependent percentage ulcer protection of 16, 42 and 66% respectively compared to the untreated disease control group with zero percentage ulcer protection. Group treated with 500 mg/kg b.w of extract has total gastric acidity of 7.80 ± 1.72 mmol/L, which showed a significant ($P < 0.05$) decrease in total gastric acidity compared to the untreated group with total acidity of 17.75 ± 4.79 mmol/L. Methanol extract of *S. mombin* stem bark possesses ulcer protective effect against mucosal damage by acidified ethanol-induced ulcer in rats. This supports the claim of traditional herbal use of *S. mombin* stem bark for ulcer treatment.

Keywords: *Spondias mombin, Gastric Ulcer Index, Mucosal Necrosis, Percentage Ulcer Protection*

INTRODUCTION

A gastric ulcer is a type of peptic ulcer disease that affects the digestive system (Bi *et al*., 2016). Muscular layer below the mucous membrane in the stomach's inner wall deteriorates due to intense wear and tear. This leads to significant discomfort. Compromised mucosal integrity leads to perforation and bleeding, potentially resulting in death (Thabrew and Arawwawala, 2016). Etiological risk factors for gastric ulcers involve Helicobacter pylori infection, extended use of NSAIDs, stress, alcohol consumption, and other chemicals (Minyaylo *et al.,* 2021). The drugs currently used for treating ulcers in Nigeria are: Cimetidine, Omeprazole, and antacids. Surviving on synthetic drugs can be expensive, costing more than people might expect. Not all these drugs are entirely safe because of their potential side effects, including kidney issues (Essex *et al* 2013). To solve this issue, we must look for cheap alternatives with less adverse effects from natural plant sources (Nethaji *et al*., 2013). *Spondias mombin* Linnaeus. (Hog plum) belongs to the Anacardiaceae family and can be found mainly in tropical rain forests and along coastlines (Mabeku *et al*., 2017). *Spondias mombin* is a vital African herbal remedy for treating stomach pain and promoting wound recovery (Ayoka *et al*., 2008; Ibegbu *et al*. 2018).

Numerous studies indicate that *S. mombin* possesses beneficial effects in treating bacterial infections (Corthout *et al*., 1994); antimalarial (Caraballo *et al*., 2004); abortifacents were mentioned by (Uchendu *et al.*, 2008); enhanced memory (Asuquo *et al*., 2013); improved respiration (Bussman and Glenn, 2010) and ulcer-fighting qualities (Akinlolu *et al.*, 2014).

Despite the growing use of various parts of *S. mombin* for therapy, there remains scarce scientific data regarding its stem bark's pharmacological effects on ulcers or similar studies employing acidified ethanol-induced ulcer models. The objective of this paper is to evaluate the ulcer protective potential of methanol extract of *S. mombin* in acidified ethanol-induced ulcer rats.

MATERIALS AND METHODS

Preparation of methanol extract of *Spondias mombin* stem-bark

The stem bark of the plant was collected from Amorji-Nike Abakpa in Enugu State, Nigeria. The plant was identified by a taxonomist, Mr. Alfred Ozioko, at the International Centre for Ethnomedicine and Drug Development (INTERCEDD) Nsukka, Enugu State, Nigeria and the Voucher number is INTERCEDD/D11/001.

The plant samples were air-dried at room temperature (27 ± 2 °C), then pulverized using a mill grinder. The powdered sample (600g) was extracted by cold maceration with 2.5L of methanol for forty-eight (48) hours and then filtered with fine muslin cloth followed by filter paper (Whatman No.1). The filtrate was concentrated at 60°C in a water bath which yield 31.5g of methanol extract in air tight plastic bottle and was stored in fridge at 4°C until needed for use.

Experimental animals

A total of twenty-four (24) adult Wistar rats of body weights (82.00 g – 130.00 g) were obtained from the animal house of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka. Animals were divided into six groups of four rats per group. The animals were maintained in clean cages to prevent coprophagy. Grower feeds and water were given *ad libitum* under standard conditions (27 ± 2°C) at 12-hour light/dark cycle. All animals were acclimatized for two weeks before use. Ethical clearance was obtained from the Faculty of Biological Sciences Ethics and Biosafety Committee, University of Nigeria Nsukka, with the approval number UNN/FBS/EC/1021.

Qualitative phytochemical screening

Qualitative phytochemical screening aimed at detecting bioactive constituents present in the plant using the procedure outlined by Sofowora (1993), Trease and Evans (2002) and Harborne (1998).

Effects of *Spondias mombin* stem bark on the gastric indices of acidified ethanol-induced gastric ulcer in rats

Stomach ulcer was induced on the experimental animals according to the modified method of Mizui and Doteuchi (1983). Animals were grouped into six groups of four rats in each group. Group 1 is the normal control rats received 5 ml/kg b.w of normal saline, group 2 is disease control received 5 ml/kg b.w of normal saline before ulcer induction, group 3 is the standard control orally received omeprazole (50 mg/kg b.w) and group 4 - 6 were the test groups received (100, 200 and 500 mg/kg b.w) of methanol extract of *Spondias mombin* stem bark respectively for two weeks. Animals were afterwards fasted for 24hrs leaving water *ad libitum*. Water was removed 2 hrs before treatment with different doses of extract. Then, one hour after drug administration, animals (Group 2 - 6) were induced by oral administration of 5 ml/kg b.w of acidified ethanol (0.15M HCL/ absolute ethanol; volume ratio of 40 ml of 0.15M HCL: 60 ml of ethanol).

One hour after induction with acidified ethanol, blood samples were collected for some biochemical tests. The experimental animals were sacrificed, the stomachs opened along the greater curvature and rinsed with clean water. The stomach was pinned on a board to score the ulcer lesions. The ulcer index was then calculated. Ulcer scores were graded as described by Anosike and Ofoegbu (2013). Protection index (% inhibition) was calculated according to the method (Ribeiro *et al*., 2015). Gastric ulcer protection was evaluated thus;

$$\% \text{ Ulcer inhibition} = \frac{(\text{Ulcer index in Control} - \text{Ulcer index in test})}{\text{Ulcer index in Control}} X\ 100.$$

Determination of the gastric pH and volume

The stomach was excised carefully, and the luminal contents were removed. The stomach content was drained into a centrifuge tube, the tube and its content were spun at 1000 rpm for 10 minutes to obtain the supernatant. The volume of the supernatant was measured and recorded. The pH of the supernatant was also recorded using a digital pH meter (Katary and Salahuddin 2017).

Determination of the gastric total acidity

This was carried out according as described by (Kulkarni 1999). The stomach was excised, and the luminal contents were removed. The gastric content was drained into a centrifuge tube and spun at 1000 rpm for 10 minutes to obtain the supernatant. The supernatant obtained after centrifugation was titrated with 0.01N NaOH using thymol blue as an indicator until a yellow color was observed. Titration continued until blue color appeared, and the total volume of the alkaline solution used (0.01N NaOH) was recorded. This indicates total acidity.

Preparation of serum/plasma from blood sample

The collected blood sample was centrifuged at a relative centrifugal force (RCF) at 4°C for 10 minutes to separate the upper layer (serum) for biochemical analysis.

Analysis of liver, kidney and oxidative stress parameters

The activities of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) were estimated by the method (Reitma and Frankel, 1957) and (Klein *et al*., 1960), respectively. Superoxide dismutase (SOD) (Misra and Fridovich, 1972). Catalase assay (Aebi, 1983); Urea, creatinine and total protein (Wybenga *et al*., 1971). All were determined using Randox reagents.

Histology of gastric lesion

The stomach samples were fixed in 10% phosphate buffered formalin for 48 hours before tissue preparation processes. The tissues were cut to a required size and dehydrated in 4 grades of alcohol from lower to higher grade. Then tissues were cleared in 3 grades of xylene and placed in molten wax. The blocks were sectioned on solidification and incubated at 60˚C for 30 minutes. The sectioned tissues were cleared in 3 grades of xylene, and then rinsed with another 3 grades of alcohol from higher concentration to lower concentration and stained with Haematoxylin. This was followed by bleaching and differentiation with ammonium chloride and 1% acid alcohol, respectively. The slides were examined with a Motic™ compound light microscope. The photomicrographs were taken using a Motic™ 9.0 megapixel microscope camera at x 160 magnification.

Statistical analyses

Data obtained were analyzed by one-way analysis of variance (ANOVA) using statistical product and service solutions (SPSS) Version 20. The results were expressed as Mean ± SD. P < 0.05 was considered to be statistically significant.

RESULTS

Methanol Extract of *Spondias mombin* stem bark contains phytochemicals in varying amounts. However, Phenols and Flavonoids were not detected (Table 1). In addition, treatment of ulcerated animals with methanol extract of *Spondias mombin* stem bark was able reduce the level of stomach injury, total acidity and hence improved the gastric pH level and overall gastric percentage protection against acidified ethanol; an attacking agent (Table 2).

Table 1: The Phytochemical Screening of Methanol Extract of *Spondias mombin* Stem Bark

Constituents	Availability
Tannins	+ + + +
Saponins	+ + +
Alkaloids	+ +
Terpenoids	+
Steroids	+
Glycosids	+
Hydrogen cyanide	+
Reducing sugar	+
Carbohydrates	+
Phenols	-
Flavonoids	-

+ + + + (abundantly present)
+ + + (present in very high concentration)
+ + (present in moderately concentration)
+ (present in low concentration)
- (Not detected).

There is no damaging effect on the liver and kidney markers observed in the treatment of ulcerated animals with *Spondias mombin* stem bark. Treatment of ulcerated rats with methanol extract of *Spondias mombin* stem bark showed a significant ($p < 0.05$) decrease in the ALT, AST, ALP, urea, total protein and creatinine levels when compared to the ulcer untreated rats in group 2 (Table 3).

Treatment of acidified-ethanol induced ulcer rats with methanol extract of Spondias mombin stem bark significantly ($p < 0.05$) increased the superoxide dismutase, catalase, and glutathione peroxidase activities when compared to the ulcer untreated rats. Also, a significant ($p < 0.05$) increase in Malondialdehyde concentration was observed among the treated groups when compared to the untreated rats (Table 4). Treatment of ulcerated animals with different doses of methanol extracts of *Spondias mombin* stem bark produced varying degrees of gastric protection (Fig.1).

Table 2: Effect of Methanol Extract of *Spondias mombin* Stem Bark on Gastric Secretion in Acidified Ethanol-induced Ulcer in Rats

Group	Treatment	Dose (mg/kg)	Gastric volume (ml)	Gastric pH values	Total acidity (mmol/l)	Ulcer index	Ulcer protection (%)
1	Normal control	5ml/kg	3.98 ± 0.24^{a}	6.11 ± 0.71^{ab}	4.75 ± 2.36^{a}	0.00 ± 0.00^{a}	-
2	Positive control	5ml/kg	7.91 ± 2.95^{b}	4.41 ± 2.62^{a}	17.75 ± 4.79^{c}	1.38 ± 1.51^{b}	-
3	Omeprazole	50	3.63 ± 1.07^{a}	6.63 ± 0.20^{b}	4.75 ± 2.36^{a}	0.45 ± 0.37^{ab}	67
4	Extract	100	4.47 ± 0.10^{a}	5.93 ± 0.56^{ab}	14.00 ± 4.08^{bc}	1.15 ± 1.14^{ab}	16
5	Extract	200	4.20 ± 0.87^{a}	6.20 ± 0.45^{ab}	9.00 ± 4.55^{ab}	0.80 ± 0.55^{ab}	42
6	Extract	500	4.03 ± 0.57^{a}	6.41 ± 0.44^{b}	7.80 ± 1.72^{a}	0.48 ± 0.21^{ab}	66

The values are shown as mean ± SD, with n = 4. Mean values containing different alphabetic superscript letters in the column exhibit a significant difference (p < 0.05)

Table 3: Effect of Methanol Extract of *Spondias mombin* Bark on Liver and Kidney Markers of Acidified Ethanol-induced Ulcer in Rats

Group	Treatment	Dose (mg/kg)	ALT (iu/l)	AST (iu/l)	ALP (iu/l)	Urea (mg/dl)	Total protein (mg/dl)	Creatinine (mg/dl)
1	Normal control	5 ml	11.10±1.40^{ab}	10.69±3.01^{a}	53.00±4.30^{a}	30.60±3.11^{a}	2.09±0.43^{a}	0.81±0.16^{a}
2	Positive control	5 ml	14.54±1.01^{b}	13.88±0.85^{b}	80.83±4.41^{c}	63.93±12.49^{b}	4.12±0.76^{b}	1.78±0.11^{b}
3	Omeprazole	50	11.85±0.32^{ab}	11.26±0.85^{ab}	63.00±20.46^{ab}	33.14±5.05^{a}	2.71±0.60^{a}	1.00±0.25^{a}
4	Extract	100	12.04±3.27^{ab}	13.53±2.12^{b}	70.03±9.8^{bc}	35.22±2.33^{a}	2.33±0.15^{a}	0.78±0.07^{a}
5	Extract	200	11.56±4.79^{ab}	12.74±0.41^{ab}	61.29±5.72^{ab}	38.46±9.31^{a}	2.47±0.08^{a}	0.87±0.14^{a}
6	Extract	500	10.39±0.55^{a}	11.78±0.45^{ab}	52.88±2.50^{a}	40.21±11.08^{a}	2.56±0.11^{a}	0.96±0.10^{a}

The values are shown as mean ± SD, with n = 4. Mean values containing different alphabetic superscript letters in the column exhibit a significant difference ($p < 0.05$)

Table 4: Effect of Stem Bark Extract of *Spondias mombin* on Activities of Antioxidant Enzymes and MDA Concentration in Acidified Ethanol-induced Ulcer in Rats

Groups	Treatment	Dose (mg/kg)	SOD (iu/l)	CAT (u/l)	GPX (u/l)	MDA (mg/dl)
1	Normal	5ml	11.50±0.57^{c}	1.15±0.06^{bc}	10.70±2.54^{b}	1.22±0.16^{a}
2	Ulcer control	5ml	6.69±2.00^{a}	0.76±0.15^{a}	7.31±3.32^{a}	3.09±1.08^{b}
3	Omeprazole	50	11.35±0.14^{c}	1.15±0.12^{bc}	10.13±0.43^{ab}	1.27±0.14^{b}
4	Extract	100	8.39±0.41^{b}	0.94±0.82^{ab}	8.42±1.06^{ab}	2.43±0.41^{b}
5	Extract	200	10.32±0.41^{c}	1.07±0.41^{bc}	9.76±2.04^{ab}	1.29±0.24^{a}
6	Extract	500	11.40±0.53^{c}	1.36±0.15^{c}	10.31±1.16^{ab}	1.24±0.16^{a}

The values are shown as mean ± SD, with n = 4. Mean values containing different alphabetic superscript letters in the column exhibit a significant difference ($p < 0.05$)

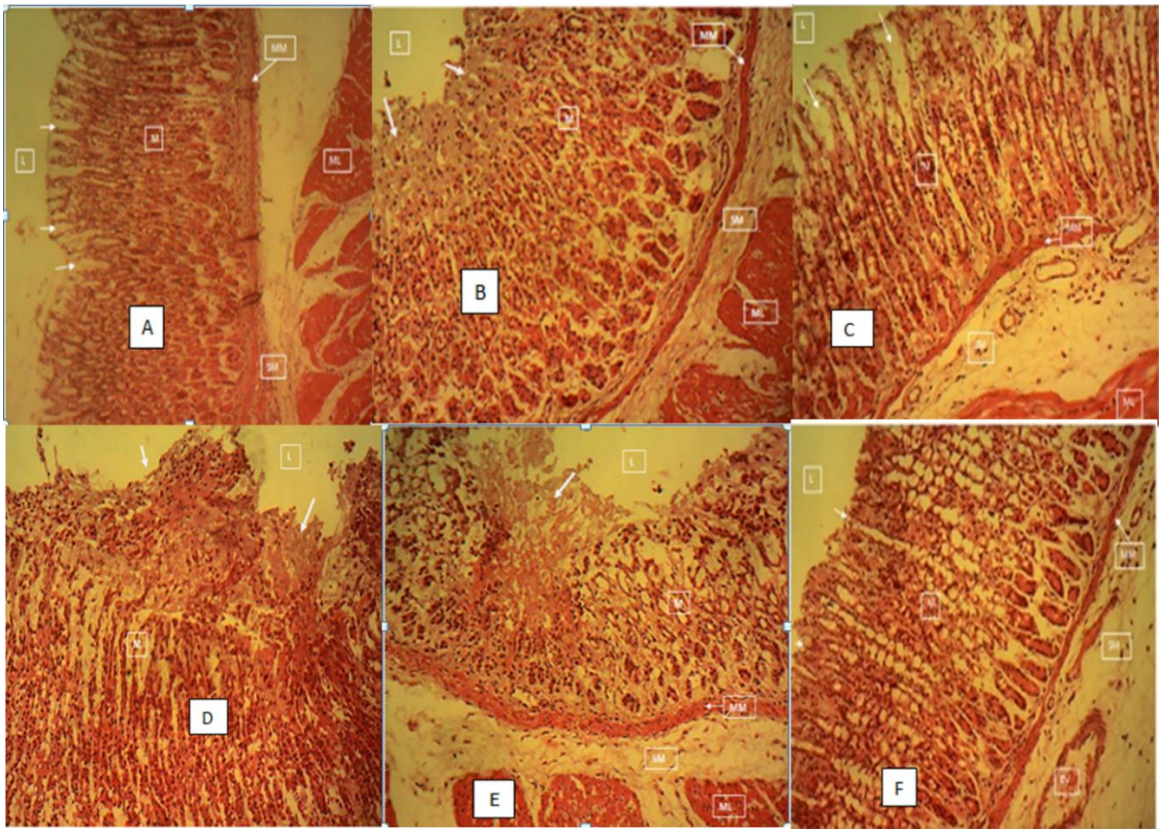

Figure 1: Histological Evaluation of Stomach Ulcer. (Magnification x 160)

A represents group 1: normal histomorphology, B is group 2: induced without treatment showed wide, areas of mucosal necrosis, involving one-third to two-thirds of the mucosa, C is group 3: received omeprazole, and it showed the relatively normal histomorphology but, a widened gastric pit was observed (white arrows), D is group 4: received 100 mg/kg b.w of extract, and it showed a wide area of mucosal necrosis, involving one-third to two-third of the mucosa. The affected areas consist of irregular necrotic cellular debris (white arrows), E is group 5: received 200 mg/kg b.w of extract and it showed multifocal areas of mucosal necrosis (arrow). Lumen (L); Mucosa (M); Muscularis mucosa (MM); Submucosa (SM); Muscularis layer (ML), F, is group 6: Received 500 mg/kg b.w of extract and it showed a widened gastric pit. Mucosa (M); Muscularis mucosa (MM); Submucosa (SM); Lumen (L). Blood vessel (BV)

DISCUSSION

Plants contain phytochemical compositions which are responsible for the potency found in plants in treating diseases. Table.1 shows the phytochemical screening on the methanol extract of *Spondias mombin* stem bark. The presence of alkaloids, tannins, saponins are in agreement with the reports of (Ogunro *et al.,* 2023; Okonkwo *et al*., 2024). Acidified ethanol-mediated ulceration seen in this study is ulceration caused by the oral ingestion of 5 ml/kg body weight of acidified ethanol (0.15M HCL/ absolute ethanol; 40:60 v/v respectively). This triggers the activation of the aggressive factors that lead to the imbalance between the membrane protective factor and the aggressive factors, and subsequently results in the mucosal ulceration. Gastric ulcers caused by ethanol can result from changes in blood flow to the stomach, leading to tissue damage. Also, ethanol causes solubilization of the mucosal tissue, increasing Na^+ and K^+ flow to the lumen and pepsin secretion, thereby increasing histamine and H^+ ions. Ulceration can be caused by cyclooxygenase inhibition, and this prevents prostaglandin synthesis, leading to epithelial cell infiltration or generation of free radicals that disrupt membrane integrity and cause gastric mucus depletion **(**Ukwuani-kwaja and Zakari, 2018**)**. Biochemical evaluation of gastric secretions and mucosal integrity are usually parameters employed to ascertain stomach status after ingestion of ulcerogenic agents (Biplab *et al*., 2011). In this study, there is a significant decrease ($p < 0.05$) in the volume of mucosal contents among the treated groups when compared to the ulcer untreated. This implies that the anti-secretory property of *S. mombin* extract was able to prevent acid and pepsin secretions in the mucosa which are potent aggressive factors that lead to ulceration. Decreased gastric pH value implies an increased hydrogen ion concentration of the gastric juice which has been linked to etiology of gastric ulcer in animals (Dauda *et al*., 2017). The significant ($P < 0.05$) increase in pH values among the groups treated with methanol extract of *S. mombin* when compared to the untreated group implies that methanol extract of *S. mombin* improved the mucosal integrity by restoration of the normal hydrogen ion level in the experimental rats. This could be due to the presence of the phytochemical constituents helpful in ulceration treatment which include tannins, Quercetin and they had also been reported to be present in *S. mombin* (Nworu *et al*., 2007; Siqueira *et al*., 2020), respectively.

The increased total acidity observed in the ulcerated untreated rats could be due to the increase in free radicals and inhibition of prostaglandin synthesis, which helps in the regulation of mucosal acid balance. This could also be due to the release of histamine in the enterochromaffin-like cells that will activate the proton pump into production of acids. The significant ($P<0.05$) reduction in gastric acid secretion in the groups treated with *S. mombin* might be due to the action of tannin, which was reported to be present in the leaf extract of *S. mombin*. Tannin tends to compete with adenosine triphosphate at the ATP hydrolysis site, thereby causing the inhibition of gastric H^+- K^+ ATPase that is necessary for gastric acid secretion (Murakami *et al*., 1992). Tannin also has the potential of reacting with proteins in the stomach tissue layers by precipitation of micro proteins at the site of the ulcer, forming protective thin films that prevent irritation from gastric mucosa (Vasconcelos *et al*., 2010).

The significant decrease ($p<0.05$) in the total acidity as observed in the treated groups (100, 200 and 500 mg/kg b.w) is an indication that *S. mombin* has the capacity to reduce acid secretion in the stomach. This action could also be attributed to the alkaloids present in the plant, which are known to stimulate prostaglandin formation. Apart from an increase in prostaglandin synthesis, endothelial cell renewal, *S. mombin* extract may bring this effect by inhibition of histamine from binding to the H_2-receptor. These activities will reduce the activation of the proton pump in the parietal cell and hence inhibit acid secretion (Ayada *et al*., 2003).

The activity of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) in serum are generally indicators of liver function (Table 3). Significant decrease ($p < 0.05$) in ALT, AST and ALP activities was observed among the groups treated with *S. mombin* when compared to the ulcer untreated group. The increase in the activities of these liver enzymes in the ulcerated rats may have been caused by the dissolution of the mucosal membrane by the acidified ethanol which led to increased mucosal permeability and the generation of free radicals which resulted in compromised membrane integrity. Activities of liver enzymes increase in the plasma when there is a disruption of the membrane which leads to the leakage of these enzymes into the blood plasma (Hwang *et al*., 1996). The decrease in the activities of liver enzymes observed among the *S. mombin*-treated groups is an indication that *Spondins mombin* pose no potential danger to hepatic damage.

The increase in the total protein as observed among the untreated group was an indication of high protein metabolism and decreased glomerular filtration rate (GFR) that led to a high concentration of total protein in the plasma of the untreated rats. However, treatment of rats with methanol extract of *S. mombin* stem bark significantly ($P<0.05$) decreased and improved the urea, creatinine and total protein levels of the rats. The improvement in the urea and creatinine levels observed among the treated groups implies that the body was able to excrete this drug efficiently. This result is in agreement with the findings of (Eze-Steven *et al*., 2019), who had also reported improved kidney function in the treatment of Leiomyoma with *S. mombin* extract.

Superoxide dismutase (SOD), Catalase (CAT) and Glutathione peroxidase (GPx) are inherent biological enzymes that have been reported to protect the cell from oxidative damage. Kunwar and Priyadarsini (2011) also reported that these enzymes help to neutralize and prevent damage caused by reactive oxygen species (ROS) during

disease conditions. A significant increase ($p<0.05$) in the activities of these antioxidant enzymes (SOD, CAT and GPX) and converse significant ($p<0.05$) decrease in Malondialdehyde concentration (MDA) observed among the groups treated with varying doses of extract implies that the *S. mombin* extracts were able to prevent lipid peroxidation and mop up the generated free radicals that resulted in protection of mucosal damage. This research is in agreement with the report of **(**Musa *et al.,* 2017) where the administration of plant extract of *Phoenix dactylifera* fruit offered gastro-protection against ethanol-induced ulcer in rats.

Different degrees of mucosal tissue damage were observed in the experimental animals. Figures A-F represent groups, and the best form of protection of tissue damage was found in F, which represents the group treated with 500 mg/kg body weight of stem bark of *S. mombin* when compared to the D and E groups treated with 100 and 200 mg/kg b.w respectively.

Group treated with Omeprazole also showed a good mucosa protection.

CONCLUSION

This study showed that oral administration of methanol extract of *Spondias mombin* stem bark possesses gastro-protective effect against acidified ethanol-induced ulcer in rats. This ulcer protective property exhibited by this plant in this study against acidified ethanol-induced ulcers in rats suggests that this plant product could be used in the treatment and protection of damage against stomach ulcers. However, more research is needed for further investigation, such as identification and characterization of the bioactive ingredients in the methanol extract of *S. mombin* stem bark. This will help to provide more knowledge and insights in the understanding the mechanisms of action of these bioactive molecules.

AUTHORS' CONTRIBUTIONS

Writing – Original draft preparation, IUE; Investigation – EBA, CCA and CJA; Data analysis and interpretation – EBA; Design and Supervision – CAA. All the Authors have read and approved the final manuscript.

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CONFLICT OF INTEREST

The authors hereby state that there is no conflict of interest in the publication of this research.

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