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## Study of aqueous leaf extracts of *Spondias mombin* Linn. (Anacardiaceae) in gastric ulcer models and the possible mechanisms of action

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### ABSTRACT

*Spondias mombin* L. (Anacardiaceae) commonly known as 'yellow mombin' is a multipurpose herb cultivated in parts of the Brazilian Northeast for its edible fruits, oil and leaves. The bark is used to carve figures and the leaves and roots used as medicine. The objective of this study was to evaluate the effect of aqueous leaf extract of *Spondias mombin* Linn. on gastric ulcers. The effect was evaluated in ibuprofen, alcohol and pylorus ligation-induced gastric ulcer models. The extract was administered orally at three different doses of 50, 100 and 200 mg/kg. The antiulcer activity was accessed in rats by comparing the ulcer index in the test group with that of the control (distilled water) and standard (omeprazole, misoprostol) groups. The involvements of endogenous nitric oxide, prostaglandins and non-protein sulfhydryl groups in the cytoprotective action of *Spondias mombin* L. were also investigated. The extract showed a significant ( $p < 0.05$ ,  $p < 0.01$ ,  $p < 0.001$ ) antisecretory and gastric cytoprotective effects in pylorus ligation, alcohol and ibuprofen-induced ulcer model respectively. Antioxidant analysis showed significant scavenging of free radical using nitric oxide, reducing power activity and DPPH assay. *Spondias mombin* L. showed presence of flavonoids, tannins, reducing sugar, cardiac glycosides and terpenes. The experiment suggests a possible participation of NO synthase and NP-SH pathways in the gastroprotective effect of *Spondias mombin*.

**Keywords:** Gastric ulcer, extract, *Spondias mombin*, antioxidant, cytoprotection, antisecretory.

### INTRODUCTION

Peptic ulcer (PUD), a public health problem with high prevalence in the global population has been identified as the most common gastrointestinal problem in clinical practice, due to its high rate of morbidity and substantial mortality. It affects about 60 % of human adults and nearly 80 % of child population in tropical countries [1, 2]. Peptic ulcer disease is characterized by mucosal damage secondary to pepsin and gastric acid secretion. It usually occurs in the stomach and proximal duodenum; less commonly, it occurs in the lower oesophagus, the distal duodenum or in the jejunum. It is one of the major gastrointestinal disorders that occurs due to an imbalance between offensive and defensive factors along with weakness of the mucosal barrier [3]. The major offensive factors are gastric acid, non-steroidal anti-inflammatory drugs (NSAIDs), pepsin, *Helicobacter pylori* (*H-pylori*), and bile salts, while the defensive factors involve bicarbonate secretion and prostaglandins. The several side effects (arrhythmias, impotence, haematopoietic changes among others, necessitated the need for a better alternative possessing fewer side effects among indigenous drugs [4].

*Spondias mombin*, commonly known as "Iyeye" in the South-Western part of Nigeria is a fructiferous tree in the family Anacardiaceae. It is commonly used in folk medicine to cure many diseases due to its potent bioactive principles including tannins, saponins, flavonoids, phenolics and anthraquinone glycosides [5]. The leaf extract of the plant has been outstandingly advocated for use in speedy wound healing processes, haemorrhoids and inflamed mucous membrane due to its tannin content [6]. Antioxidant vitamins; alpha-tocopherol and ascorbic acid have also been detected in its leaf extracts [7]. Tea from its flowers and leaves is taken as an analgesic and anti-inflammatory cure against stomach ache and discomfort [8]. Abdulkabir *et al.*, (2016) [9] reported the cytotoxic activity of *S. mombin*.

The present study was designed to evaluate the antiulcer potentials of *Spondias mombin* and to elucidate the possible mechanism(s) of actions.

## MATERIALS AND METHODS

### Collection of Plant and Extraction

The fresh leaves of *Spondias mombin* L. were collected in Ogun state, identified and authenticated by Prof. J.D. Olowokudejo, Department of Botany and Microbiology, University of Lagos, Lagos-Nigeria.

Leaves were rinsed under a running tap, air-dried and separated from stems and thereafter oven-dried at 40°C till a constant weight was obtained at three consecutive weighing days apart. Ground dried leaves weighing 517.7g were soaked in 2.50 L of boiled distilled water for 3 days. It was then filtered with a clean white handkerchief. The filtrate was oven dried at a temperature of 40°C to obtain granules referred to as the extract, from which appropriate concentrations were made in distilled water for experimentation. Percentage yield was calculated, and extract stored at room temperature in an air-tight bottle.

### Drugs and Chemicals

Omeprazole (Sterling Healthcare, India), Ibuprofen (Unicare Pharmaceutical Ltd, Nigeria), Misoprostol (Pfizer, USA), Ethanol (Sigma Chemical, CO, USA), N<sup>G</sup>-nitro L-arginine methyl ester (L-NAME) (Sigma Chemical, CO, USA).

### Phytochemical analysis of the leaf extracts of *Spondias mombin* Linn

A 10 % stock solution of extract in water was prepared and used for the study [10].

### Total Antioxidant Assay

#### DPPH Radical Scavenging Activity Assay

The free radical scavenging activity of the extract, based on the scavenging of the stable 1, 1-diphenyl-2-picrylhydrazyl (DPPH) free radical was estimated according to the procedure described by early workers [11]. An aliquot of 0.5 ml of extract in ethanol (95%) at different concentrations (25, 50, 75, 100µg/ml) was mixed with 2.0 ml of reagent solution (0.004g of DPPH in 100 ml methanol). The control contained only DPPH solution in place of the sample while methanol was used as blank. The mixture was vigorously shaken and left to stand at a room temperature. After 30 minutes, the decrease in absorbance test mixture (due to quenching of DPPH free radicals) was read at 517 nm. The scavenging effect was calculated using the expression:

$$\% \text{ inhibition} = \frac{[A0-A1] \times 100}{A0}$$

Where A0 is the absorption of the blank sample and A1 is the absorption of the extract.

#### Nitric Oxide Scavenging Activity Assay

The compound sodium nitroprusside is known to decompose in aqueous solution at physiological pH (7.2) producing NO•. Under aerobic condition, NO• reacts with oxygen to produce stable products (nitrate and nitrite), which can be determined using Griess reagent. The absorbance of the chromophore that formed during diazotization of the nitrite with sulphanilamide and subsequent coupling with Naphthylethylenediamine dihydrochloride can be immediately read at 550 nm. A 4ml sample of plant extract or standard solution of different concentrations (25, 50, 75, 100 µg/ml) were taken in different test tubes and 1 ml of Sodium nitroprusside (5 mM in phosphate buffered saline) solution was added into the test tubes. They were incubated for 2 hours at 30°C to complete the reaction. A 2 ml sample was withdrawn from the mixture and mixed with 1.2 ml of Griess reagent (1% Sulphanilamide, 0.1% naphthylethylene diamine dihydrochloride in 2% H<sub>3</sub>PO<sub>4</sub>). The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling

with naphthylethylene diamine was measured at 550 nm [12]. Ascorbic acid was used as standard.

The percentage (%) inhibition activity was calculated from the following equation:

$$\frac{[A0-A1] \times 100}{A0}$$

### Reducing Power Assay

Various concentrations of the extracts (20 to 100µg/ml) in 1.0 ml of deionized water were mixed with phosphate buffer (2.5 ml) and potassium ferricyanide (2.5 ml). the mixture was incubated at 50°C for 20 min. Aliquots of trichloroacetic acid (2.5 ml) were added to the mixture, which was then centrifuged at 3000 rpm for 10 min. The upper layer of the solution (2.5 ml) was mixed with distilled water (2.5 ml) and a freshly prepared ferric chloride solution (0.5 ml). The absorbance was measured at 700 nm. A blank was prepared without adding extract. Ascorbic acid at various concentrations (1 to 6µg/ml) was used as standard. % Increase in reducing power = (A<sub>test</sub> / A<sub>blank</sub>) - 1 × 100. A<sub>test</sub> is absorbance of test solution; A<sub>blank</sub> is absorbance of blank.

### Experimental Animals

Albino mice and rats of both sexes obtained from the Laboratory Animal Centre of the College of Medicine, University of Lagos, Idi-Araba, Lagos, Nigeria were used. The animals were allowed to acclimatize for one week under standard environmental conditions; provided with standard pellet diet (Neimeth Livestock Feeds Ikeja, Lagos, Nigeria), and maintained under standard laboratory conditions, as approved by the United States National Institute of Health (NIH) guide for Care and Use of laboratory animals and recommendation of IASP [13].

### Acute Toxicity Test

Albino mice were fasted 24 hours prior to test, and were divided into five groups of three animals each. Each group was administered doses of 10, 100, 500, 1000 and 2000 mg/kg *Spondias mombin* intraperitoneally respectively. Oral route administration of 1000, 2000 and 4000mg/kg extract to separate groups of animals followed afterwards. The animals were closely observed for signs of toxicity as well as mortality for the first 24 hours and LD<sub>50</sub> was calculated. The animals were thereafter observed for another 14 days to observe any delayed toxicity [14]. The lethal dose via intraperitoneal route was calculated using the formula:

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

Where D<sub>0</sub> = Highest dose that gave no mortality  
D<sub>100</sub> = Lowest dose that produced mortality.

### Induction of ulcer in Experimental Animals

#### Ibuprofen-induced Gastric ulcer Model

Animals were randomly selected to six groups containing four animals each. They were pretreated for 15 days as follows;

- Group 1 – Distilled water (10 ml/kg)
- Group 2 – Omeprazole (20 mg/kg)
- Group 3 – Misoprostol (200 µg/kg)
- Group 4 – Aqueous extract of *S. mombin* (50 mg/kg)
- Group 5 – Aqueous extract of *S. mombin* (100 mg/kg)
- Group 6 – Aqueous extract of *S. mombin* (200 mg/kg).

A non-steroidal anti-inflammatory drug, ibuprofen, was given in two doses of 300 mg/kg orally at 15-hour intervals, to 24-hour fasted animals [15]. The plant extract was given 30 minutes before each dose of ibuprofen, by oral administration. Animals were sacrificed six hours

after the second dose of ibuprofen. The stomachs were removed, cut along the greater curvature, washed with normal saline, and the ulcer index was scored using the Magistretti scoring scale [16].

#### Alcohol-induced Ulcer Model

Animals were randomly selected to five groups containing four animals each. They were pretreated for 15 days as follows:

- Group 1 – Distilled water (10 ml/kg)
- Group 2 – Omeprazole (20 mg/kg)
- Group 3 – Aqueous extract of *S. mombin* (50 mg/kg)
- Group 4 – Aqueous extract of *S. mombin* (100mg/kg)
- Group 5 – Aqueous extract of *S. mombin* (200 mg/kg).

Gastric ulcers were induced by administration of absolute ethanol at a dose of 1 ml / animal, orally, after 45 minutes of oral administration of the standard and test drugs to all groups of animals [17]. Animals were sacrificed one hour after the administration of ethanol. The stomach was removed, cut along the greater curvature, washed with normal saline, and the ulcer index was scored using the Magistretti scoring scale [16].

#### Pylorus ligation-induced Gastric Ulcer Model

Animals were randomly selected to five groups containing four animals each. They were pretreated for 15 days as follows:

- Group 1 – Distilled water (10 ml/kg)
- Group 2 – Omeprazole (20 mg/kg)
- Group 3 – Aqueous extract of *S. mombin* (50 mg/kg)
- Group 4 – Aqueous extract of *S. mombin* (100mg/kg)
- Group 5 – Aqueous extract of *S. mombin* (200 mg/kg).

This is the oldest animal model [18] of gastric ulcers. Rats were fasted for 24-hours prior to pyloric ligation. Under light ether anaesthesia the abdomen was opened by a small midline incision below the Xiphoid process. The pyloric position of the stomach was lifted out slightly and ligated, avoiding traction to the pylorus or damage to its blood supply. The stomach was replaced carefully and the abdominal wall was closed by interrupted sutures. The animals were deprived of both food and water during the postoperative period and were sacrificed at the end of six hours, after the operation. The stomachs were dissected out, the contents were drained into tubes. The volume of the gastric juice was measured after centrifugation at 2000 rpm for 10 minutes. From the supernatant, aliquots were taken for the determination of pH and titrable acidity [18]. The stomachs were then cut along the greater curvature and the inner surfaces were examined for ulcerations, and the ulcer index was calculated using the Magistretti scoring scale [16].

#### Titration acidity

The volume of gastric secretions (VGS) of rats in all groups was recorded and percentage reduction in volume when compared to the control group was calculated by the equation:  $[(VGS \text{ control} - VGS \text{ treated}) / VGS \text{ control}] \times 100$ . The titration acidity in mEq/ml was obtained by the following equation:

$$\frac{\text{Titre value} \times 0.1N \text{ NaOH} \times 36.5 \text{ Eq HCl}}{10 \times 1000}$$

#### Estimation of Nonprotein Sulphydryl Group (NP-SH)

Gastric mucosal was measured according to method reported by Sedlak and Lindsay, 1968 [19]. The glandular stomachs of control and treated rats were removed and homogenized in ice-cold 0.02 M ethylenediaminetetraacetic acid. The homogenate was mixed with distilled water and 50% TCA and centrifuged; the supernatants were mixed with Tris buffer, 5,5'-dithiobis(2-nitrobenzoic acid). 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB) was added and the sample was

shaken. The absorbance was measured, within 5 min of addition of DTNB, at 412nm, against a reagent blank with no homogenate.

#### Investigation of the Possible involvement of Prostaglandins in the Cytoprotective Action of *Spondias mombin*

In order to examine the effect of PG in the cytoprotective activity, Ibuprofen is administered subcutaneously to one group of animals followed by oral administration of the test drug 30 min later. Ethanol was given to each animal 30 min after drug administration and animals were sacrificed 1 h later. The stomachs were subsequently removed to measure the ulcer index.

#### Investigation of the Possible involvement of Endogenous Nitric Oxide (NO) in the Cytoprotective Action of *Spondias mombin* LINN

In another group of animals, N<sup>G</sup>-nitro L-arginine methyl ester (L-NAME; 25 mg/kg), an inhibitor of NO synthase activity, was administered subcutaneously 15 min before the administration of the test drug orally. Ethanol and Ibuprofen were given orally 30 min later and animals were sacrificed 1 and 6h after the administration of ethanol and ibuprofen respectively and the stomach ulcer index was scored.

#### Rating of Ulcer Lesion [16]

- 0 = No lesion
- 0.5 = Haemorrhage
- 1 = 1-3 small lesions
- 2 = 1-3 large lesions
- 3 = 1-3 thickened lesions
- 4 = more than 3 small lesions
- 5 = more than 3 large lesions
- 6 = more than 3 thickened lesions

#### Calculation of Ulcer Index and percentage inhibition

The extent of ulceration in the excised stomach was compared in each model and expressed as an ulcer index obtained from the ulcer score in each group using the equation (1)

$$UI = DU/n$$

Where DU = Degree of ulceration for each group (ulcer score).  
n = number of rats in each group

Percentage inhibition was determined as in the equation (2) [20]:

$$\text{Inhibition (\%)} = [(UIC - UIt) / UIC] \times 100 \dots (2)$$

Where UIC = the ulcer index for the control group;

UIt = the ulcer index for the treatment group.

#### Statistical Analysis

The results are expressed as mean  $\pm$  S.E.M (standard error of mean). Data were analyzed using One-way ANOVA followed by Dunnett's and Turkey's multiple comparisons tests using GraphPad Prism 6 (GraphPad Software Inc., CA, USA). Values were considered significant at  $p < 0.05$ .

## RESULTS

#### Physicochemical Properties of the Extract

- Colour of extract - Dark brown
- Taste of extract - Sour
- Smell of extract - Medicinal
- Texture of extract - Coarse

Nature of extract - Deliquescent  
 Solubility in water - High  
 pH - 4.9  
 Percentage yield - 14.68 %

**Acute Toxicity**

In the oral acute toxicity test carried out in mice, the extract did not produce any toxic symptoms or mortality up to dose level of 4000 mg/kg, hence, the extract was considered safe for further pharmacological screening.

Doses 10, 100, 500, 1000 and 2000 mg/kg were administered via intraperitoneal route. At doses lesser than 1000 mg/kg, the mice showed abdominal contraction and frequent bowel movements at a mean onset of action of fifteen minutes and sedation at a mean onset of action of 45 minutes. At doses of 1000 mg/kg and 2000 mg/kg, behavioural patterns included abdominal contraction at mean onset of action of fifteen minutes, frequent bowel movements, sedation at a mean onset of action of 45 minutes and an actual time of death above 3 hours onset of action.

The lethal dose via intraperitoneal route was calculated as 707.107 mg/kg.

**Phytochemical Screening**

The extract contained flavonoids, tannins, reducing sugar, cardiac glycosides, steroids, and terpenes, while alkaloids, saponins and anthraquinones were absent.

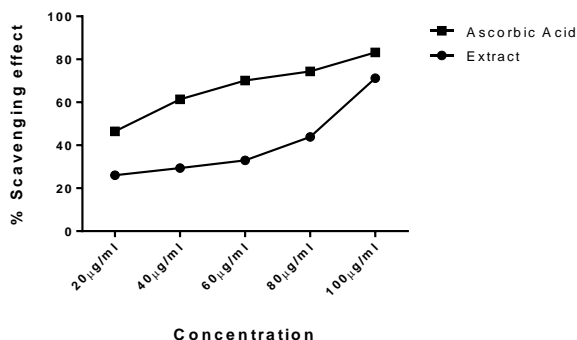
**Antioxidant Capacity**

**1,1-diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Activity**

The DPPH radical quenching of the extract indicated potent radical scavenging activities.

Values on graph (Fig. 2) are mean±S.E.M. Extract 20µg/ml: 26.04±1.11, 40µg/ml: 29.37±0.28, 60µg/ml: 32.96±0.28, 80µg/ml: 43.90±0.42, 100µg/ml: 71.90±0.83.

Ascorbic acid 20µg/ml: 46.46±0.25, 40µg/ml: 61.38±0.09, 60µg/ml: 70.17±1.30, 80µg/ml: 74.40±0.12, 100µg/ml: 83.25±0.52.



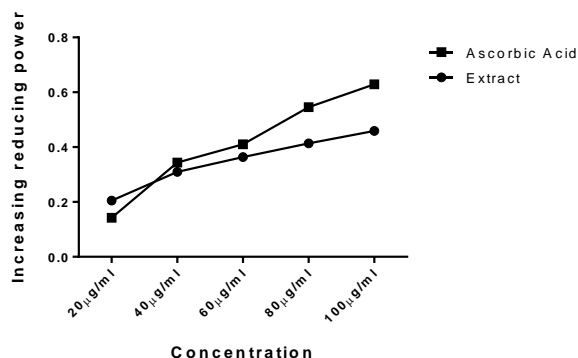
**Figure 2:** 1,1-diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Activity

**Reducing Power Assay**

In the reducing power assay, the extract possess a potent reducing power activity.

Values on graph (Fig. 3) are mean±S.E.M. Extract 20µg/ml: 0.205±0.001, 40µg/ml: 0.310±0.001, 60µg/ml: 0.363±0.002, 80µg/ml: 0.414±0.001, 100µg/ml: 0.459±0.003.

Ascorbic acid 20µg/ml: 0.142±0.003, 40µg/ml: 0.344±0.003, 60µg/ml: 0.410±0.001, 80µg/ml: 0.545±0.002, 100µg/ml: 0.629±0.003.



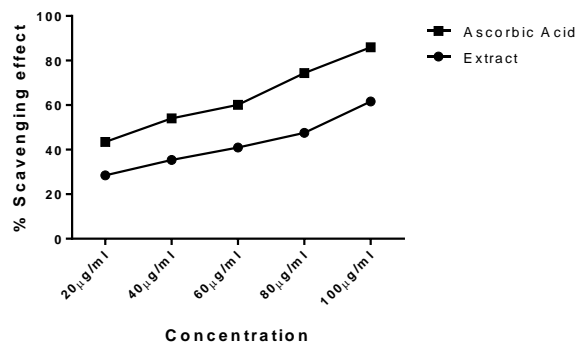
**Figure 3:** Reducing Power Assay

**Nitric Oxide Scavenging Activity Assay**

In this assay, the extract possess a potent NO<sup>-</sup> scavenging activity as an antioxidant property.

Values on graph (Fig. 4) are mean±S.E.M. Extract 20µg/ml: 28.44±0.26, 40µg/ml: 35.40±0.175, 60µg/ml: 40.98±0.26, 80µg/ml: 47.51±0.26, 100µg/ml: 61.60±0.43.

Ascorbic acid 20µg/ml: 43.47±1.87, 40µg/ml: 54.08±2.81, 60µg/ml: 60.12±0.34, 80µg/ml: 74.35±1.12, 100µg/ml: 85.94±0.39.



**Figure 4:** Nitric Oxide Scavenging Activity Assay

**Ibuprofen-induced ulcer**

Oral administration of aqueous leaf extract of *Spondias mombin* L. for 15 days produced significant (p<0.05, p<0.01) reduction in ulcer index when compared to control. The maximum effect occurred at 200 mg/kg (90.60 % protection).

Similar effect was produced by extract 50 mg/kg and misoprostol of 81.30% protection each.

Effect produced is non-dose dependent with extract 200 mg/kg producing the highest percentage protection (90.60 % protection) followed by extract 50 mg/kg (81.30 % protection). (Fig. 5; Fig. 6a-6f).

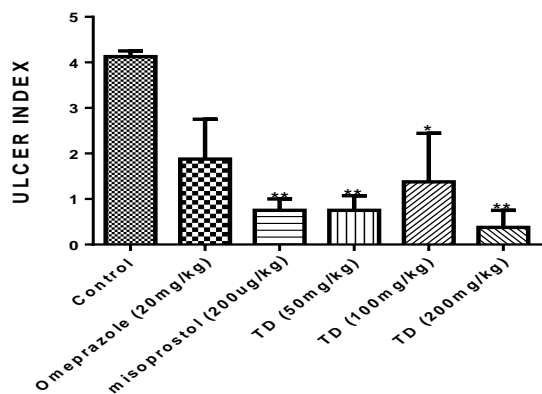


Figure 5: Bar chart showing Ibuprofen induced gastric ulcer results

Values are mean ± S.E.M (n=4) \*p<0.05, \*\*p<0.01 when compared to control. (One way ANOVA followed by Dunnett's multiple comparisons test).



Figure 6e: Misoprostol 200µg/kg



Figure 6f: Omeprazole 20mg/kg



Figure 6a: Distilled water 10ml/kg



Figure 6b: Extract 50mg/kg



Figure 6c: Extract 100mg/kg



Figure 6d: Extract 200mg/kg

Alcohol-induced ulcer

Oral administration of *Spondias mombin* L. leaf extract produced significant (p<0.05, p<0.01) reduction in ulcer index.

The peak effect occurred at 200 mg/kg dose (74.20 % protection), followed by (45.20 % protection) in extract 100 mg/kg.

Extract at 200 mg/kg produced a higher effect (74.20 % protection) than the standard drug omeprazole (67.70 % protection).

The antiulcer effect produced is dose-dependent, with least effect produced by 50 mg/kg (37.10 % protection). (Fig. 7; Fig. 8a-8e).

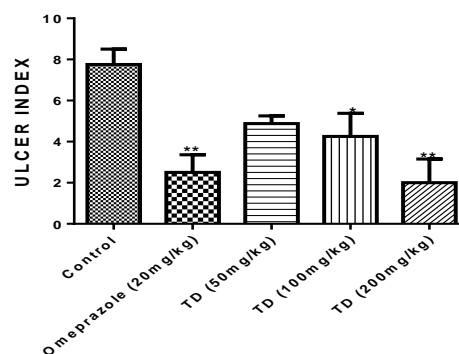


Figure 7: Bar chart showing Alcohol induced gastric ulcer results.

Values are mean ± S.E.M (n=4) \*p<0.05, \*\*p<0.01 when compared to control. (One way ANOVA followed by Dunnett's multiple comparisons test).



Figure 8a: Distilled water 10ml/kg



Figure 8b: Extract 50mg/kg



Figure 8e: Omeprazole 20mg/kg



Figure 8c: Extract 100mg/kg



Figure 8d: Extract 200mg/kg

**Pylorus ligation-induced ulcer**

In the pylorus ligation model, the oral administration of *Spondias mombin* Linn. leaf extract produced a significant reduction of ( $p < 0.05$ ,  $p < 0.01$ ) reduction in ulcer index. It produced a dose-dependent effect with peak effect of (64.30 %) protection at 200 mg/kg followed by (52.40 %) protection at 100 mg/kg.

The extract at 50 mg/kg produced an effect similar to that produced by Omeprazole (47.60%) protection each.

There was also a significant ( $p < 0.001$ ,  $p < 0.0001$ ) reduction in the volume of gastric juice when compared to control. The peak effect was seen at 50 mg/kg (61.81%).

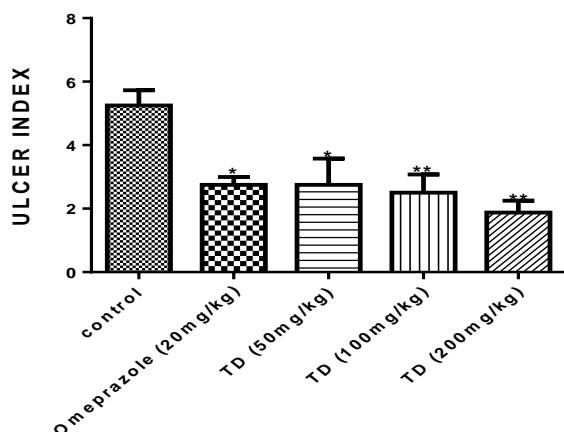
There was no significant change in the pH of all groups when compared to the control.

The titrable acidity of a solution is an approximation of the solution's total acidity, there was also a significant reduction of titrable gastric acid when compared to control of  $p < 0.001$ ,  $p < 0.0001$ . (Fig. 9; Fig 10).

**Table 1:** Effect of *Spondias mombin* L. on Pylorus ligated-induced ulcer in rats.

Treatment	Volume (ml)	% Reduction	pH	Titrable Acidity	Ulcer Index	% Protection
Group 1 (Distilled water 10ml/kg)	3.208±0.25	0	3.050±0.57	3.798±0.31	5.250	0
Group 2 (Extract 50mg/kg)	1.775±0.17 <sup>a</sup>	44.67	4.448±0.23	2.208±0.03 <sup>d</sup>	2.750 <sup>*</sup>	47.60
Group 3 (Extract 100mg/kg)	1.50±0.08 <sup>d</sup>	53.34	4.50±0.44	2.153±0.07 <sup>d</sup>	2.50 <sup>**</sup>	52.40
Group 4 (Extract 200mg/kg)	1.225±0.15 <sup>d</sup>	61.81	4.675±0.09	2.455±0.22 <sup>c</sup>	1.875 <sup>**</sup>	64.30
Group 5 (Omeprazole 20mg/kg)	0.93±0.20 <sup>d</sup>	71.01	4.450±0.18	2.198±0.02 <sup>d</sup>	2.750 <sup>*</sup>	47.60

Values are mean ± S.E.M (n=4) <sup>a</sup>  $p < 0.05$ , <sup>c</sup>  $p < 0.001$ , <sup>d</sup>  $p < 0.0001$  when compared to control; <sup>\*</sup>  $p < 0.05$  when compared to standard (Omeprazole). (One way ANOVA followed by Dunnett's and Turkey's multiple comparisons test).



**Figure 9:** Bar chart showing Pylorus ligation induced gastric ulcer results.

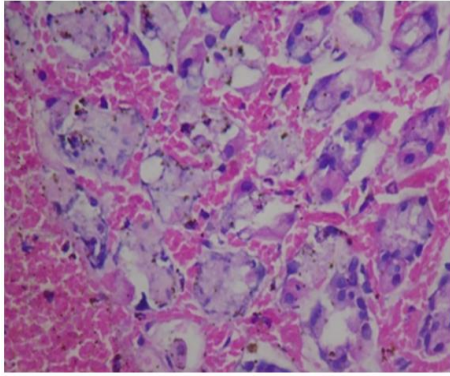
Values are mean ± S.E.M (n=4) <sup>\*</sup>  $p < 0.05$ , <sup>\*\*</sup>  $p < 0.01$  when compared to control. (One way ANOVA followed by Dunnett's multiple comparisons test).

**Table 2:** Estimation of non-protein sulfhydryl groups ((NP-SH) in alcohol- induced model.

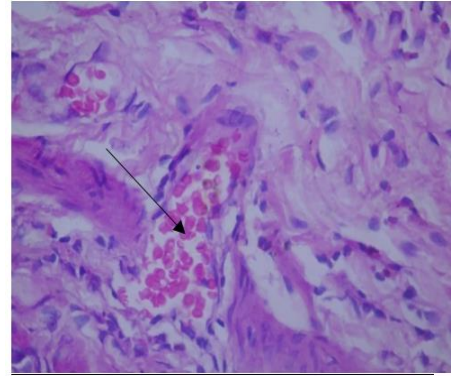
Treatment	NP-SH (µmol/ml)
Distilled water (10 ml/kg)	22.50 ± 0.75
Omeprazole (20 mg/kg)	9.250 ± 1.25
Extract (200 mg/kg)	39.50 ± 6.00 <sup>*</sup>

Values are mean± S.E.M <sup>\*</sup>  $p < 0.05$  when compared to control. (One way ANOVA followed by Dunnett's multiple comparisons test).

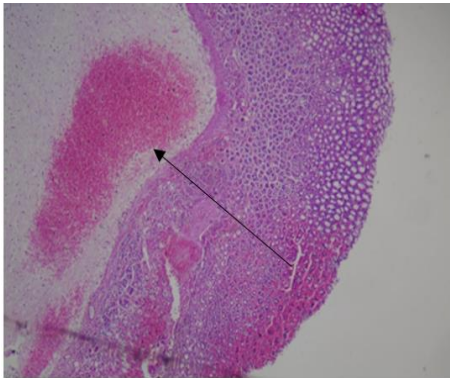
**Histopathology**



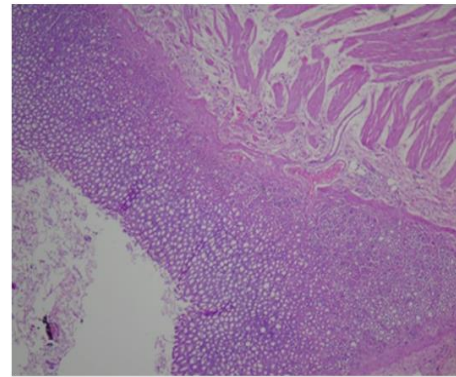
A) Distilled water 10ml/kg – ulceration with submucosa congestion and haemorrhage, extending to the submucosa and serosa. (x400 Magnification)



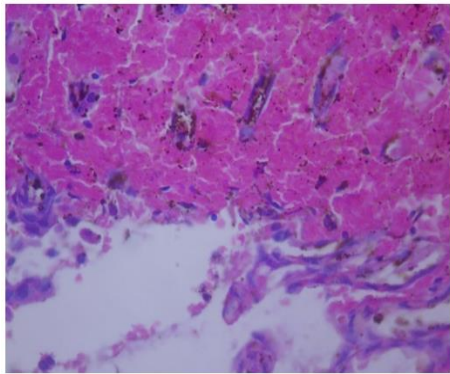
E) Extract 100 mg/kg – mild to moderate congestion of submucosa vessels. (x400 Magnification).



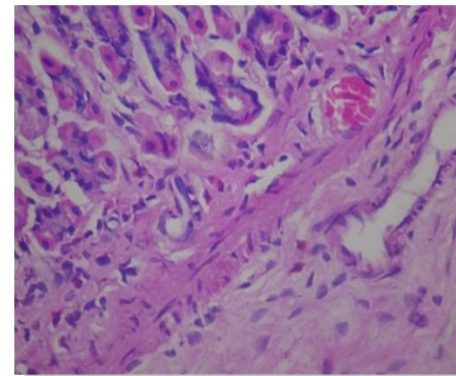
B) Distilled water 10 ml/kg – arrow points to ulcerated area. (x100 Magnification)



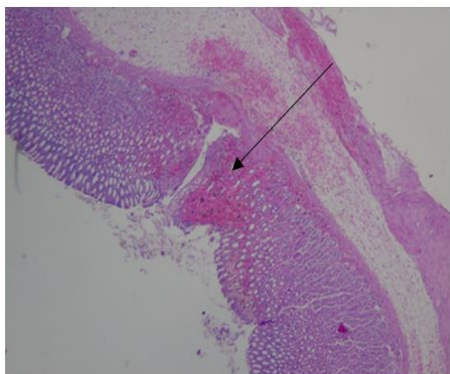
F) Extract 100mg/kg – mild to moderate congestion of submucosa vessels. (x100 Magnification).



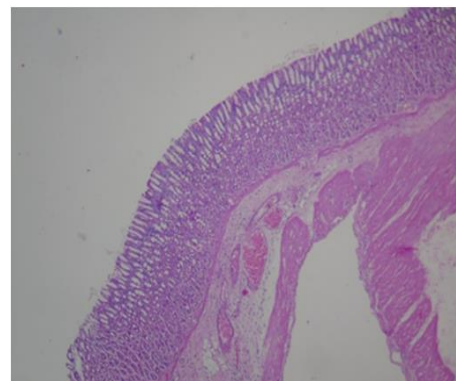
C) Extract 50 mg/kg – extensive mucosal congestion and haemorrhage, extending to the submucosa and serosa.



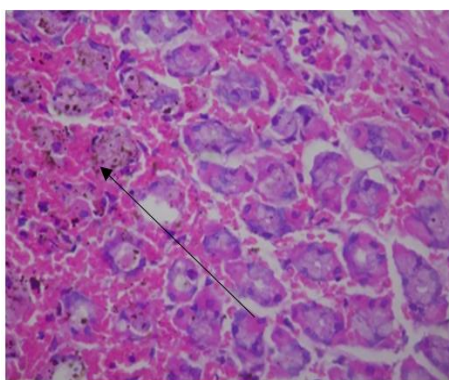
G) Extract 200mg/kg – well generated mucosa (x400 Magnification).



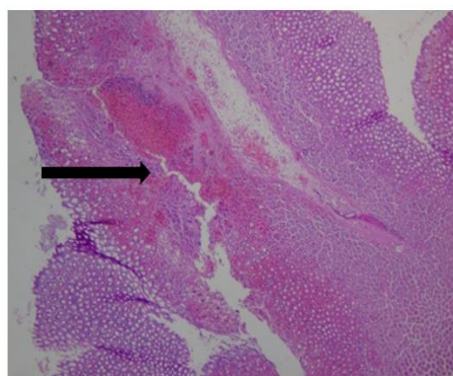
D) Extract 50 mg/kg – arrow points to ulcerated area with extensive congestion and haemorrhage. (x100 Magnification)



H) Extract 200mg/kg – well generated mucosa (x100 Magnification).



I) Omeprazole 20mg/kg – extensive mucosal congestion and haemorrhage (×400 Magnification).



J) Omeprazole 20mg/kg – arrow points to ulceration with congestion. (×100 Magnification).

Figure 10: Histopathology of gastric induced ulcer in albino rats.

### Investigation of the Possible Involvement of Endogenous Nitric Oxide (NO) in the Cytoprotective action of *Spondias mombin*

#### Ibuprofen induced gastric ulcer model; animals pre-treated with L-NAME (25 mg/kg)

Oral pre-treatment with N<sup>G</sup>-nitro L-arginine methyl ester (L-NAME 25 mg/kg) produced a significant (p<0.05) increase in the ulcer index of extract at 200 mg/kg compared to the untreated group. Pre-treatment with L-NAME produced an effect of (31.25 %) protection while the untreated group produced (77.78 %) protection.

Pre-treatment with L-NAME didn't alter the effect produced by the standard drugs omeprazole and misoprostol. (Fig. 11).

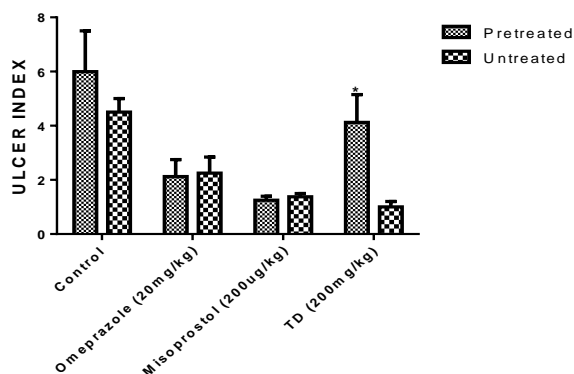


Figure 11: Bar chart showing Ibuprofen induced gastric ulcer model animals pre-treated with L-NAME results.

Values are mean ± S.E.M (n=4) \*p<0.05 when compared to the untreated group. (Two way ANOVA followed by Sidak's multiple comparisons test).

#### Alcohol-induced gastric ulcer model animals pre-treated with L-NAME (25mg/kg)

In the alcohol-induced gastric ulcer model, pre-treatment with L-NAME produced a significant (p<0.01) increase in the ulcer index of extract at 200 mg/kg (37.10 % protection) when compared to the untreated group (77.41 % protection).

In the standard group (omeprazole), there was a significant (p<0.01) increase in the ulcer index of pre-treated group (20.96 % protection) when compared to the untreated group (61.2 % protection). (Fig. 12).

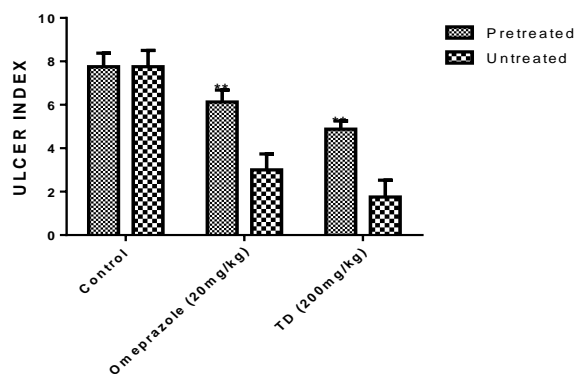


Figure 12: Bar chart showing Alcohol induced gastric ulcer model pre-treated with L-NAME results.

Values are mean ± S.E.M (n=4) \*\*p<0.01 when compared to the untreated group. (Two way ANOVA followed by Sidak's multiple comparisons test).

#### Investigation of the Possible Involvement of Prostaglandins in the Cytoprotective action of *Spondias mombin*

At 200 mg/kg extract, oral pre-treatment with Ibuprofen (100 mg/kg) in alcohol-induced ulcer model did not produce a significant difference between the pre-treated group (77.42 % protection) and the untreated group (82.81 %).

In the standard group (Omeprazole), there was a significant (p<0.05) increase in the ulcer index of the pre-treated group (21.88 % protection) compared to the untreated group (61.29 % protection). (Fig. 13).

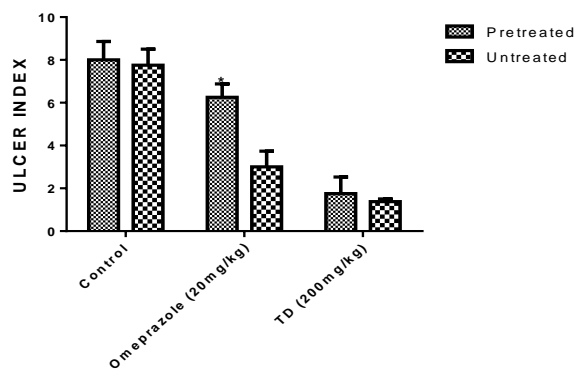


Figure 13: Bar chart showing Alcohol-induced gastric ulcer model pre-treated with Ibuprofen results.



Values are mean  $\pm$  S.E.M (n=4) \*p<0.05 when compared to the untreated group. (Two way ANOVA followed by Sidak's multiple comparisons test).

## DISCUSSION

The antiulcer activity of *S. mombin* has been explored using ibuprofen, ethanol and pylorus ligation ulcer models, which represent some of the most common causes of gastric ulcer in humans. Many factors and mechanisms are implicated in the ulcerogenesis and gastric mucosal damage induced by different models employed in the present study. Among these are depletion of gastric wall, mucosal damage induced by non-steroidal anti-inflammatory drugs and free radical production [21].

Ethanol-induced gastric injury is associated with significant production of oxygen free radicals leading to increased lipid peroxidation, which causes damage to cell and cell membrane [22]. The aqueous leaf extract of *S. mombin* significantly protected the gastric mucosa against the ulcerogen as shown by reduced values of lesion index as compared to the control group, suggesting its potent cytoprotective and free radical scavenging activity. The herbal drug recorded a superior activity to the standard drug, omeprazole.

Ibuprofen, a non-steroidal anti-inflammatory drug, induce gastric mucosal damage by decreasing prostaglandin levels through inhibition of prostaglandin synthesis [23].

*S. mombin* extract was significantly effective, especially at the highest dose of 200 mg/kg in protecting the gastric mucosa against the assault through ibuprofen. This further suggests its potent cytoprotective activity. The herbal drug effect supersedes that of both misoprostol and omeprazole at 200 mg/kg dose.

It has been proposed that in pyloric ligation, the digestive effect of accumulated gastric juice and interference of gastric blood circulation are responsible for induction of ulceration [24].

The antiulcer activity of the extract in this model is evident from its significant reduction of gastric volume, total acidity, free acidity and ulcer index.

Histopathology of the stomach (fig. 10) shows *Spondias mombin* L. produced reduction in distortion and submucosa congestion when compared to control. Few or no ulceration was observed, with prominent effect produced by extract 200 mg/kg with well generated epithelium of the mucosa of the tissues followed by extract 100 mg/kg with mild to moderate congestion of mucosal vessels.

Non-protein sulfhydryls (NP-SHs) are thought to be involved in protecting gastric mucosa against various chemicals [25, 26]. (Rogers *et al.*, 1988; Ibrahim *et al.*, 2006). NP-SHs are important constituents of the intracellular protective mechanism against a number of noxious stimuli, including oxidative stress and are capable of binding reactive free radicals. The excessive generation of oxygen radicals in the extracellular space and depletion of NP-SHs are responsible for oxidative tissue damage of the gastric mucosa after the administration of alcohol, as suggested by various studies. [27] In this study, significant (p<0.05) increase in the NP-SH levels produced by extract 200 mg/kg when compared to control was observed, indicating that *Spondias mombin* L. prevented the depletion of non-protein sulfhydryl groups caused by alcohol treatment.

As the aqueous extract of *Spondias mombin* L. produced significant gastroprotective effects in all the experimental induced ulcer models in this study, the possible pharmacological mechanism of action was therefore investigated. For this purpose, involvement of endogenous nitric oxide (NO) and endogenous prostaglandins in the protective effect of *Spondias mombin* L. were investigated.

Nitric oxide (NO) is considered one of the most important defensive

endogenous agents in the gastric mucosa [28]; it is synthesized by nitric oxide synthase (NOS) from L-arginine [29]. Nitric oxide is involved in the modulation of gastric mucosal integrity and is important in the regulation of acid and alkaline secretion, mucus secretion and gastric mucosal blood flow [30]. In accessing the involvement of endogenous NO in the alcohol and ibuprofen-induced gastric ulcer models, rats were pre-treated subcutaneously with N<sup>G</sup>-nitro L-arginine methyl ester (L-NAME; 25 mg/kg), an inhibitor of NO synthase activity fifteen minutes prior to the administration of the standard drugs and test drug (extract 200 mg/kg).

L-NAME pretreatment significantly caused a reduction of (p<0.01) in the protective effect produced by the extract against alcohol-induced ulcer when compared to the untreated 200 mg/kg group. In this model, L-NAME pretreatment also produced a significantly (p<0.01) reduction in the gastroprotective effects exerted by the standard drug omeprazole.

In the ibuprofen-induced ulcer model, pretreatment with L-NAME produced a significant (p<0.05) reduction in the protective effect of the extract.

These findings indicate the possible participation of NOS pathway in the gastroprotection exerted by *Spondias mombin* L., supporting the premise of the free radical scavenging effect of this extract.

Prostaglandins are found in high concentration in the gastric mucosa and gastric juice. Prostaglandins inhibit acid secretion, stimulate mucus and bicarbonate secretion, alter mucosal blood flow, and provide dramatic protection against a wide variety of agents which cause acute mucosal damage [31]. To check for the involvement of endogenous prostaglandins in alcohol-induced ulcer model, animals were pretreated with ibuprofen subcutaneously at a dose (50 mg/kg) that did not cause ulcer, thirty minutes prior to the administration of test drug. The essence of this ibuprofen is to inhibit the biosynthesis of endogenous prostaglandins which enhance mucosal protection.

Pre-treatment with ibuprofen did not alter the gastroprotective effect produced by extract 200 mg/kg when compared to the untreated extract 200 mg/kg group.

In phytomedicine, various phytoconstituents such as flavonoids, alkaloid, tannins saponins and terpenes have been reported to possess antiulcer property [32].

Presence of flavonoids, tannins, cardiac glycosides, reducing sugar, steroids and terpenes are shown in the phytochemical analysis of *Spondias mombin*. Presence of flavonoids facilitates the increase in mucosal prostaglandin levels and inhibition of histamine release thus exhibiting a protective effect of the extract [33]. Tannins, another group of constituents in *Spondias mombin* L. prevent ulcer formation as a result of their protein precipitating and vasoconstricting effects [34]; their anstringent action helps to precipitate microproteins on the ulcer site, thereby, forming an impervious layer over the lining, which hinders induced gastric ulcer in rats [34]. The effect of aqueous leaf extract of *Spondias mombin* L. in this study may be due to one or a combination of these phytoconstituents.

Since free radicals have been reported to be implicated in the pathophysiology of gastric ulcers, antioxidants, which can scavenge free radicals are expected to heal or prevent gastric ulcers [35].

DPPH radical is widely used as the model system to investigate the scavenging activities of several natural compounds [36]. In solution, 1,1-diphenyl-2-picrylhydrazyl (DPPH) generates free radicals; the potent radical scavenging activity of the extract is recorded in figure 2. Furthermore, the extract also possesses potent NO<sup>-</sup> scavenging activity (Fig. 4), an antioxidant property also.

## CONCLUSION

The study has shown that the aqueous leaf extract of *Spondias mombin* L. has antiulcer property, which compared effectively with the standard drugs used. *Spondias mombin* L. showed protective effect on gastric mucosa in ibuprofen and alcohol-induced ulcer models and an inhibitory effect on gastric acid secretion in the pylorus-ligated models, hence, the speculated mechanisms of action of *Spondias mombin* L. include antisecretory, cytoprotective and antioxidant activities. The study has also been able to suggest some of the possible pathways for *Spondias mombin* L. cytoprotective activity which includes the NP-SH and Nitric oxide synthase pathways.

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