Gastroprotective And Antisecretory Properties Of The Hydroethanolic Leaf Extract Of *Blighia Sapida* (Sapindaceae) In Rats

Oreagba I. A., Ishola I.O., Oremule B.O.

(Department of Pharmacology, Therapeutics and Toxicology, University of Lagos, Nigeria)

Abstract: The objective of this study was to investigate the antiulcer activity of hydroethanolic extract of leaf extract of *Blighia sapida* (HeBS) using absolute ethanol, HCl-Ethanol, indomethacin, cold-restraint stress, and pylorus ligation ulceration in rats. The extract was primarily evaluated for acute toxicity test. Antiulcer potential was studied for at HeBS doses of 50, 100, or 200 mg/kg, p.o. HeBS (50-200 mg/kg) produced dose-dependent and significant anti-ulcer effects with 91.97, 50.00 and 83.26% protection against ulcer at 200 mg/kg in ethanol-, indomethacin-, and HCl-ethanol-induced ulcer, respectively which were comparable to the antiulcer effects of misoprostol (91.97 and 68.42% protection, respectively for ethanol- and indomethacin-induced ulcer models) and cimetidine (93.55% protection in HCl-ethanol-induced ulcer). Also, in the cold restraint stress- and pyloric ligation-induced ulcer models, the extract dose-dependently and significantly (p<0.05) reduced ulcer index (using cimetidine and omeprazole respectively as standard drugs) by 68.00 and 65.79% against ulcer at 200 mg/kg, respectively. Ulcer scores and ulcer index were measured in all the ulcer-induced models. Also, the pH, volume of gastric juice, free acidity, and total acidity was exclusively measured in the pyloric ligation-induced ulcer model. Treatment with hydroethanolic leaf extract of *Blighia sapida* prior to administration of ulcerogen significantly (p<0.05) protected gastric mucosa by significant reduction of ulcer index, and decrease in acid secretion in gastric mucosal injury. The mechanism through which the extract produces its anti-ulcer activity could said to be through its gastro-protective and anti-secretory as well as free radical scavenging activity.

Keywords: Antiulcer, anti-secretory, *Blighia sapida*, cimetidine, gastro-protective, indomethacin, omeprazole, pylorus ligation.

I. Introduction

Peptic ulcer is the most common gastro-intestinal tract (GIT) disorder in clinical practice, which affects approximately 5-10% of the people during their life time [1]. Peptic ulcer affects approximately 4.5 million people annually. The pathophysiology of these disorders has focused on an imbalance between aggression and protective factors in the stomach, such as acid-pepsin secretion, mucus barrier, mucus secretion, blood flow, cellular regeneration, prostaglandins and epidermal growth factors [2]. The drugs currently used in the treatment of gastric ulcers are antacids, anticholinergics, proton pump inhibitors, and H2-receptor antagonists [3,4]. However, most of these drugs produce adverse reactions such as: hypersensitivity, arrhythmia, impotence, gynaecomastia, and hematopoietic changes [5]. A search for ulcer treatment that is affordable, effective and devoid of side effects seen in currently available drugs is being intensified and current research has turn to natural resources [6].

*Blighia sapida* K.D. Koenig (syn. Cupania sapida Voigt,) belongs to the soapberry family (Sapindaceae): it is a pantropical distribution family with many edible fruits species exploited commercially. Ethnopharmacologically, it is used in making soap, dysentery, relieving pain, severe headache, epilepsy, yellow fever, conjunctivitis, oedema, yaws, ulcers, hepatitis, cirrhosis, and amygdalitis. Though *Blighia sapida* has been used in the treatment of gastrointestinal disorders in folk medicine, there are no scientific evidences on its anti-ulcer properties. Hence, this present study was designed to investigate the antiulcer potential of HeBS in ethanol, HCl-Ethanol, indomethacin, cold-restraint stress, and pylorus ligation-induced rat ulcer model.

II. Materials And Method

The fresh leaves were collected on the 15th of May, 2013 from Abatadu village (about 3km to Ikire township), Osun State by Mr. T.K. Odewo (Forestry expert) in the Department of Botany, University of Lagos with specimen number (LUH 5802). A specimen was also preserved in their herbarium for reference.

2.1 Preparation of Plant Extract: Fresh leaves of *Blighia sapida* were thoroughly washed with tap water followed by sterile distilled water. They were then air-dried in shade for seven days which was then pulverized...
into a fine powder. 500g of the milled powdered leaves of *Blighia sapida* were soaked in 70% ethanol for 72 h. It was thereafter decanted and filtered. The filtrate was dried in an oven at 40°C. The dried extract was weighed and stored in sample bottles and kept in the refrigerator until ready for use. The percentage yield calculated was 13.68%.

2.2 Animals: Young adult albino mice of both sexes weighing 15-30g were obtained from the Laboratory Animal Centre of the College of Medicine, University of Lagos. Adult albino rats weighing (150-200 g) of both sexes were also collected from the Laboratory Animal Centre of the College of Medicine, University of Lagos. The animals were given clear water, fed on pellets obtained from Animal care Livestock Feeds, Lagos and were kept under standard conditions of light, darkness, temperature, and humidity. The animals were housed in cages which had wire net tops while they were acclimatized for 7 days; they were fasted but allowed water for a period of 24 hours prior to the experiment.

2.3 Phytochemical Analysis of Extract: Preliminary phytochemical screening was carried out using Tease’s method [7] to test for alkaloids, flavonoids, tannins, saponins, cardiac glycosides.

2.4 Acute Toxicity Studies: Acute toxicity profile of the extract was determined using Miller’s method [8]. Mice were fasted for 12 hours prior to the study, graded doses of the extract was orally administered up to 5000 mg/kg while the control group received 10ml/kg of distilled water. Doses of 1000, 1500, 2000, 3000, and 4000 mg/kg were administered to different groups of animals to determine the LD$_{50}$ of the plant extract through the intra-peritoneal route. The control group was given distilled water (10 ml/kg). All the mice will be closely observed for toxic symptoms for the first 2 hours and mortality recorded 24 hours post administration. Also delayed toxicity was further observed for a period of 14 days.

2.4.1 Ethanol-induced gastric ulcer model: Rats were divided into five groups, n=5; animals were fasted for 24 h before the study. First group served as control and received distilled water (10 ml/kg). Second, third, and fourth groups served as treated groups and administered orally HeBS at the dose levels of 50, 100, 200 mg/kg, respectively. The fifth group was treated with misoprostol (200 µg/kg) which served as the standard drug. After 1 h of drugs treatment, absolute ethanol (10 ml/kg) was administered [9]. After 1 h, the animals were sacrificed by cervical dislocation and the abdomen was opened. The stomach was incised along the greater curvature and examined for ulcers [10]. Ulcer score was determined using the Magistreni Scores XXS221 scoring scale [11].

2.4.2 Indomethacin-Induced gastric ulcer model: The rats were divided into five groups, n=5; and were fasted for 24 h before experiment. First group served as control and received distilled water, while the second, third, and fourth groups served as treated groups and administered orally HeBS at doses of 50, 100, 200 mg/kg, respectively. The fifth group served as the standard group and was treated with misoprostol (200 µg/kg). After 1 h of drug treatment, indomethacin (50 mg/kg, p.o) was administered to all groups [12]. The animals were sacrificed 5 h after treatment. Stomach was cut along the greater curvature and examined for ulcers [10]. The number of ulcers were counted and ulcer scoring was done according to [11].

2.4.3 Cold restraint stress-induced gastric ulcer model: The experimental rats in each group were fasted for 24 hours and were divided into five groups, n=5. First group served as control and received distilled water, while the second, third, and fourth groups served as treated groups and administered orally HeBS at doses of 50, 100, 200 mg/kg, respectively. The fifth group served as the standard group and was treated with cimetidine (400 mg/kg). The animals were immobilized by strapping the fore and hind limbs on a flat packaging foam; and kept in the refrigerator for 2 h at a temperature of 4-6°C [13]. Two hours later, each rat was sacrificed by cervical dislocation, and the stomach was incised along the greater curvature. The lumen were rinsed with normal saline and examined. The ulcer score was determined according to the Magistreni scoring scale [11].

2.4.4 HCl-Ethanol-induced gastric ulcer model: Animals in all the groups were fasted for 24 hours and divided into five groups, n=5. First group served as control and received distilled water, while the second, third, and fourth groups served as treated groups and administered orally HeBS at doses of 50, 100, 200 mg/kg, respectively. The fifth group served as the standard group and was treated with cimetidine (400 mg/kg). Following the method of Okabe with slight modifications, 1 ml of HCl–ethanol mixture (0.15M of HCL in 70% v/v ethanol) was given orally for the induction of acute gastric mucosal damage [14]. The rats were sacrificed by cervical dislocation one hour after the administration of HCL– ethanol mixture, by cervical dislocation (under 1.5M Ketamine ) [15]. Each stomach was excised and the extent of gastric mucosal damage was determined by measuring each lesion in mm along its greater curvature. The ulcers in this model were localized in the glandular portion of stomach mucosa and various ulcer indices were determined [16].

2.4.5 Pyloric ligation-induced gastric ulcer model: Rats were divided into five groups, n=5; and fasted for 24 h but had free access to little quantity of water. First group served as control and received distilled water, while the second, third, and fourth groups served as treated groups and administered orally HeBS at doses of 50, 100, 200 mg/kg, respectively. The fifth group served as the standard group and was treated with omeprazole (20 mg/kg). After 1 h of drugs treatment, they were anaesthetized using 25 % Urethane and 1 % Chloralose at a...
dose of 1 ml of mixture/200g weigh of the rats used. The abdomen was opened by small incision below the xiphoid process. The pyloric portion of the stomach was slightly lifted out and ligated avoiding traction of the pylorus or damage to its blood supply [17]. The stomach was replaced carefully and the abdomen was closed in two layers with a moist swab of normal saline. After 4 h, each stomach was dissected out and cut open along the greater curvature. Ulcer score were determined using the Magistreni scoring scale [11].

2.4.6 Titration of Gastric Juice:

2.4.6.1 Collection of gastric juice: Gastric juice was collected from the pylorus of ligated rats. The gastric juice collected was centrifuged at 1000 rpm for 10 minutes and the supernatant was obtained. Volume of gastric juice was measured.

2.4.6.2 Determination of free acidity and total acidity: 1 ml of the gastric juice obtained was taken into a 100 ml conical flask. 2-3 drops of phenolphthalein indicator was added and it was titrated with 0.01 M NaOH until the colour turned pink (end point).

2.4.6.3 Calculation of Ulcer Index: The stomachs were spread out after removing them from 0.9% w/v saline and examined with the aid of a transparent graph sheet for lesion. The extent of ulceration in the excised stomach is compared to that of the controls in each experiment. The presence of spots and scoring of gastric ulceration was done according Magistretti’s method [11]. The extent of ulceration was expressed as an ulcer index.

2.4.7 Statistical Analysis of Results: This was done and statistical significance was evaluated by one-way ANOVA followed by Tukey Comparisons Test. Significance of difference was accepted as P<0.05.

III. Results

3.1 Physicochemical Properties of the Extract: The extract appeared sticky, with a dark brown coloration. It had a pungent smell and it was tasteless and would readily absorb moisture when exposed to air. It completely dissolved in water and had a pH of 8.0.

3.2 Acute Toxicity Test in Mice: There was no mortality nor visible signs of toxicity observed during the 14 days of observation in the group treated with the aqueous leaf extract of Blighia sapida up to a dose of 5000 mg/kg, p.o. However, intraperitoneal administration of the extract produced dose-dependent mortality; the calculated LD₅₀ was 1288.25 mg/kg. The observed toxicity behaviors include; writhes (abdominal contraction), increased respiratory rate, mild sedation, calmness, stooling, and decreased motion activity within the first few hours of administration.

3.3 Phytochemical Analysis: Preliminary phytochemical screening revealed the presence of flavonoid, saponin, alkaloids, phenols, tannin, cardiac glycoside, reducing sugar and carbohydrates (Table 2).

3.4 Ethanol-induced Gastric Ulcer in Rat: Oral administration of ethanol induced extensive visible hemorrhagic necrosis in gastric mucosa (Plate 3). However, misoprostol (prostaglandin analogue) pretreatment prevented the gastric mucosa damage-induced by ethanol (Plate 4). Also, significantly (P<0.001) decreased

---

www.ijmdsi.org

40
ulcer index by 91.97% (Plate 3). Similarly, hydroethanolic leaf extract of Blighia sapida (HeBS) at graded doses of 50, 100 and 200 mg/kg also improved healing of gastric ulceration (Plates 5, 6, 7 respectively) significantly ($P < 0.01; 0.001$) in rats when compared with ethanol control group by 60.58%, 55.47% and 91.97%, respectively (Table 3). However, there were no significant ($P > 0.05$) difference between the HeBS treated rats at the doses used (50, 100 and 200mg/kg) in comparison to misoprostol treated.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Ulcer index</th>
<th>% protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>10 ml/kg</td>
<td>27.40±9.80</td>
<td>-</td>
</tr>
<tr>
<td>HeBS</td>
<td>50</td>
<td>10.80±3.00</td>
<td>60.58</td>
</tr>
<tr>
<td>HeBS</td>
<td>100</td>
<td>12.20±3.50</td>
<td>55.47</td>
</tr>
<tr>
<td>HeBS</td>
<td>200</td>
<td>2.20±0.70</td>
<td>91.97</td>
</tr>
<tr>
<td>Misoprostol</td>
<td>200 mcg/kg</td>
<td>2.20±0.90</td>
<td>91.97</td>
</tr>
</tbody>
</table>

Table 3: Effect of hydroethanolic leaf extract of Blighia sapida

Values are expressed as Mean ±SEM (n = 5); **$P < 0.01$, ***$P < 0.001$ as compared with distilled water treated control group, ^$P < 0.001$ as compared with 50 mg/kg B. sapida treated group. Statistical analysis of level of significance was done using one way ANOVA followed by Tukey post hoc multiple comparison test.

Plate 1: Pictorial Illustration of the gross appearances of pre-treated groups of the rat stomach and control group for the ethanol induced ulcer model.

<table>
<thead>
<tr>
<th>EMG</th>
<th>EEG 1</th>
<th>EEG 2</th>
<th>ECG 3</th>
<th>ECG 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>Moderate congestion and inflammation</td>
<td>Moderate congestion and inflammation</td>
<td>Mild congestion and inflammation</td>
<td>Moderate inflammation</td>
</tr>
</tbody>
</table>

Table 4: HISTOPATHOLOGICAL ANALYSIS:

H&E  EMG = Ethanol model, misoprostol (200mcg/kg) group
a x 40  ECG = Ethanol model, control (10ml/kg) group
b x 100  EEG 1 = Ethanol model, extract (50mg/kg) group
c x 400  EEG 2 = Ethanol model, extract (100mg/kg) group
          EEG 3 = Ethanol model, extract (200mg/kg) group

3.4.1 Stomach:
Normal - Mucinous gastric glands displayed on a muscular layer.
Congestion- Presence of dilated vessels containing blood.

www.ijmdsi.org 41
Inflammation - Presence of inflammatory cell infiltrates which includes eosinophils and plasma.

Plate 2: Histological section of ethanol induced gastric mucosa ulcer in animal pretreated with HeBS (50mg/kg, H & E stain 400 x magnification) showing inflammatory cells and congested vessels.
Plate 3: Histological section of ethanol induced gastric mucosa ulcer in animal pretreated with HeBS (100mg/kg, H & E stain 400 x magnification) showing inflammatory cells.
Plate 4: Histological section of ethanol induced gastric mucosa ulcer in animal pretreated with HeBS (200mg/kg, H & E stain 400 x magnification) showing inflammatory cells and congested vessels.
Plate 5: Histological section of ethanol induced gastric mucosa ulcer in animal pretreated with Misoprostol (200mcg/kg, H & E stain 400 x magnification) showing congested vessels.
Plate 6: Histological section of ethanol induced gastric mucosa damaged in control animal (10ml/kg H & E stain 400 x magnification) showing inflammatory cells.

3.5 Indomethacin-Induced Gastric Ulcer in Rat: Oral administration of indomethacin induced visible hemorrhagic necrosis in gastric mucosa (Plate 7). However, misoprostol (prostaglandin analogue) pretreatment prevented the gastric mucosa damage-induced by indomethacin (Plate 7). Also, significantly (P< 0.05) decreased ulcer index by 68.42% (Table 4). Similarly, hydroethanolic leaf extract of Blighia sapida (HeBS) at graded doses of 100 and 200 mg/kg also improved healing of gastric ulceration (Plates 17 and 18 respectively) significantly (P< 0.05) in rats when compared with indomethacin control group by 60.52% and 50.00%, respectively (Table 4). However, HeBS at 50 mg/kg (Plate 7) did not show significance (P> 0.05) in gastric ulceration healing (26.31%) when compared with indomethacin control group (Table 4). Futhermore, there were no significant (P> 0.05) difference between the HeBS treated rats at the doses used (50, 100 and 200mg/kg) in comparison to misoprostol treated.

Table 5: Effect of hydroethanolic leaf extract of Blighia sapida (HeBS) on indomethacin induced ulcer model.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Ulcer index</th>
<th>% protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>10 ml/kg</td>
<td>3.80±0.50</td>
<td>-</td>
</tr>
<tr>
<td>HeBS</td>
<td>50</td>
<td>2.80±0.70</td>
<td>26.31</td>
</tr>
<tr>
<td>HeBS</td>
<td>100</td>
<td>1.50±0.60</td>
<td>60.52</td>
</tr>
<tr>
<td>HeBS</td>
<td>200</td>
<td>1.90±0.80</td>
<td>50.00</td>
</tr>
<tr>
<td>Misoprostol</td>
<td>200 mcg/kg</td>
<td>1.20±0.80</td>
<td>68.42</td>
</tr>
</tbody>
</table>

Values are expressed as mean±Standard Error of Mean; *P<0.05 when compared with distilled water treated control group. One way ANOVA followed by Tukey post hoc multiple comparison test

www.iijmdsi.org
Plate 7: Pictorial Illustration of the gross appearances of pre-treated groups of the rat stomach and control group for the indomethacin induced ulcer model.

3.6  HCl-Ethanol-Induced Gastric Ulcer in Rat: Oral administration of HCl-ethanol induced visible hemorrhagic necrosis in gastric mucosa. However, cimetidine (H₂ antagonist) pretreatment prevented the gastric mucosa damage-induced by HCl-ethanol. Also, significantly (P< 0.05) decreased ulcer index by 93.55% (Table 6). Similarly, hydroethanolic leaf extract of Blighia sapida (HeBS) at graded dose of 200 mg/kg also improved healing of gastric ulceration significantly (P< 0.05) in rats when compared with HCl-ethanol control group by 83.26% (Table 6). Although there was healing of gastric ulceration in HeBS doses of 50 and 100 mg/kg by 55.65 and 58.87%, there was no significance (P> 0.05). Also, there was no significance (P> 0.05) when the graded of HeBS used (50, 100, 200 mg/kg) with the cimetidine group.

Table 6: Effect of hydroethanolic leaf extract of Blighia sapida (HeBS) on HCl-ethanol-induced ulcer model.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Ulcer index</th>
<th>% protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>10 ml/kg</td>
<td>12.40±5.30</td>
<td>-</td>
</tr>
<tr>
<td>HeBS</td>
<td>50</td>
<td>5.50±2.90</td>
<td>55.65</td>
</tr>
<tr>
<td>HeBS</td>
<td>100</td>
<td>5.10±3.20</td>
<td>58.87</td>
</tr>
<tr>
<td>HeBS</td>
<td>200</td>
<td>2.20±1.10</td>
<td>83.26</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>400</td>
<td>0.80±0.40</td>
<td>93.55</td>
</tr>
</tbody>
</table>

Values expressed as Mean±SEM; *p< 0.05 when compared with HCl-Ethanol (control) group. HCl = Hydrochloric acid

3.7 Cold restraint stress-induced gastric ulcer in rat: Cold restraint induced visible hemorrhagic necrosis in gastric mucosa. However, cimetidine (H₂ antagonist) pretreatment prevented the gastric mucosa damage-induced by cold restraint. Also, significantly (P< 0.05) decreased ulcer index by 84.00% (Table 7). Similarly, hydroethanolic leaf extract of Blighia sapida (HeBS) at graded doses of 50, 100 and 200 mg/kg also improved healing of gastric ulceration significantly (P< 0.05) in rats when compared with cold restraint control group by 62.00, 62.00 and 68.00%, respectively (Table 7). However, there were no significant (P> 0.05) difference between the HeBS treated rats at the doses used (50, 100 and 200 mg/kg) in comparison to cimetidine treated.

Table 7: Effect of hydroethanolic leaf extract of B. sapida (HeBS) on cold restraint stress-induced ulcer model.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Ulcer index</th>
<th>% protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>10 ml/kg</td>
<td>5.00±1.90</td>
<td>-</td>
</tr>
<tr>
<td>HeBS</td>
<td>50</td>
<td>1.90±0.30</td>
<td>62.00</td>
</tr>
</tbody>
</table>
Values expressed as Mean±SEM; *P< 0.05 when compared with cold restraint (control) group.

3.8 Pyloric ligation-induced gastric ulcer in rat: Pylorus ligation caused accumulation of gastric secretions and hence, intense lesions in the mucosa of the stomach in control rats (Fig. 16). However, omeprazole (proton pump inhibitor) pretreatment prevented the gastric mucosa damage induced by pylorus ligation (Fig. 17). Also, significantly (P< 0.001) decreased ulcer index by 50.00% (Table 8). Similarly, hydroethanolic leaf extract of *Blighia sapida* (HeBS) at graded doses of 50, 100 and 200 mg/kg also improved healing of gastric ulceration (Fig. 18, 19, 20 respectively) significantly (P< 0.01; 0.001) in rats when compared with pyloric ligation control group by 47.37%, 64.47% and 65.79%, respectively (Table 8). There were significant (P< 0.01; 0.001) change in the average volume of gastric juice only in HeBS treated rats at dose of 100 and 200 mg/kg while pH was significantly (P<0.01; 0.001) modified in the 200 mg/kg dose HeBS when compared to result observed in the pylorus ligation control group (Table 8). There were no significant (P> 0.01; 0.001) modifications in pH of other HeBS treated doses of 50, 100 mg/kg as well as the omeprazole treated group when compared to the pylorus ligation control group (Table 8). The total acidity output was significantly affected (P<0.01; 0.001) only in HeBS dose at 50 mg/kg when compared with both the pylorus ligation control group and the omeprazole treated group. However, there were no significant (P> 0.05) difference between the HeBS treated rats at the doses used (50, 100 and 200 mg/kg) in comparison to omeprazole treated.

### Table 8: Effect of hydroethanolic leaf extract of *B. sapida* (HeBS) on Pyloric Ligation-induced ulcer model.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Ulcer Index</th>
<th>Percent-age protection (%)</th>
<th>Average Volume of gastric juice (ml)</th>
<th>Average pH</th>
<th>Free Acidity (ml)</th>
<th>Total Acidity (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>10 ml/kg</td>
<td>7.6±0.60</td>
<td>-</td>
<td>0.89±0.80</td>
<td>3.70±0.03</td>
<td>9.02±0.70</td>
<td>9.9±0.80</td>
</tr>
<tr>
<td>HeBS 50</td>
<td>5</td>
<td>4.00±0.04</td>
<td>47.37</td>
<td>1.12±3.00</td>
<td>4.04±0.12</td>
<td>13.44±1.10</td>
<td>14.57±1.18</td>
</tr>
<tr>
<td>HeBS 100</td>
<td>100</td>
<td>2.70±0.30</td>
<td>64.47</td>
<td>0.50±3.50</td>
<td>5.82±0.58</td>
<td>5.68±1.93</td>
<td>6.17±2.09</td>
</tr>
<tr>
<td>HeBS 200</td>
<td>200</td>
<td>2.60±0.60</td>
<td>65.79</td>
<td>0.57±0.70</td>
<td>6.78±1.96</td>
<td>6.02±0.70</td>
<td>7.34±2.13</td>
</tr>
<tr>
<td>Omeprazole 20</td>
<td>20</td>
<td>3.80±4.90</td>
<td>50.00</td>
<td>0.81±0.90</td>
<td>6.36±0.16</td>
<td>7.12±0.80</td>
<td>7.93±0.90</td>
</tr>
</tbody>
</table>

Result Expressed As Mean±Standard Error of Mean: *p<0.01; 0.001 when compared with Pylorus ligation (control) group. *P< 0.05 when compared with omeprazole.

3.9 Effects of the Hydroethanolic Leaf Extract of *Blighia Sapida* (HeBS) Reduced Glutathione (GSH), Superoxide Dismutase (SOD), Catalase (CAT) and Lipid Peroxidation (Malondialdehyde [MDA]) Activities on Pyloric Ligated-Induced Ulcer Rat Model: In Table 9, antioxidant activities including reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT) and lipid peroxidation (MDA) were investigated in the pyloric ligated-induced ulcer model. From the results obtained, GSH levels increased significantly (P< 0.05) by 68.85 and 90.16% in 50 and 200 mg/kg treatment respectively, however, there were increase in HeBS treated animals 100 mg/kg and omeprazole 20 mg/kg by 80.33 and 85.25% respectively without significance (P> 0.05) when compared with control. Also, the activity of SOD changed significantly (P< 0.05) in the HeBS treated groups of 50 and 200 mg/kg, and which increased by 3.24 and 5.01% respectively when compared to the control group. However, HeBS at 100 mg/kg and omeprazole 20 mg/kg showed increased SOD level by 3.83 and 7.96% respectively when compared with control group. CAT levels increased significantly (P< 0.05) in the HeBS pretreated groups of 100 and 200 mg/kg by 6.35 and 9.40% respectively; however, there were increase in HeBS pretreated groups of 50 mg/kg and omeprazole 20 mg/kg by 4.00 and 11.96% respectively with no significance (P> 0.05). More so, lipid peroxidation measured as MDA activity changed significantly (P< 0.05) in pretreated animals at graded doses 100mg/kg and omeprazole 20 mg/kg by 33.33 and 40.00% respectively; however, there were increase in 50 and 200 mg/kg treated animals by 26.67 and 53.33% without significance (P> 0.05) group respectively compared to the free radical activities of the control group.

### Table 9: Effects of hydroethanolic leaf extract of *Blighia sapida* (HeBSs) on Reduced Glutathione, Superoxide Dismutase, Catalase and Malondialdehyde Activities in Pyloric ligated-induced ulcer rat model.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>GSH</th>
<th>% diff</th>
<th>SOD</th>
<th>% diff</th>
<th>CAT</th>
<th>MDA</th>
<th>% diff</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>0.61±0.47</td>
<td>-</td>
<td>3.39±0.24</td>
<td>-</td>
<td>19.99±1.62</td>
<td>-</td>
<td>0.15±0.00</td>
</tr>
<tr>
<td>HeBS 50 mg/kg</td>
<td>1.03±0.13</td>
<td>-68.85</td>
<td>3.50±0.06</td>
<td>-3.24</td>
<td>20.79±0.94</td>
<td>-4.00</td>
<td>0.11±0.03</td>
</tr>
<tr>
<td>HeBS 100 mg/kg</td>
<td>1.10±0.57</td>
<td>-80.33</td>
<td>3.52±0.06</td>
<td>-3.83</td>
<td>21.26±0.99</td>
<td>-6.35</td>
<td>0.14±0.05</td>
</tr>
<tr>
<td>HeBS 200 mg/kg</td>
<td>1.16±0.70</td>
<td>-90.16</td>
<td>3.56±0.38</td>
<td>-5.01</td>
<td>21.87±3.58</td>
<td>-9.40</td>
<td>0.07±0.08</td>
</tr>
</tbody>
</table>

www.ijmdsi.org 44
Table 1: Effect of different doses of HeBS and cimetidine on gastric acid secretion of rats (mg/kg)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Acid Decrease (%)</th>
<th>pH Increase (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Omeprazole 20 mg/kg</td>
<td>-85.26 ± 0.22</td>
<td>3.66 ± 0.04</td>
</tr>
<tr>
<td>HeBS 50 mg/kg</td>
<td>-7.96 ± 0.04</td>
<td>22.38 ± 0.26</td>
</tr>
<tr>
<td>HeBS 100 mg/kg</td>
<td>-11.96 ± 0.04</td>
<td>0.09 ± 0.04</td>
</tr>
</tbody>
</table>

Values are expressed as mean±Standard Error of Mean. *p<0.05 when compared with the control group.

Plate 8: Pictorial Illustration of the gross appearances of pre-treated groups of the rat stomach and control group for the Pylorus ligation induced ulcer model.

IV. Discussion

There are several factors that may induce ulcer in human being such as stress, chronic use of anti-inflammatory drugs and continuous alcohol ingestion, among others [19]. Although in most cases the etiology of ulcer is unknown, it is generally accepted that it is the result of an imbalance between aggressive factors and maintenance of the mucosal integrity through the endogenous defense mechanism [20, 21]. A significant reduction in the number of lesions in HeBS pretreated rats with 200 mg/kg dose showing equal protective capacity as misoprostol. This protective capacity of HeBS may be due to the acid neutralizing capacity of the drug, thereby reducing the gastric tissue damage and ulcer formation, revealing its antacid property.

Pretreatment with cimetidine (positive control) and HeBS (50, 100, 200 mg/kg) significantly increased mucosal NO level when compared to indomethacin treated rats. This caused a reduction in the damage caused by indomethacin. The possible mechanism of the increased NO action of HeBS may be due to its flavonoids content. Reports by Matsuda [22] revealed that flavonoids are the major secondary metabolites class with several descriptions of antiulcer, antioxidant and gastroprotective properties. Flavonoids could scavenge free radicals, inhibit lipid peroxidation, and increase prostaglandins and mucosal content of the gastric mucosa; showing cytoprotective effects [23]. HeBS hadprotective effect against indomethacin-induced inflammatory infiltration and congestion at the ulcer sites. It prevented gastric mucosal lesions through its flavonoid content.

The prior administration of HeBS (50, 100, 200 mg/kg) dose had a gastro-protective activity on the mucosal cells by reducing the ulcer index level (Table 6) suggesting prevention of loss of protein. The study showed that only HeBS 200 mg/kg as well as cimetidine (positive control) showed significance (P< 0.05) compared to the negative control (HCl-ethanol) group.

Exposure of the animals to the cold restraint stress may have caused severe imbalance in the normal physiological conditions that might have resulted in a stressful condition leading to ulcers. Pre-treatment with HeBS 50, 100, 200 mg/kg produced a significant decrease in the intensity of gastric mucosal damage induced by the stress as compared with control. A significant increase in the ulcer index and mean score in the control group was observed, however both the parameters were significantly decreased in the HeBS 50, 100, 200 mg/kg treated groups.

In this study, antiulcer activity of HeBS (50, 100, 200 mg/kg) showed significance (P< 0.05) in ameliorating the corrosive effect of the gastric acid. All the different doses of the extracts showed decrease in gastric acidity on
comparison with control group and indicated their anti-secretory effort compared to control group, all the test extract showed elevation in pH indicating their capacity to reduce the acidity of the gastric juice.

The results of the present investigation further showed that omeprazole significantly decreased gastric acidity and ulcer index. The effect of omeprazole is mediated by inhibition of the proton pump (H⁺K⁺ATPase - the enzyme responsible for H⁺ secretion by the gastric parietal cells) on the parietal cells which reduces the release of gastric acid from these cells into the gastric mucosa. The tested extracts have an effective antisecretory and antulcer activity against pyloric ligation-induced gastric ulcer in rats. HeBS has been revealed in this study to attenuate elevated mucosal H⁺K⁺ATPase in gastric mucosal injury induced by ischaemic-reperfusion.

Study of oxidative stress parameters showed that pyloric ligation resulted in increase in gastric mucosal MDA content along with a decrease in gastric mucosal GSH content and SOD activity [24, 25]. The reduction of SOD activity could be attributed to the increase in free radical generation [26] where SOD protects organisms from ROS-mediated damage to cell components [27]. Results of the present study showed that HeBS decreased gastric mucosal MDA content as well as omeprazole. MDA activity decreased significantly in 100mg/kg and omeprazole 20 mg/kg doses but decreased insignificantly in 50 and 200 mg/kg treatment. The present data showed that HeBS increased gastric mucosal GSH content increased significantly in the 50 and 200 mg/kg treatment but it increased insignificantly in the 100 mg/kg and omeprazole treatment. GSH detoxifies hydrogen peroxide (H₂O₂) and/or organic acids chemically; H₂O₂ accumulates in the absence of GSH [28] and in the presence of transition metals, H₂O₂ reacts with superoxide anion resulting in the formation of hydroxyl radical, the most reactive and cytotoxic form of reactive oxygen species (ROS) [29]. The activity of SOD increased significantly in the HeBS treated groups of 50 and 200 mg/kg however increased significantly in the 100 mg/kg and omeprazole treatment. CAT plays a significant role in the elimination of hydrogen peroxide. It is the most efficient enzyme known and so efficient that it cannot be saturated by H₂O₂ at any concentration [30]. In this study, CAT levels increased significantly in treated groups of 100 and 200 mg/kg, however increased insignificantly in the 50 mg/kg and omeprazole treatment.

V. Conclusion

Based on the results obtained from this study, it could be concluded that:

- administration of HeBS in the treatment of peptic ulcer has revealed its gastro-protective properties; in addition to its anti-secretory activity.
- it possesses antioxidant properties by its free radical scavenging activity.
- the anti-gastric ulcer activity of HeBS 200 mg/kg showed a higher gastroprotective activity compared to the other graded doses of the HeBS. However, there was no significance (P> 0.05) when compared to the different standard doses employed. Hence, it is not dose-dependent.

References

Gastroprotective And Antisecretory Properties Of The Hydroethanolic Leaf Extract Of Blighia ...


