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Antiulcerogenic Potentials of Fermented Aqueous Extract of *Pentaclethra macrophylla* (Benth) Seeds

Ugbogu A.E.[1,*], Arunsi U.O.[1], Uche-Ikonne O. O[2], Ude V.C[3], Okezie E[1]

[1]Department of Biochemistry, Abia State University, Uturu, Abia State, Nigeria
[2]Department of Statistics, Abia State University, Uturu, Abia State, Nigeria
[3]Department of Medical Biochemistry, College of Medicine Enugu State University of Science and Technology, Enugu-Nigeria
*Corresponding author: amasryal@yahoo.com

Abstract *Pentaclethra macrophylla* popularly known as African oil bean is a member of the Leguminosae family. A decoction of fermented extract of the plant has been known to be effective in the management of malnutrition, gastrointestinal disorders and dental caries. This study evaluated the antiulcerogenic potentials of aqueous extract of fermented *P. macrophylla* seeds using acetic acid, aspirin, ethanol, indomethacin and pyloric ligation of ethanol induced ulcer models at the doses of 400 and 800mg/kg body weight. Omeprazole at 5mg/kg was used as a standard reference drug. The result of the acute toxicity test showed that up to 5,000mg/kg body weight of the extract did not cause any mortality of the animals. The different doses of the extract and the reference drug decreased significantly ($p<0.05$) the ulcer parameters in a dose-dependent manner in all the ulcer models. The degree of ulcer index for the negative control groups is in the order: Indomethacin (11.10±0.10) < Pyloric-ligation (11.57±0.06) < Aspirin (11.60±0.10) <Acetic acid (15.85±0.13) < Ethanol (16.30±0.18). Similarly, the percentage gastro-protective activity increased from 0% in the negative control up to 29.85% at the dose of 800mg/kg body weight of the extract. The degree of percentage gastro-protection is in the order: Acetic acid (0.21%) <Indomethacin (3.89%) < Aspirin (5.17%) < Pyloric-ligation (8.64%) < Ethanol (29.85%). The enhanced cessation of gastric erosions could be attributed to the synergistic role of biochemicals and microbiomes residents in fermented aqueous extract of *P. macrophylla* seeds. However, the decoction of the plant could be employed in ethnomedicine for the treatment of peptic ulcer.

Keywords: *antiulcerogenic, gastro-protective activity, Pentaclethra macrophylla*

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1. Introduction

Peptic ulcers, otherwise known as "ulcus pepticum", are sores or open wounds that occur along the lining of the gastrointestinal tract due to loss of tissues. Although the etiopathology of ulcers is still questionable, Odenbreit [1] and Nartey *et al*. [2] opined that peptic ulcers are caused by an imbalance between aggressive factors (*Helicobacter pylori*, stomach acid and pepsin production, non-steroidal anti-inflammatory drugs, ethanol, bile salts, free radicals, chronic alcohol consumption, smoking) and protective factors (mucin secretion, cellular mucus, prostaglandins, bicarbonate secretion, increased mucosal blood flow, cell turn over and impermeability to hydrogen ions), thus leading to an interruption in the mucosal integrity. Several conventional drugs have been employed over the years to arrest this "red devil" called peptic ulcers. Such drugs include H_2-blockers (Cimetidine, ranitidine), M_1-blockers (porenzepine, telezepine), prostaglandin analogue (misoprostol), proton pump antagonists (omeprazole, lansoprazole), antacids (calcium tetraoxosulphate (iv) [$CaSO_4$], sodium hydrogen trioxocarbonate (iv) [$NaHCO_3$), triple drug therapy (to eradicate *Helicobacter pylori*). The successes of these drugs are limited by several adverse effects: darkening of the stool and/or tongue, metallic taste in the mouth, hypotension, loss of libido, impotence, relapse of the disease, drug resistance, constipation and nausea [3,4]. These shortcomings have led to the search for more effective therapeutic targets with better protection and decrease in incidence of relapse [5].

Mankind relies upon products from medicinal plants for the management of diseases including ulcers. The wide use of medicinal plants in the management of diseases is due to their bioavailability, higher safety margin, efficacy, quality, affordability [6,7] and most importantly, the ability to target biochemical pathways. Several plants have been tested in the laboratory for antiulcerogenicity. Included in the pharmacopeia are extracts of the roots and barks of *Cassia sieberiana* in rats [2] extracts of the roots and leaves of *Flabellaria paniculata Cav* in rats [8], extracts of the stem of *Synclisa scabrida* in mice [9], extracts of *Cochlospermum planchonii* in both male and female wistar rats [10], extracts of *Enicosanthelum pulchrum* in *Sprague Dowley* rats [11] and extract of unripe *Musa paradisiaca* Linn. peel in wistar rats [6]. *Pentaclethra macrophylla* (Benth) is a leguminous plant native to tropical Africa and belongs to the family

Leguminosae (sub-family Mimosoideae) in the order Fabales. The trees of *P. macrophylla* thrive in the Eastern and Southern parts of Nigeria [12]. The local names include "Congo acacia" in Congo, "Duala Kombola" in Cameroon and "Ugba", Ukpkala" in South Eastern part of Nigeria [13]. In the traditional setting, the plant is used as salt substitute, charcoal, carvings, seed craft, dye, mild poison, medicine (against convulsion, abortion, diarrhoea, infertility), wound treatment, lactogenicity, ornamental as well as fencing, timber and structural work [14]. The participation of probiotics (products of fermentation) in the management of infectious diseases has not been given proper attention over the years. Probiotics are live microorganisms that, when administered in adequate amounts, confer health benefits on the host [15]. It is on this backdrop that this present study was developed. Therefore, the study was aimed at investigating the antiulcerogenic potentials of aqueous extract of fermented *P. macrophylla* (Benth) seeds on different ulcer models (acetic acid, aspirin, ethanol, indomethacin and pyloric ligation-induced) in wistar rats.

2. Materials and Methods

2.1. Sample Collection and Identification

Pentaclethra macrophylla seeds were purchased from Eke Okigwe, Okigwe Local Government Area of Imo State, Nigeria. Eke Okigwe lies between latitude $5^0 50^1 0.9^{II}$N and $5^0 49^1 55^{II}$N and longitude $7^0 21^1 32.6^{II}$E and $7^0 23^1 17^{II}$E.The plants were identified in the Department of Plant Science and Biotechnology, Abia State University, Uturu.

2.2. Sample Preparation

P. macrophylla seeds upon purchased were sorted to remove rotten seeds, dust and extraneous materials and washed with clean water. Exactly 100g of the fermented *P. macrophylla* (ugba) was weighed using G & G® Electronic Scale into a beaker containing 270ml of distilled water. The beaker was covered with foil and allowed to stand for 15 hours. The extract was filtered using cheese cloth and the membrane filter paper of 47.0mm diameter and the pore size approximately 0.45µm. The filtrate was stored in the refrigerator prior to use.

2.3. Acute Toxicity Study

Lorke [16] method was adopted for the determination of median lethal dose of fermented aqueous extract of *P. macrophylla* seed. In the pilot study, twelve rats weighing between 150-200g were randomly divided into four groups (A, B, C and D) of three rats each and were administered aqueous extract of fermented *P. macrophylla* seeds at 500, 1000, 2000 and 5000mg/kg body weight respectively. The animals were then observed for behavioural changes and mortality for 24 hours. The LD_{50} is usually calculated as the geographic mean of the least lethal dose that killed a rat and the highest dose that did not kill a rat.

Figure 1. Diagram of *P. macrophylla* seed extract

2.4. Experimental Animals

The albino wistar rats were obtained from University of Nigeria, Nsukka, and were allowed to acclimatize for two (2) weeks in the Animal House of the Department of Biochemistry, Abia State University, Uturu. These animals were fed on grower mash. All the animals used had free access to clean water. They were kept in well ventilated rooms with 12/12 h light/dark condition and ambient room temperature. The experimental procedures used in this study conformed to the United States National Institutes of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research [17].

2.5. Experimental Design

2.5.1. Acetic Acid-induced Ulcer Study

The anti-ulcerogenic potentials of fermented aqueous extract of *P. macrophylla* seed on acetic acid-induced ulcer model in wistar rats was investigated using modified method [18]. A total of fifteen wistar rats (150-200g body weight) was starved for 24 hours and grouped into five

groups (Group 1 to V) of three rats each based on their body weights. Each administration in the various groups was done according to the specification below:

Group I: Normal Control (n=3) given orally distilled water (0.25ml/kg) body weight.

Group II: Negative control (n=3) given orally acetic acid (0.4ml/kg) body weight

Group III: Reference drug (n=3) given orally omeprazole (5mg/kg) body weight.

Group IV: Plant extract (n=3) given orally plantain extract (400mg/kg) body weight.

Group V: Plant extract (n=3) given orally plantain extract (800mg/kg) body weight.

After 30 minutes of treatment, each animal in Groups II- V received 0.5ml of 80% acetic acid orally. After 1 hour, all the animals were anaesthetized with chloroform and dissected. The stomachs were excised and carefully opened along the line of greater curvature to expose the walls. The stomachs' contents were then washed off and the stomach walls viewed with the aid of hand lens (x10) to determine the ulcer scores using the method [19]. The ulcerative lesions were counted and scored as follows:

Normal stomach	-		0
Pinhole	-	-	1.0
Spot ulceration	-	-	1.5
haemorrhagic streaks	-	-	2.0
Small erosion	-	-	2.5
Large erosion	-	-	3.0
Perforation	-	-	3.5.

2.5.2. Aspirin-induced Ulcer Study

The anti-ulcerogenic potentials of fermented aqueous extract of *P. macrophylla* seeds on aspirin induced ulcer model in wistar rats was investigated using modified method [19]. A total of fifteen wistar rats (150-200g body weight) was starved for 24 hours and grouped into five groups (Group 1 to V) of three rats each based on their body weights. Each administration in the various groups was done according to the specification below:

Group I: Normal Control (n=3) given orally distilled water (0.25ml/kg) body weight.

Group II: Negative control (n=3) given orally aspirin (1000mg/kg) body weight.

Group III: Reference drug (n=3) given orally omeprazole (5mg/kg) body weight.

Group IV: Plant extract (n=3) given orally plantain extract (400mg/kg) body weight.

Group V: Plant extract (n=3) given orally plantain extract (800mg/kg) body weight.

After 30 minutes of treatment, each animal in Groups II-V received 1000mg/kg of aspirin orally. After 1 hour, all the animals were anaesthetized with chloroform and dissected. The stomachs were excised and carefully opened along the line of greater curvature to expose the walls. Their stomachs were removed and treated as mentioned and the ulcers scored.

2.5.3. Ethanol-induced Ulcer Study

The anti-ulcerogenic potentials of fermented aqueous extract of *P. macrophylla* seed on ethanol-induced ulcer model in wistar rats was investigated using the method [20]. A total of fifteen wistar rats (150-200g body weight) was starved for 24 hours and grouped into five groups (Group 1 to V) of three rats each based on their body weights. Each administration in the various groups was done according to the specification below:

Group I: Normal Control (n=3) given orally distilled water (0.25ml/kg) body weight.

Group II: Negative control (n=3) given orally ethanol (absolute) (1.2ml/kg) body weight

Group III: Reference drug (n=3) given orally omeprazole (5mg/kg) body weight.

Group IV: Plant extract (n=3) given orally plantain extract (400mg/kg) body weight.

Group V: Plant extract (n=3) given orally plantain extract (800mg/kg) body weight.

After 30 minutes of treatment, each animal in Groups II- V received 1.2ml/kg of absolute ethanol orally. After 1 hour, all the animals were anaesthetized with chloroform and dissected. Their stomachs were removed and treated as previously mentioned and the ulcer scores determined.

2.5.4. Indomethacin-induced Ulcer Study

The anti-ulcerogenic potentials of fermented aqueous extract of *P. macrophylla* seeds on indomethacin-induced ulcer model in wistar rats was investigated using the method [21]. A total of fifteen wistar rats (150-200g body weight) was starved for 24 hours and grouped into five groups (Group 1 to V) of three rats each based on their body weights. Each administration in the various groups was done according to the specification below:

Group I: Normal Control (n=3) given orally distilled water (0.25ml/kg) body weight.

Group II: Negative control (n=3) given orally indomethacin (100mg/kg) body weight

Group III: Reference drug (n=3) given orally omeprazole (5mg/kg) body weight.

Group IV: Plant extract (n=3) given orally plantain extract (400mg/kg) body weight.

Group V: Plant extract (n=3) given orally plantain extract (800mg/kg) body weight.

After 30 minutes of treatment, each animal in Groups II- V received 100mg/kg indomethacin orally. After 1 hour, all the animals were anaesthetized with chloroform and dissected. Their stomachs were removed and treated as previously mentioned and the ulcer scores determined.

2.5.5. Pyloric Ligation of Ethanol Induced Ulcer Study

The anti-ulcerogenic potentials of fermented aqueous extract of *P. macrophylla* seeds was studied using the pyloric ligation method [19]. A total of fifteen wistar rats (150-200g body weight) was starved for 24 hours and grouped into five groups (Groups I-V) of three rats each based on their body weights. The abdomen was slightly opened under mild chloroform anesthesia, and their pylorous carefully lifted and ligated. The stomachs were quickly replaced and the abdomen sutured. Each administration in the various groups was done according to the specification below:

Group I: Normal Control (n=3) given orally distilled water (0.25ml/kg) body weight.

Group II: Negative control (n=3) given orally ethanol absolute (1.2ml/kg) body weight.

Group III: Reference drug (n=3) given orally omeprazole (5mg/kg) body weight.

Group IV: Plant extract (n=3) given orally plantain extract (400mg/kg) body weight.

Group V: Plant extract (n=3) given orally plantain extract (800mg/kg) body weight.

After 30 minutes of treatment, each animal in Groups II- V received 1.2ml of absolute ethanol orally. After 1 hour, all the animals were anaesthetized with chloroform and dissected. Their stomachs were removed and treated as previously mentioned and the ulcer scores determined.

2.6. Calculation of Ulcer Index and Percentage Inhibition

Ulcer Index (UI) = Mean of ulcer scores per rats

Percentage Ulcer inhibition:

The percentage ulcer protection was determined using the formula from reference [22].

$$\text{Protection Index} = 1 - \frac{\text{Ulcer index with extract}}{\text{Ulcer index with distilled water}} \text{x100.}$$

2.7. Statistical Analysis

Results were expressed as mean ± SD (standard deviation). Statistical analysis was performed by One-way analysis of variance (ANOVA) with the Graph Pad Prism® Statistic software package, version 7.01. One-way ANOVA with a Tukey's multiple comparisons test was used to identify statistical differences among groups. A *p*-value of $\leq$0.05 was considered statistically significant.

3. Results

The result of acute oral toxicity (LD_{50}) in experimental rats (Table 1) showed that doses of fermented aqueous extract of *P. macrophylla* seed as high as 5,000mg/kg body weight did not cause any behavioural change or mortality in the animals.

The gastro-protective effect of omeprazole and treatments at different doses of aqueous extract of fermented *P. macrophylla* seed on acetic acid induced ulcer in wistar rats after one hour is presented in Table 2 below. The result showed that fermented aqueous extract of *P. macrophylla* seeds prevented ulcerogenesis. Group II (negative control) has the highest ulcer score of 39.17±0.76 while Group V (800mg/kg) has the lowest ulcer score of 37.00 ±0.00. There was significant difference ($p<0.05$) in ulcer score and ulcer index between the negative control and pretreated groups. The highest percentage inhibition was observed in Group V. The reference drug (omeprazole) offered more percentage gastro-protection of 1.47% when compared with fermented aqueous extract of *P. macrophylla* seeds at 400 and 800mg/kg body weight, which offered percentage gastro-protective activities of 0.95 and 0.21% respectively.

The gastro-protective effect of omeprazole and treatments at different doses of aqueous extract of fermented *P. macrophylla* seeds on aspirin induced ulcer in wistar rats after one hour is presented in Table 3 below. The injection of Aspirin (1000mg/kg body weight) to the experimental rats in Groups II to V produced mucosal erosions with ulcer index of 11.60±0.10, 11.35±0.09, 11.50±0.10 and 11.00±0.10 respectively. The result revealed that ulcer indices among the test groups were not statistically significant at $p<0.05$ level of significance. The mean percentage ulcer inhibition (gastro-protection) obtained for different groups revealed that Group V (800mg/kg) of aqueous extract of fermented *P. macrophylla* seeds extract offered the highest percentage gastro-protection activity of 5.17% whereas Group III (5mg/kg body weight of omeprazole) offered percentage gastro-protection of 2.14%. The result revealed that the mean percentage gastro-protective activity between the test groups was statistically significant ($p<0.05$).

Table 1. Determination of acute toxicity (LD_{50}) value of fermented aqueous extract of unripe *P. macrophylla* seeds

Group	Dose (mg/kg)	Death	% Mortality	Behavioural Pattern
I	500	0.00	0.00	No observable sign seen
II	1000	0.00	0.00	Cool and healthy post 24 h examination
III	2000	0.00	0.00	So calm, but agile after sometime
IV	5000	0.00	0.00	Highly uncomfortable and could not eat enough food.

Table 2. Gastro-protective effect of omeprazole and treatment at different doses of fermented *P. macrophylla* on acetic acid induced ulcer in wistar rats after one hour

Test	Group I (Normal Control 5ml/kg)	Group II (Negative Control 0.4ml/kg)	Group III (Omeprazole 5mg/kg)	Group IV (400 mg/kg)	Group V (800 mg/kg)
Ulcer score	0.00±0.00	39.17±0.76^{b}	38.17±0.76^{a}	38.00±0.00^{a}	37.83±1.53^{a}
Ulcer Index	0.00±0.00	15.85±0.13^{a}	15.58±0.10$_{a}$	15.67±0.06$_{a}$	15.85±0.10^{a}
Inhibition (%)	-	-	1.47	0.95	0.21

Values represent the mean ± SD for N=3. Values in the same row bearing the same letter of alphabets are not significantly different from each other ($P < 0.05$).

Table 3. Gastro-protective effect of omeprazole and treatment at different doses of aqueous extract of fermented *P. macrophylla* seed on aspirin induced ulcer in wistar rats after one hour

Test	Group I (Normal Control 5ml/kg)	Group II (Negative Control 1000mg/kg)	Group III (Omeprazole 5mg/kg)	Group IV (400 mg/kg)	Group V (800 mg/kg)
Ulcer score	0.00±0.00	11.00±1.00^{c}	8.83±0.29^{b}	10.67±0.58^{c}	6.33±0.58^{a}
Ulcer Index	0.00±0.00	11.60±0.10^{s}	11.35±0.09^{s}	11.50±0.10^{s}	11.00±0.10^{s}
Inhibition (%)	-	-	2.14	0.86	5.17

Values represent the mean ± SD for N=3. Values in the same row bearing the same letter of alphabets are not significantly different from each other ($P < 0.05$).

The gastro-protective effect of omeprazole and treatments at different doses of fermented aqueous extract of *P. macrophylla* seeds on ethanol induced ulcer in wistar rats after one hour is presented in Table 4 below. The injection of ethanol (1.2ml/kg body weight) to the experimental rats in Groups II to V produced haemorrhagic streaks and mucosal erosions with ulcer index of 16.30±0.18, 14.50±0.05, 11.62±0.08 and 11.43±0.06 respectively. The result revealed that ulcer indices among the test groups were not statistically significant at $p<0.05$ level of significance. The mean percentage ulcer inhibition (gastro-protection) obtained for different groups revealed that Group V (800mg/kg) body weight of aqueous extract of fermented *P. macrophylla* seeds extract offered the highest percentage gastro-protection activity of 29.85% whereas Group III (5mg/kg body weight of omeprazole) offered percentage gastro-protection of 11.04%. The result further revealed that the mean percentage gastro-protective activity between the groups pretreated with omeprazole (5mg/kg) and those with 400 and 800mg/kg body weight of fermented aqueous extract of *P. macrophylla* seeds was statistically significant ($p<0.05$).

The gastro-protective effect of omeprazole and treatments at different doses of fermented aqueous extract of *P. macrophylla* seeds on indomethacin induced ulcer in wistar rats after one hour is presented in Table 5 below. The administration of indomethacin (100mg/kg body weight) to the experimental rats in Groups II to V produced mucosal erosions with ulcer index of 11.10±0.10, 10.87±0.06, 10.67±0.12 and 10.67±0.12 respectively. The result revealed that ulcer indices among the test groups were not statistically significant at $p<0.05$ level of significance. The result showed that fermented aqueous extract of *P. macrophylla* seeds produced a dose dependent protection post one hour administration when compared with the reference drug (omeprazole). The mean percentage ulcer inhibition (gastro-protection) obtained for different groups revealed that Group V (800mg/kg) of aqueous extract of fermented *P. macrophylla* seeds extract offered the highest percentage gastro-protection activity of 3.89% whereas Group III (5mg/kg body weight of omeprazole) offered percentage gastro-protection of 2.09%. This suggests that if the dose of fermented aqueous extract of *P. macrophylla* seeds is increased above 800mg/kg, it will have a higher antiulcer effect than omeprazole and the gastro-protective activity will be statistically significant at ($p< 0.05$).

The result of gastro-protective effect of different doses of fermented aqueous extract of *P. macrophylla* seeds on pylorous ligation of ethanol induced ulcer in wistar rats after one hour is presented in Table 6 below. The results showed that the different doses of fermented aqueous extract of *P. macrophylla* seeds were effective in protecting the gastro-mucosa and decreasing the severity of the ulcer and the ulcer index when compared to those pretreated with omeprazole. The negative control has the highest ulcer index of 11.57±0.06, because it was not pretreated and the severity of the ulcer increases thereby promoting ulcer formation, which includes, among other, haemorrhagic streak and mucosal erosion. The ulcer indexes for groups pretreated with omeprazole, 400 and 800mg/kg were 11.15±0.00, 10.73±0.06 and 10.57±0.12 respectively. There was no significant difference ($p<0.05$) in ulcer index between the negative control and the pretreated groups. The mean percentage ulcer inhibition (gastro-protection) obtained for different groups revealed that Group V (800mg/kg) of aqueous extract of fermented *P. macrophylla* seeds extract offered the highest percentage gastro-protection activity of 8.64% whereas Group III (5mg/kg body weight of omeprazole) offered percentage gastro-protection of 3.60%. The result further revealed that there was a significant increase ($p<0.05$) in gastro-protective activity between the different doses of the extract and omeprazole group.

Table 4. Gastro-protective effect of omeprazole and treatment at different doses of fermented *P. macrophylla* on ethanol induced ulcer in wistar rats after one hour

Test	Group I (Normal Control 5ml/kg)	Group II (Negative Control 1.2ml/kg)	Group III (Omeprazole 5mg/kg)	Group IV (400 mg/kg)	Group V (800 mg/kg)
Ulcer score	0.00±0.00	37.33±1.26^{c}	28.67±0.29^{b}	9.83±0.29^{a}	8.33±0.58^{a}
Ulcer Index	0.00±0.00	16.30±0.18^{c}	14.50±0.05^{b}	11.62±0.08^{a}	11.43±0.06^{a}
Inhibition (%)	-	-	11.04	28.73	29.85

Values represent the mean ± SD for N=3. Values in the same row bearing the same letter of alphabets are not significantly different from each other ($P < 0.05$).

Table 5. Gastro-protective effect of omeprazole and treatment at different doses of fermented *P. macrophylla* on Indomethacin induced ulcer in wistar rats after one hour.

Test	Group I (Normal Control 5ml/kg)	Group II (Negative Control 100mg/kg)	Group III (Omeprazole 5mg/kg)	Group IV (400 mg/kg)	Group V (800 mg/kg)
Ulcer score	0.00±0.00	6.83±0.58^{b}	5.67±0.58^{b}	4.33±0.58^{a}	3.67±1.15^{a}
Ulcer Index	0.00±0.00	11.10±0.10^{a}	10.87±0.06^{a}	10.67±0.12^{a}	10.67±0.12^{a}
Inhibition (%)	-	-	2.09	3.89	3.89

Values represent the mean ± SD for N=3. Values in the same row bearing the same letter of alphabets are not significantly different from each other ($P < 0.05$).

Table 6. Gastro-protective effect of omeprazole and treatment at different doses of fermented *P. macrophylla* on pylorous ligation of ethanol induced ulcer in wistar rats after one hour

Test	Group I (Normal Control 5ml/kg)	Group II (Negative Control 1.2ml/kg)	Group III (Omeprazole 5mg/kg)	Group IV (400 mg/kg)	Group V (800 mg/kg)
Ulcer score	0.00±0.00	10.67±0.58^{c}	6.50±0.00^{b}	4.33±0.58^{a}	3.33±0.58^{a}
Ulcer Index	0.00±0.00	11.57±0.06^{a}	11.15±0.00^{a}	10.73±0.06^{a}	10.57±0.12^{a}
Inhibition (%)	0.00	0.00	3.60	6.94	8.64

Values represent the mean ± SD for N=3. Values in the same row bearing the same letter of alphabets are not significantly different from each other ($P < 0.05$).

4. Discussion

Peptic ulcer is caused by distortion of the equilibrium that exists between the aggressive factors such as gastric acid, pepsin, NSAIDs, *Helicobacter pyloric*, ethanol; and the defensive factors (mucin, mucus, bicarbonate, prostaglandins) which are responsible for the maintenance of the integrity of gastrointestinal membrane [6,19]. Acetic acid, aspirin, ethanol, stress, indomethacin are possible risk factor responsible for the pathogenesis of peptic ulcer [18,19,20,21]. Omeprazole is a proton pump inhibitor used to treat peptic ulcer. It is effective in suppressing gastric secretion by blocking enzyme; hydrogen-potassium ATPase responsible for acid secretion [23]. The present study was aimed at investigating the antiulcerogenic potentials of aqueous extract of fermented *P. macrophylla* (Benth) seeds on acetic acid, aspirin, ethanol, indomethacin and pyloric ligation of ethanol induced ulcer models in wistar rats. The result of our studies revealed that the oral administration of acetic acid, aspirin, ethanol and indomethacin led to the manifestation of ulceration, and these were epitomized by severe gastric lesions or erosions and haemorrhagic streaks on the experimental animals. The etio-pathology of peptic ulcers by acetic acid, aspirin, ethanol, indomethacin and pylorous ligation of ethanol could be due to the following reasons: blockage of the enzyme cyclooxygenase [24], which converts arachidonic acid to prostaglandins, expression of histamine, gastrin and acetycholine receptors [23], which are responsible for gastric acid secretion and generation of reactive oxygen species [25], which are implicated in the inflammation of the mucosal cells of the gastrointestinal tract. The result of the acute toxicity study showed no mortality with a higher dose of up to 5,000 mg/kg body weight of fermented aqueous extract of *P. macrophylla* seeds, indicating that the LD_{50} of the extracts are above 5,000 mg/kg (Table 1). This result shows that the extract is safe for oral usage and can be characterized as non-toxic [26].

Acetic acid is a weak acid, which is able to induce ulcerogenesis by increasing the stomach acid. The administration of fermented aqueous extract of *P. macrophylla* seeds prevented ulceration of the wall of gastrointestinal tract. The result showed that the reference drug (omeprazole) offered better gastro-protection when compared to that offered by the different doses of the extract of the plant (Table 2). The findings are in agreement with the works of [27], who evaluated the antiulcerogenic and antiulcer properties of *Ocimum sativum* (Linn.). Acetylsalicyclic acid (aspirin) is a non-steroidal inflammatory drug (NSAID), which produced ulcer lesions in the glandular part of the rat's stomach. Aspirin is able to cause ulcerogenesis by direct injuries to the stomach walls [28] and suppression of prostaglandin synthesis [29]. In this study, fermented aqueous extract of *P. macrophylla* seeds at different doses interfered with ulcerogenesis by aspirin (Table 3). This suggests that the extract of the plant might have the capability of activating cyclooxygenase, thus cancelling the effect due to aspirin. This observation is not unconnected with the work of [10], who reported the antiulcerogenic activities of methanol root-bark extract of *Cochlospermum planchonii* on both male and female wistar rats. Ethanol is highly implicated in the etiopathology of peptic ulcer. The oral administration of ethanol produces severe gastric lesions and haemorrhagic streaks. The first pass metabolism of ethanol in the stomach is responsible for the activation of tumor necrosis factor-alpha (TNF-α), mitogen activated protein kinase (MAPK) and release of reactive oxygen species [25]. These cause lipid peroxidation, inflammation of mucosal cells of the stomach, lowering of the concentration of non-protein sulphydryl especially glutathione, lowering of bicarbonate secretion, activation of endothelin-1 [30] and finally apoptosis. Ulcerogenesis by ethanol was prevented significantly ($p<0.05$) by the oral injection of fermented aqueous extract of *P. macrophylla* seeds (Table 4). This suggests that the fermented aqueous extract of *P. macrophylla* was able to regain the balance between the aggressive and defensive factors, by scavenging reactive oxygen species. Ngbolua *et al.* [31] earlier reported that the extract of the plant, *P. macrophylla* is rich in antioxidants, which are responsible for preventing the generation of reactive oxygen species. The findings of this study is also in line with the work of [20], who reported the antiulcer activity of ethanolic leaf extract of *Musa paradisiaca* in rats. Indomethacin is also an example of non-steroidal anti-inflammatory drugs which are used to relieve pain, fever, and to manage rheumatoid arthritis [32]. The administration of indomethacin resulted in the manifestation of severe gastric lesions (Table 5). There was no significant difference ($p<0.05$) in the percentage gastro-protective activity between the reference drug and the doses of fermented aqueous extract of *P. macrophylla*. The mechanism of action of indomethacin and aspirin are quite similar. They cause peptic ulcer by inhibiting the enzyme cyclooxygenase. This blockage will therefore result in the release of endothelin-1 (ET-1), generation of reactive oxygen species (ROS) [33]), marked reduction in mucosal blood flow, mucus-bicarbonate secretion, impaired platelet aggregation, reduced epithelial cell renewal and increased leukocyte adherence [32]. Pylorous ligation of ethanol induced ulcer model is also another parameter to study the antiulcer activities of conventional and alternative drugs. The increased gastric acid of the stomach due to pylorous ligation interferes with gastric blood circulation, thus inducing ulcerogenesis [34]. The administration of the extract of *P. macrophylla* interferes with the activation of gastric acid secretion (Table 6). The observation is in agreement with the work of [18], who investigated the antiulcer of *Qualae grandiflora*. The antiulcerogenic potentials could be due to the preponderance of probiotics and phytochemicals resident in fermented aqueous extract of *P. macrophylla* seeds. Probiotics have been known over the years to enhance gut health [15] and treatment of gastrointestinal infections and diseases [35]. Phytochemicals are compounds such as flavonoid, saponins, tannins and flavonoids [6] found in medicinal plants, which confer health benefits on the host. The synergistic roles of phytochemicals and probiotics are responsible for the enhanced cessation of gastric erosions as observed in the present study.

5. Conclusion

The present study was undertaken to ascertain whether the fermented aqueous extract of *P.macrophylla* seeds

could offer antiulcerogenic activity on acetic acid, aspirin, ethanol, indomethacin and pyloric ligation of ethanol-induced ulcer models in wistar rats. The results showed that the doses of the extract of the plant effectively inhibited ulceration of the wall of the gastrointestinal tract comparable to that of omeprazole. However, the decoction of the plant could be employed in ethnomedicine for the treatment of peptic ulcer.

Conflict of Interest

The authors have not declared any conflict of interests.

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