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Evaluation of The Antiulcer Activity of Aqueous Seed Extract of *Moringa Oleifera* Lamarck (Moringaceae)

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ABSTRACT

Moringa oleifera Lam (Family: *moringaceae*) is a multipurpose tree with reported medicinal properties. The seeds are used traditionally to treat peptic ulcer. However, no pharmacological evidence to support the use of the aqueous seed extract of *M. oleifera* on peptic ulcer exists. The study therefore aimed at evaluating the antiulcer activity of the aqueous seed extract of *M. oleifera*. Isolated rabbit jejunum and guinea pig ileum were used to investigate its spasmolytic activity. The protective effect of the aqueous seed extract of *M. oleifera* was evaluated against pylorus ligated and indomethacin-induced ulcers in rats. The acute toxicity was determined by administering a single dose of 2,000 mg/kg orally to Wistar rats and observed 1 h post-dosing and once daily for 14 days. This dose did not produce mortality or acute signs of toxicity throughout the observation period. In the pylorus ligated studies, ulcer index and gastric juice volumes were not significantly different from the control, although, the extract at 500 mg/kg and omeprazole impacted positively on gastric juice pH and total acidity. However, all the doses used produced a significant ($p < 0.01$) inhibition of lesion in indomethacin induced-ulcer (40.84 - 45.02%) when compared with the control, but higher percentage inhibition was recorded with cimetidine (66.72%). On isolated tissue, the extract produced a relaxant effect alone, a slight inhibition of histaminergic contractions and no effect on Acetylcholine contractions. The results suggest that the extract lacks antisecretory properties but it is effective against indomethacin induced-ulcer in rats.

Keywords: *Moringa oleifera*, antiulcer, ulcer index, pylorus ligation, indomethacin-induced ulcer.

Introduction

Peptic ulcers are sores that develop on the inside mucosal lining of the digestive tract, specifically the initial portion of the small intestine (duodenum), stomach and less commonly the oesophagus. It is defined as mucosal erosion equal or greater than 5 mm.[1] An acid peptic disorder occurs when the injurious effects of acid and pepsin overwhelm the mucosal barrier. Peptic ulcers occur worldwide[2] with a life time prevalence of 5 to 10% and equal prevalence among men and women.[3] The incidence of ulcer increases with age because of excessive use of non-steroidal anti-inflammatory drugs (NSAIDs) and the reduction in tissue prostaglandins.[3] Other risk factors for the development of peptic ulcer disease (PUD) include *Helicobacter pylori (H. pylori)*, genetics, smoking, emotional stress and excessive alcohol consumption. In Nigeria, the true prevalence rate of peptic ulcer among the populace is not certain, although Nigeria had been listed as an area of high incidence.[4] Also, the prevalence of duodenal ulcers has declined while that of gastric ulcer has increased.[5] The modern approach to control gastric ulceration is to inhibit gastric acid secretion, promote gastro-protection, block apoptosis and stimulate epithelial cell proliferation for effective healing.[6] This is achieved by the use of antacids, proton pump inhibitors, H_2-receptor antagonists and mucosal protective agents. The high cost of treatment and limitations of conventional anti-ulcer drugs present a clear need for newer agents. The plant kingdom may provide a useful source of new anti-ulcer compounds for development as pharmaceutical entities or simple dietary adjuncts to existing therapies. The leaves, fruits, bark and roots of *Moringa oleifera* Lam (Family: *moringaceae*) have been extensively studied for their anti-ulcer activities.[7] However, despite the medicinal usefulness of the seeds, they fall among the lesser known and highly under-utilized part of the plant. The effect of the aqueous seed extract of the plant on gastric and duodenal ulcers is not known. The current study was undertaken to evaluate the effect of the aqueous seed extract of *M. oleifera* (ASEMO) on experimentally-induced peptic ulcers.

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Materials and Methods

Chemicals and Equipment

All reagents and solvents used in this experiment were of analytical grade. The chemicals used include: histamine dihydrochloride (Sigma-Aldrich Germany), acetylcholine chloride (Sigma-Aldrich Germany), atropine sulphate (Shandong Shendlu Pharmaceutical Company, China), mepyramine (Chemos GmbH & Co,KG, Germany), indomethacin (Greenfield Pharmaceutical Company, Jiangsu, China), cimetidine (Krishat Pharma Ltd, Ibadan, Nigeria), omeprazole (Cipla, Miami, USA), 0.1% Tween 80, formol-saline (2% v/v), tyrode solution (composition in mM- NaCl, 137; $CaCl_2$, 1.8; KCl, 2.7; glucose, 5.55; $NaHCO_3$, 11.9; $MgCl_2$, 1; NaH_2PO_4, 0.4). Microdynamometer 7050 (Ugo Basile), Mettler P162 analytical balance and bench centrifuge (Baird and Tatlock London Ltd).

Plant Collection and Identification

The seeds of *M. oleifera* plant were collected from a farm in Katsina Local Government Area of Katsina State, Nigeria in April 2015 The plant was identified and authenticated in the Botany Department of Bayero University, Kano with accession number BUKHAN0011. The seeds were

dried under shade to constant weight then crushed to a coarse powder using pestle and mortar. The powder was soaked in distilled water for 24 hours after which it was filtered with a fine muslin cloth followed by Whatman filter paper No1. The filtrate was collected into a porcelain dish and oven dried at 59ºC. The dried extract was weighed and the percentage yield calculated. The resultant extract was stored in an airtight container for use.

Experimental Animals

Wistar rats of both sexes weighing 120 - 150 g were procured from the University of Ibadan, Oyo State, Nigeria. The rats were housed in cages at Animal House, Department of Pharmacology, Bayero University, Kano, Nigeria. The animals were maintained at a temperature of approximately 25^{owith}C. They were fed standard dry pellets and tap water *ad libitum*. The rats were allowed to acclimatize to the environmental conditions for 14 days before the experiments commenced. Rabbits (2.0 - 2.5 kg) and guinea pigs (400 – 600 g) of either sex were purchased from Samaru and housed in standard conditions of temperature and light, as mentioned earlier prior to the experiment. All animals were handled in accordance with the Ahmadu Bello University, Zaria Guidelines for Laboratory Animal Use and Care.

Phytochemical Screening

Preliminary phytochemical screening was carried out on the dried aqueous seed extract of *M. oleifera* using standard methods.[8,9]

Acute Toxicity Study

The acute oral toxicity study was performed according to the OECD guideline.[10] The method used a pre-defined limit dose of 2000 mg/kg body weight in a step wise procedure with the use of three animals per step at intervals of 48 h. The rats were fasted overnight (only food was withdrawn, with free access to water). The animals were then weighed and the test substance administered orally as a single dose of 2000 mg/kg body weight. Food was withheld for a further 2 h. The animals were observed individually for acute toxicity signs and behavioral changes 30 min, 1 h and 4 h post-dosing, and at least once daily for 14 days.

Pylorus Ligation-Induced Ulcer Model in Rats

Gastric ulcers were produced in rats according to the methods of Vogel.[11] Wistar rats of either sex were starved of food for 18 h but having access to drinking water *ad libitum*. During this time, they were housed in starvation cages with raised bottoms of wide wire mesh to avoid coprophagy and cannibalism. Animals were divided into five groups with six animals per group). Group I served as negative control, receiving only distilled water (10 mL/kg). Groups II, III and IV received graded doses of 500, 1000 and 1500 mg/kg respectively of the aqueous seed extract of *M. oleifera* (ASEMO) and Group V received 20 mg/kg omeprazole as the positive control. The standard, control and various doses of ASEMO were administered orally immediately after ligation. Under light anaesthesia with chloroform, the abdomen was dissected by midline incision below the xiphoid process. The pyloric portion of the stomach was slightly lifted out and ligated, avoiding damage to its blood supply. The stomach was placed back carefully and the abdominal wall was closed with sutures. The animals were deprived of food, water and any ingestible material during the post-operative stage and sacrificed 5 h after pylorus ligation[12] by over dose of chloroform anaesthesia. The abdominal cavity was again dissected, the stomach removed and gastric juice was emptied into pre-labelled test tubes. The contents of the stomach were centrifuged for 10 min at 1000 rpm in a bench centrifuge. After centrifuging, the supernatants were subject to analysis for gastric volume, gastric pH, and total acidity. The stomachs were opened along the greater curvature, inner surface was washed with distilled water and examined for ulceration. Ulcer-index (UI) and percentage protection were calculated.

Measurement of Ulcer Index (UI)

The opened stomachs were examined for ulceration under a 3-fold magnifier. The number of ulcers was recorded and the severity scored as follows: 0 = no ulcer; 1 = superficial ulcers; 2 = deep ulcers; and 3 = perforated or penetrated ulcer[13]. Ulcer index (UI) was calculated using the formula:

$$UI = UN + US + UP \times 10\text{-}1$$

Where UN = average number of ulcers per animal, US = average of severity score, and UP = percentage of animals with ulcer.[14] The percentage protection was calculated using the following formula:

$$\% \text{ Protection} = \frac{\text{Mean ulcer index of control} - \text{Mean ulcer index of test X 100}}{\text{Mean ulcer index of control}}$$

Determination of Total Acidity

Gastric juice was collected from the ligated-rats, the volume and pH were measured.[15] For the determination of total acidity, 0.5 mL of the supernatant fluid was pipetted into a 100 mL beaker. Two drops of phenolphthalein solution were added and the solution titrated with 0.1N NaOH until a pink colour appears. The titration was repeated where the volume of gastric juice was adequate. The total volume of alkali added was noted for each titration. Total/titratable acidity was calculated and expressed as micro Eq/L per 100 g of body weight. Acidity was calculated using the formula:[11]

$$\text{Acidity} = \frac{\text{volume of NaOH x Normality of NaOH x 100 mEq}}{0.1\ \text{L}}$$

Indomethacin Induced Ulcers in Rats

Wistar rats of either sex were fasted overnight. The animals were divided into 5 groups of 6 animals each. Group I was administered distilled water in 0.1% Tween 80 orally. The animals in Groups II, III and IV were pretreated orally with 500, 1000 and 1500 mg/kg of ASEMO in 0.1% Tween 80 respectively while Group V was pretreated with the standard drug cimetidine also dissolved in 0.1% Tween 80 at the dose of 200 mg/kg. After 30 min, 20 mg/kg indomethacin dissolved in 0.1% Tween 80 solution was administered orally to the animals. Six hours later, the rats were sacrificed in chloroform chamber and their stomachs removed. Formol-saline (2% v/v) was injected into the totally ligated stomachs and stored in plain tubes filled with formol-saline overnight. The next day, the stomachs were opened along the greater curvature, washed in warm water and examined under a 3-fold magnifier. The lengths of the longest diameters of the lesions were measured and summated to give a total lesion score (in mm) for each animal. The mean count for each group was then calculated. Inhibition (protection) of the lesion production is expressed as percentage value.[11]

Isolated Tissue Studies

According to the method of Amos *et al*.,[16] after an overnight fast, the guinea pigs were sacrificed. The abdomen was opened and a piece of ileum was dissected and placed in oxygenated Tyrode's solution at room temperature. Longitudinal strips of ileum 2 – 3 cm long were then prepared and mounted under a 2 g tension in a 25 mL organ bath filled with Tyrode's solution at 37ºC and maintained under constant aeration with carbogen gas (95% O_2 + 5% CO_2). The tissue was washed several times with fresh Tyrode's solution and allowed to rest and equilibrate for 30 min to achieve a stable basal rhythm. The contact time for each concentration was 1 min and the tissues were washed three times before new administration; the responses were recorded using a microdynamometer with speed of 24 mm/min and a sensitivity of 2.

Isolated Guinea Pig Ileum Preparation

Graded dose response of acetylcholine (ACh) alone (0.02, 0.04, 0.08 and 0.16 µg/mL) alone and that of the extract alone (0.004, 0.008, 0.016, 0.032, 0.04, 0.08, 0.16, 0.32, 0.4, 0.8, 1.6 and 3.2 mg/mL) in plain Tyrode's solution were determined. Using the dose of ACh that produced the submaximal response, the procedure was repeated in the presence of varying concentrations of the extract.

In another setup of isolated guinea pig ileum, graded dose response of histamine alone (0.02, 0.04, 0.08 and 0.16 µg/mL) in plain Tyrode solution and that of the extract alone (0.004, 0.008, 0.016, 0.032, 0.04, 0.08, 0.16, 0.32, 0.4, 0.8, 1.6 and 3.2 mg/mL) in plain Tyrode's solution were determined. Using the dose of histamine that produced the submaximal response, the procedure was repeated in the presence of varying concentrations of the extract.

Isolated Rabbit Jejunum Preparation

Dose response curve of acetylcholine and histamine in the absence and presence of the plant extract was performed as described in respect of the isolated guinea pig ileum preparation.

Statistical Analysis

The results of various studies were expressed as Mean ± SEM. The data were analyzed using one-way analysis of variance (ANOVA) followed by

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Dunnett's *t*-test to find out the level of significance. Data were considered statistically significant at minimum level of $P < 0.05$.

Results and Discussion

Gastric hyperacidity and ulcers are common disorders that affect a considerable number of people in our societies. Stress, smoking, administration of non-steroidal anti-inflammatory drugs (NSAIDs) contribute to gastric ulcer and the infection of *H. pylori*, a bacterium found in the stomach, is also implicated in gastric ulcer. The conventional treatment of gastric ulceration is usually achieved by the use of antacids, proton pump inhibitors, H_2-receptor antagonists and mucosal protective agents. The high cost of treatment and several side effects of conventional antiulcer drugs (e.g. arrhythmias, impotence, gynaecomastia and haematopoeitic changes) present a clear need for newer agents. Extracts of many plants have been shown to produce promising results for the treatment of gastric ulcers with fewer side effects.[17] The use of *Moringa oleifera* seeds as a 'cure-all' is becoming a common trend in northern Nigeria. Ethno-medicinal claims suggest that the seeds of *M. oleifera* are used in the treatment of peptic ulcer disease.[7] The leaves, fruits, bark and roots of *M. oleifera* have been reported to have potential antiulcer activities.[7] In view of these facts, the antiulcer potential of the aqueous seed extract of *M. oleifera* (ASEMO) on experimentally induced peptic ulcers was investigated in this study.

Cold maceration of the pulverized *M. oleifera* seeds yielded a honey coloured extract with a characteristic smell and highly viscous consistency. The percentage yield was 18.69%. Phytochemical screening of the extract indicates the presence of several secondary metabolites that might be responsible for its pharmacological activities. These phytoconstituents include carbohydrates, steroids, glycosides, tannins and flavonoids. Anthracenes were however absent from the seed extract (Table 1). The findings in this study agree with earlier studies which also found that, not all phytochemicals are present in all plant parts and that those present differ according to the type of extracting solvent used.[18] Alkaloids are pharmacologically active, nitrogen-containing basic compounds of plant origin. Their roles in plants include chemical defense which is supported by their wide range of physiological effects on animals. In insects, alkaloids are toxic thus acting as feeding deterrents. Flavonoids constitute a class of phenolic natural products and have been analyzed as modulators of immune and inflammatory responses, for their impact on smooth muscle function, as anticancer, antiviral, antitoxic and hepato-protective agents.[19] Flavonoids are also well-known anti-ulcer agent that protects the gastrointestinal mucosa from lesions produced by experimental ulcer models and different necrotic agents.[3] The seeds also contain steroids that are known to reduce the development of gastric ulcers.[20]

Tannins possess gastro-protective properties and are present in the seed extract used as astringent medicines for the treatment of dysentery and diarrhea.[21] Saponins are used medically for the treatment of increased blood cholesterol and are beneficial to patients with atherosclerosis and hypertension, control of post-menopausal syndrome and inflammatory conditions of the digestive tract.[21]

Median lethal dose (LD_{50}) study of ASEMO was found to be greater than 2,000 mg/kg as no mortality or significant physical or behavioural changes was observed in the animals. According to the OECD guidelines, this limit dose thus puts the median lethal dose (LD_{50}) of the aqueous extract in GHS category 5 for chemical substances and mixtures. This indicated that the extract has a high safety margin and is non-toxic. A dose-response antiulcer study was made using 500 mg/kg, 1,000 mg/kg and 1,500 mg/kg of ASEMO for both antiulcer screening models.

Pyloric ligation-induced ulcer is due to the autodigestion of gastric mucosa and break down of gastric mucosal barrier resulting in gastric acid accumulation and thus ulcer formation.[22] The model is useful for evaluating the effect of drugs that reduce the secretion of gastric aggressive factors such as acid and pepsin. Pylorus ligation for 5 h caused gastric damage with ulcer index of 8.67 ± 1.67 in the experimental control rats. Omeprazole (20 mg/kg) and ASEMO 500 mg/kg were found to reduce the ulcer index by 29.53% and 25.73%, respectively (Table 2). However, 1000 mg/kg and 1500 mg/kg of the ASEMO showed negative percentage protection of 14.42 and 3.11%, respectively. All the aggressive factors e.g. gastric volume, total acidity was decreased and gastric pH was increased in the Omeprazole and 500 mg/kg ASEMO treated groups, providing some evidence of their antiulcer activity (Table 2). In another study however, where the ethanolic extract of the seed was used in the same model, a significant reduction in the ulcer index (77.68%) was recorded.[20] This indicates that the concentration of phytoconstituents extracted using aqueous solvent may vary from that with ethanolic solvent. A study of the effect of aqueous leaf and fruit extracts on ulcer using the same model also showed no significant effect on the ulcer index.[23]

Indomethacin and other NSAIDs are known to cause gastric ulcers especially when they are abused. This provides the rationale for the development of NSAIDS- induced gastric ulcer models in rats to study the cyto-protective effect of extracts against this form of ulcer. Non-steroidal anti-inflammatory drugs induce ulcers by inhibiting prostaglandin synthesis which plays a vital protective role of stimulating the secretion of bicarbonate and mucus in the stomach. Administration of the aqueous seed extract of *M. oleifera* at all doses showed significant ($p < 0.01$) and dose-dependent reduction in ulcer index when compared with control (Table 3). Ulcer index decreased from 6.22 ± 0.29 in distilled water group to 3.68 ± 0.46, 3.58 ± 0.34 and 3.42 ± 0.39 for doses of 500 mg/kg, 1,000 mg/kg and 1,500 mg/kg body weights respectively. Increased percentage inhibition of ulcer was observed in animals treated with ASEMO when compared to animals of control group but the protection was lower than that observed in cimetidine treated group.

On the isolated tissue, the effect of the seed extract on guinea pig ileum and rabbit jejunum in the presence of agonist-induced contraction was determined in order to find its possible mechanism of action. Several mechanisms are involved in the regulation of smooth muscle contraction and intestinal motility. These include the agonistic action of the parasympathetic neurotransmitter acetylcholine (Ach) and the excitatory activity of the autacoid histamine.[24] Gastric acid secretion involves the action of gastrin, acetylcholine and histamine on parietal cells. Of the three physiological secretagogues histamine, acting through H_2-receptors, plays the dominant role, while the other two, act partly directly via their own receptors (gastrin and M_3) and to a greater extent indirectly by releasing histamine from cells called histaminocytes.[25] Therefore, drugs that can affect cholinergic and histaminergic transmission can alter gastrointestinal muscle contraction and gastric acid secretion. Results from this study showed that the extract alone showed no increase in basal activity of both guinea pig ileum and rabbit jejunum. However, the extract produced a dose-dependent inhibition of contraction induced by histamine on guinea pig ileum (Table 4) and to a lesser extent that induced by acetylcholine on rabbit jejunum (Table 5). Thus, the possible mechanism for the spasmolytic activity of the extract could be through inhibition of histamine receptors and the activity could be attributed to flavonoids and other phenolic compounds present in the extract.[26]

Conclusion

The study showed that administration of the aqueous seed extract of *M. oleifera* at 500 mg/kg decreases gastric volume, total acidity while gastric pH was increased. In indomethacin-induced ulcer model, the extract also produced a significant ($p < 0.01$) and dose-dependent reduction in ulcer index and contraction induced by histamine on guinea pig ileum when compared with control thus providing some evidence of their antiulcer activity. Thus, the possible mechanism for the spasmolytic activity of the extract could be through inhibition of histamine and the activity could be attributed to flavonoids and other phenolic compounds present in the extract.

Table 1: Phytochemical analysis of aqueous seed extract of *M. oleifera*

Phytochemical	Inference
Carbohydrates	+
Anthracene derivatives	-
Steroids and Triterpenes	+
Cardiac glycosides	+
Saponin glycoside	+
Tannins	+
Flavonoids	+
Alkaloids	+

Key: (+) = Presence (-) = Absence

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Table 2: Effect of aqueous seed extract of *M. oleifera* (ASEMO) on pylorus-ligation-induced ulcer in rats

Treatment	Ulcer Index	Protection (%)	pH of gastric juice	Total acidity mEq/L	Volume of gastric juice (mL)
Distilled Water 10 mL/kg	8.67 ± 1.67	-	5.97	6.17	1.17 ± 0.10
ASEMO 500 mg/kg	6.44 ± 2.09 ^{ns}	25.73	8.01	5.28	0.83 ± 0.30^{ns}
ASEMO 1000 mg/kg	9.92 ± 0.63 ^{ns}	-14.42	3.69	2.25	0.88 ± 0.34 ^{ns}
ASEMO 1500 mg/kg	8.94 ± 1.82 ^{ns}	-3.11	3.94	7.50	1.08 ± 0.28 ^{ns}
OMP 20 mg/kg	6.11 ± 1.96 ^{ns}	29.53	7.29	2.50	0.87 ± 0.28 ^{ns}

Ulcer index and gastric juice volume are expressed as mean ± SEM; (n = 6); ns (not statistically significant) when compared with distilled water (control) alone using one-way ANOVA; OMP – Omeprazole.

Table 3: Effect of aqueous seed extract of *M. oleifera* (ASEMO) on indomethacin-induced ulcers in rats

Treatment	Total Lesion Score (mm)	Ulcer Index (mean lesion count)	Protection (%)
Indomethacin 20 mg/kg	31.1	6.22 ± 0.29	-
ASEMO 500 mg/kg	22.1	3.68 ± 0.46**	40.84
ASEMO1000 mg/kg	21.3	3.58 ± 0.34**	42.44
ASEMO 1500 mg/kg	20.5	3.42 ± 0.39**	45.02
Cimetidine 100 mg/kg	12.4	2.07 ± 0.68***	66.72

Ulcer index is expressed in mean ± SEM; (n = 6); **p < 0.01, ***p < 0.001 when compared with control (indomethacin alone) using one-way Anova followed by Dunnett's *t*-tests.

Table 4: Effect of aqueous seed extract of *M. oleifera* (ASEMO) on histamine (0.04 µg/mL) induced contractions

Treatment (mg/mL)	Average Response (mm)	Inhibition (mm)	% Inhibition
Histamine	30.33 ± 14.9	-	-
0.4 ASEMO + Histamine	28.25 ± 17.3^{ns}	2.08	6.9
0.8 ASEMO + Histamine	24.75 ± 13.8^{ns}	5.58	18.4
1.6 ASEMO + Histamine	17.00 ± 7.0^{ns}	13.33	44.0
3.2 ASEMO + Histamine	10.75 ± 1.8^{ns}	19.58	64.6

Average response expressed as mean ± SEM; (n = 3); *ns (not significant) when compared with histamine alone using one-way ANOVA.

Table 5: Effect of aqueous seed extract of *M. oleifera* (ASEMO) on acetylcholine (0.04 µg/mL) induced contractions

Treatment (mg/mL)	Average Response (mm)	Inhibition (mm)	% Inhibition
ACh	40.5 ± 1.5	-	-
0.4 ASEMO + ACh	30.5 ± 6.5^{ns}	10.0	24.7
0.8 ASEMO + ACh	29.5 ± 3.5^{ns}	11.0	27.2
1.6 ASEMO + ACh	36.0 ± 14.0^{ns}	4.5	11.1
3.2 ASEMO + ACh	34.0 ± 6.0^{ns}	6.5	16.0

Average response expressed as mean ± SEM; (n = 3); ns (not significant) when compared with acetylcholine (ACh) alone using one-way ANOVA

Conflict of interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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