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Evaluation of *Moringa oleifera* Leaf Potential in the Prevention of Peptic Ulcer in Wistar Rats

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Abstract

This study is aimed at investigating the potential of aqueous extract of Moringa oleifera leaf in the prevention of indomethacin-induced ulcer. To achieve this, thirty adult male rats of body weight between 150 and 200g were divided into six groups of five rats each. Group I was treated with 0.8mg/ml of Omeprazole for seven days. Group II was treated with a solution of 0.8 g/ml Moringa oleifera leaf for seven days, while group III received distilled water for seven days. This group served as the control group. Groups IV, V and VI were treated similarly as groups I, II and III respectively but were treated for fourteen days. Gastric ulceration was induced in the rats by the administration of 50 mg/kg indomethacin after pre-treatment with distilled water, omeprazole and Moringa oleifera leaf for 7 and 14 days respectively. Significant ulcer inhibition was produced in the groups treated with M. oleifera and Omeprazole when compared with control groups at $p<0.05$, but omeprazole-treated group showed greater ulcer inhibition (72.60 % and 74.29 %) when compared with the Moringa oleifera leaf-treated groups (53.43% and 57.58%) after 7 and 14 days respectively. This study showed that M. oleifera leaf possesses anti-ulcerogenic properties and can be used as herbal remedy for the prevention of peptic ulcers.

Keywords: *Moringa oleifera* leaf, Ulcer inhibition, anti-ulcerogenic properties, gastro-intestinal ulcer

1. Introduction

Peptic Ulcer Disease (PUD) also known as peptic ulcer or stomach ulcer is a break in the lining of the stomach, first part of the small intestine, or occasionally the lower esophagus [1]. Peptic ulcer disease comprises heterogeneous disorders, which manifest as a break in the lining of the gastrointestinal mucosa bathed by acid and pepsin. Based on site of attack, peptic ulcer may be classified as oesophageal, duodenal, or gastric. Peptic ulcer disease (PUD) is one of the most common human ailments, affecting approximately 50% of the world population. The life time for developing a peptic ulcer is approximately 10% [2]. In western countries, the percentage of people with *Helicobacter pylori* infections roughly matches age (i.e., 20 % at age 20, 30% at age 30, 80% at age 80). Prevalence is higher in developing countries where it is estimated at about 70% of the population, whereas developed countries show a maximum of 40% ratio [3].

Moringa oleifera Lam. is the most widely cultivated species of the mono-generic family Moringaceae, which includes 13 species of trees and shrubs distributed in sub Himalayan ranges of India, Sri Lanka, North-eastern and South-western Africa, Madagascar and Arabia. Moringa is also native to

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parts of West Africa particularly Nigeria [4]. The whole *Moringa oleifera* plant is used in the treatment of psychosis, eye diseases, fever and as an aphrodisiac, the aqueous extracts of roots and barks were found to be effective in preventing implantation [5]. The Moringa tree is a multifunction plant. It has been cultivated in tropical regions all over the world for the following characteristics: high protein, vitamins, mineral and carbohydrate content of entire plants; high value of nutrition for both humans and livestock; high oil content (42%) of the seed which is edible, and with medicinal uses; the coagulant of seeds could be used for wastewater treatment [4].

Different parts of the *Moringa oleifera* (Mo) tree have been established as being good sources of unique glucosinolates, flavonoids and phenolic acids [6][7], carotenoids [8], tocopherols [9], polyunsaturated fatty acids (PUFAs) [10], highly bioavailable minerals [11], and folate [12]. Among glucosinolates, 4-O-(a-L-rhamnopyranosyloxy)-benzylglucosinolate (glucomoringin) is the most predominant in the stem, leaves, flowers, pods and seeds of *M. oleifera* [6]. Although in the roots, benzyl glucosinolate (glucotropaeolin) is the most prominent. The highest content of glucosinolate is found in the leaves and seeds. The enzymatic catabolism of glucosinolates by the endogenous plant enzyme myrosinase produces isothiocyanates, nitriles, and thiocarbamates that are known for strong hypotensive (blood pressure lowering) and spasmolytic (muscle relaxant) effects [13]. In the leaves, the amount of quercetin and kaempferol was found to be in the range of 0.07–1.26 and 0.05–0.67 %, respectively. The potent antioxidant activity of Moringa is attributed to the high concentration of these polyphenols. Medicinally, the antioxidant, wound healing, hypotensive, and diuretic effects of this plant have been reported [14, 15].

Previous studies have reported the antioxidant [16], anti-inflammatory [17] and pharmacological [6] properties of *M. oleifera*. Furthermore, Awodele *et al*., [18] worked on the toxicological evaluation of the aqueous extract of *Moringa oleifera* Lam (Moringaceae). Oyedepo *et al*., [19] evaluated the anti-hyperlipidemic effect of aqueous leaves extract of *Moringa oleifera*, while Choudhary *et al*., [20] assessed the antiulcer potential of *Moringa oleifera* root bark extract in rats. *Moringa oleifera* leaf has been reported to be potent in the treatment of peptic ulcer [21]. This study is to investigate if *Moringa oleifera* leaf can also prevent peptic ulcer.

2. Methodology

2.1. *Plant Preparation*

Moinga oleifera leaf was harvested from Institute of Agricultural Research and Training, Ibadan and was identified by a botanist. The leaves were removed from the stem and washed thoroughly with clean water to remove contamination. They were dried at room temperature until they were completely dried. It was milled into powder and stored in plastic container before use.

2.2 *Experimental Design*

Thirty (30) healthy male albino rats with body weights between 150 and 200 g were used for this study. They were bought from 'Imrat animal house' of the University College Hospital, Ibadan and were housed in Educational Advancement Centre animal house.

They were allowed 14 days to acclimatize before the commencement of treatment. The animals were maintained on a standard pellet diet throughout the acclimatization and treatment period. They were divided into six groups of five rats each. Group I was exposed to omeprazole for seven days, group II was exposed to *Moringa oleifera* leaf solution for seven days and group III was exposed to distilled water for seven days. This group served as the control group. Groups IV, V and VI were treated similarly as groups I, II and III respectively but were treated for fourteen days.

A 0.8g/ml solution of *Moringa oleifera* leaf was prepared daily and the animals in groups II and V were allowed to drink *ad libitum*. This was done because people feed on Moringa without attention to dosage. A 0.8mg/ml solution of omeprazole was prepared daily and the animals in groups I and IV were allowed to drink *ad libitum* while groups III and VI drank distilled water throughout the period of administration. All the animal treatments were carried out in accordance with the principles of laboratory animal care of the National Institute of Nutrition (NIN) guide for Laboratory Animal Welfare.

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At the end of the administration, the animals were deprived of food for 18 hours and 50 mg/kg of indomethacin was administered orally (p.o) to the rats. After 8 hours of indomethacin administration, the animals were sacrificed by chloroform anesthesia and the stomach removed and opened along the greater curvature, rinsed with copious volume of normal saline and pinned on a board.

2.3. *Parameters Measured*

2.3.1. *Ulcer Index*

Ulcer index was measured. The ulcers scores were given based on their intensity as follows

Normal stomach……. 0.0

Red coloration…… 0.5

Spot ulcer……1.0

Hemorrhagic streak……..... 1.5

Ulcers…….. 2.0

Perforation……..... 3.0

Measurement of gastric ulcerations was done by first dissecting the stomachs along their greater curvature and fixing on a board [22]. Examination was carried out macroscopically with a hand lens (x 2). The ulcer indices (UI) of the control and treated groups were calculated using the method of [23].

$$\text{Ulcer index (mm)} = \frac{\text{Number of ulcers (A) x Size of ulcers (B)}}{\text{Magnification power of the lens used (x 2)}}$$

2.4. *Percentage Ulcer Inhibition*

Percentage ulcer inhibition was calculated relative to control as follows:

$$\%\ \text{Ulcer Inhibition (\% U.I)} = \left(1 - \frac{U_t}{U_c}\right) \text{x } 100$$

Where U_t and U_c represent the ulcer index of the treated and control groups respectively.

2.4. *Statistical Analysis*

Data were subjected to analysis using the Statistical Package for Social Sciences (SPSS), version 21.0. Results were presented as Mean ± Standard deviations. Student's t-test was used for comparison of the mean. Difference between means were considered to be significant at $p<0.05$.

3. Result

Indomethacin induced gastric ulcer in 22 out of 30 (73.33 %) rats used in this study.

Table 1: Effect of different Treatments on Indomethacin-Induced Ulcer with the Values of Gastric Ulcer Index and Percentage Ulcer Inhibition after 7days' Treatment

Treatment	Gastric Ulcer Index (mm)	% Ulcer Inhibition (%UI)
Control	60.83 ± 6.29^{a}	0.00^{a}
Omeprazole	16.67 ± 2.89^{b}	72.60^{b}
Moringa oleifera	28.33 ± 2.89^{c}	53.43^{c}

Results are presented as mean ± standard deviation where n=5. Values with different superscript along the same column are said to be significant at $p<0.005$

Table 2: The Effect of Different Treatment on Indomethacin-Induced Ulcer with the Values of Gastric Ulcer Index and Percentage Ulcer Inhibition 14 Days' Treatment

Treatments	Gastric Ulcer Index (mm)	% Ulcer Inhibition (%UI)
Control	64.83 ± 4.19^{a}	0.00^{a}
Omeprazole	16.67 ± 2.89^{b}	74.29^{b}
Moringa oleifera	28.33 ± 2.89^{c}	57.58^{c}

Results are presented as mean ± standard deviation where n=5. Values with different superscript along the same column are said to be significant at $p<0.005$

4. Discussion

Peptic ulcer is a common illness in internal medicine which affects a considerable number of people worldwide [24]. Although, many products are available for the treatment of gastric ulcers (e.g., antacids and antihistaminics), most of these drugs

produce several adverse effects, such as arrhythmias, impotence, gynecomastia, and hematopoeitic changes [25]. The extracts of many herbal plants have been shown to produce promising results for the treatment of gastric ulcers with fewer or negligible side effects [26]. Almost all parts of the *Moringa oleifera* plant have been reported to have medicinal values for the treatment of various ailments, such as gastrointestinal disorders, tumors, diabetes, hypertension, renal disorders, bacterial and fungal diseases, and fever [27, 28]. In lieu of this, the present study was performed to investigate the prophylactic efficacy of *M. oleifera* leaf by using indomethacin to induce gastric ulcer in wistar rats.

Moringa oleifera leaf used in this study exhibited anti-ulcerogenic effect against indomethacin-induced gastric ulcers with percentage ulcer inhibition that were significantly lower than that obtained for omeprazole, the reference anti-ulcer drug used (Tables 1 and 2). Prolonged use of indomethacin and other non-steroidal anti-inflammatory drugs are associated with gastrointestinal bleeding and ulceration. The ulcer formation can occur either by direct mucosal injury which involves the breaking of the mucosal barrier and exposure of the underlying tissue to the corrosive action of excess acid and pepsin or by a decrease in endogenous gastric prostaglandin production and release through COX-1 and COX-2 inhibition [29]. These naturally occurring prostaglandins are important for the production of gastric bicarbonate and mucous which are key components of the stomach protective barrier and in the maintenance of submucosal blood flow.

Most non-steroidal anti-inflammatory drugs including indomethacin, used in the control of inflammation have been reported to cause gastric erosions and abdominal ulcers after prolonged use. Reduction of the indomethacin-induced ulcers shown by Moringa could be attributed to the high flavonoid content of *Moringa oleifera* leaf. This result is in agreement with the study of Airaodion *et al.* [30] who reported that *Curcuma longa* was able to prevent peptic ulcer due to the presence of flavonoid. *Zingiber officinale* root has also been reprted to prevent peptic ulcer due to the presence of flavonoids [31]. Phytochemical analyses of Moringa leaf showed that it is rich in flavonoids [6, 7], this could be the active constituent exerting the anti-ulcerogenic effect. Halliwell *et al*., [32] proposed that the antioxidant and other protective effects of plant flavonoids could occur before absorption, within the gastrointestinal tract and could account for the ability of flavonoid-rich foods to protect against gastric and colon ulcers.

In this study, indomethacin produced a marked increase in gastric lesions and damaged the stomach mucosal layer in the experimental rats. Ulcer produced in this model was seen as black sores. The stomachs of rats in the control (distilled water-treated) group showed higher inductions of gastric ulcers due to increased levels of gastric juice in the rat's stomachs. *Moringa oleifera* leaf-treated rats showed **s**ignificant protection (53.43%) against gastric ulceration caused by indomethacin compared to the ulcers produced in the control group after seven days' treatment ($p<0.05$). Omeprazole treated rats showed significant protection (72.6%) against gastric ulceration caused by indomethacin administration compared to the ulcers produced in the control group after seven days' treatment ($p<0.05$). Although the protective effect of omeprazole and Moringa against indomethacin-induced ulcer is significant for both seven and fourteen respectively, the values of percentage Ulcer inhibition of omeprazole-treated animals (72.60 %, 74.29 %) was significantly higher than that of Moringa-treated animals (53.43%, 57.58 %) after seven and fourteen days respectively.

Seven and fourteen days' rats treated with *M. oleifera* leaf were compared and there was no significant difference in protection against gastric ulceration caused by indomethacin at $p< 0.05$. Also seven and fourteen days' rats treated with Omeprazole were compared and there was no significant difference in protection against gastric ulceration caused by indomethacin at $p< 0.05$. Therefore, further treatment with *Moringa oleifera* leaf and omeprazole does not guarantee greater protection as far as the treatment is ongoing before ulcer induction.

The significant difference observed between Omeprazole and *Moringa oleifera* leaf-treated group might be due to the fact that high percentage of Moringa active components did not dissolve in water used in the preparation. According to Verma *et al*., [21], an ethanolic extract of *M. oleifera* leaf has been reported to have potential for the treatment of gastric lesions.

5. Conclusion

The results of this study indicate that *Moringa oleifera* leaf is potent in the prevention of indomethacin-induced ulcer. Ulcer patients are advised to consume Moringa leaf as much as possible due to its high flavonoid and antioxidant content. Using methanoic or ethanoic extract of *Moringa oleifera* leaf in the prevention and treatment of indomethacin-induced ulcer and other types of ulcer induction can be further confirmed.

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