Moringa oleifera Lam extract attenuates gastric ulcerations in high salt loaded rats

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ABSTRACT

Moringa oleifera Lam is a plant used extensively both in traditional and orthodox medicine to treat myriad ailments, including gastrointestinal disorders. This study was carried out to investigate the effect of leaf extract of *M. oleifera* some gastrointestinal function parameters in high salt loaded rats. Acute toxicity study was done using 70 male white mice (18-20 g) were used for the study. They were randomly selected and assigned to 7 cages of 10 animals per cage. Percentage mortalities were converted to probits and plotted against the $\log_{10}$ of the dose of the extract from which the LD_{50} value was calculated. Fresh leaf extract of *M. oleifera* was Soxhlet extracted. 24 albino Wistar rats were randomly assigned into 4 main groups of 6 rats each. Fed on normal rat chow, high salt (8% NaCl) diet + 1% NaCl drinking water and/or *M. oleifera* extract (600 mg/kg bw). The feeding regimens lasted for 42 days. Results obtained revealed that the extract had an LD_{50} value of 1,872.22 mg/kg from which a test dose of 600 mg/kg was derived for the feeding regimen. The salt fed rats had significantly (p<005) raised basal gastric acid output (9.03 ± 0.17 mmol/L/hr) compared with control (7.27 ± 0.17 mmol/L/hr), but had blunted response to administered histamine and cimetidine, while treatment with the extract enhanced the sensitivity of histamine in high salt loaded rats. Gastric mucus concentration was significantly (p<0.05) higher in the salt untreated group (0.25 ± 0.004 g) compared with other groups. The salt fed untreated group also had significantly (p<0.05) raised gastric ulcers (10.83 ± 0.70) compared with other groups, these were reversed following *Moringa* treatment. In conclusion, *Moringa oleifera* extract reverses gastric ulcers and blunted histaminergic receptors in high salt fed rats. The mechanism by which high salt increases gastric secretion is independent of the histaminergic mechanism.

Keywords: *Moringa oleifera* Lam; Gastric acid secretion; Ulcers; Mucus; Rats.

1. INTRODUCTION

In spite of tremendous development in the field of orthodox medicines during the 20th century, plants still remain the first line of medication in modern and traditional system of medicine [1-4]. Among these medicinal plants is *Moringa oleifera* Lam. *Moringa oleifera* has been utilized to manage variety of ailments for many centuries [5-7].

The plant is known by common names like Miracle Tree, Horseradish tree, drumstick tree, never die tree, kelormarango, moonga etc., it local names include Zogalegandi in Hausa, Eweigbale in Youruba and Okweyibo in Igbo indicating its world-

wide significance. The trees originated from North Western region of India [8, 9].

M. oleifera leaves are edible and of high nutritive value and possesses analgesic, anti-diabetic, anti-hypertensive and anti-inflammatory effects [10-14]. This plant also has biological effects on the thyroid hormone, central nervous system and digestive system. The leaves of *M. oleifera* are also used traditionally to treat hepatotoxicity, rheumatism, venomous bites, and wounds; influenza, fever, nervous weakness, hysteria, pains, bowel disorders and worms [15]. Phytochemicals screening of the leaves of *M. oleifera* reveals that it contains some active ingredients like flavonoid, alkaloid, glycoside, niazirin, niazirinin, 4-benzl isothiocynate, benzyl glucosinolate, and carotenoids [16, 17].

Obviously, the first contact of ingested drugs and other substances in the body is the digestive system [18], different substances ingested into the body affect the functions of the digestive system in different ways. The digestive system is made up of the gastrointestinal tract (GIT) or alimentary canal and the accessory organs like the liver and pancreas, which help in the process of digestion, absorption, motility, secretion and excretion [19-21]. These parameters like gastrointestinal motility, gastric acid secretion, mucous output etc. are used to assess gastrointestinal function and could be deranged or enhanced by ingested substances.

Gastrointestinal functions have been shown to be altered by high salt intake in both man and experimental animals, high salt loading damages the lining of the GIT most especially the stomach. It decreases jejunal sodium reabsorption in young rats, and impairs intestinal Na^+/K^+ ATPase activity [22]. High salt diets interfere with normal food digestion (especially protein) by its ability to reduce the production of pepsin that enhance protein digestion. High salt diet also enhances vasoconstriction of mesenteric arteries, contributing to elevated blood pressure in rats [23].

There is paucity in scientific literature on the effect of *M. oleifera* extract on gastric ulcers, gastric acid and mucus secretion. The study is therefore aimed to investigate the effect of leaf extract of *M. oleifera* on gastric acid output, ulceration and mucus secretion in high salt loaded rats.

2. MATERIALS AND METHODS

2.1. Experimental animals

Forty-eight (48) male albino Wistar rats weighing initially between 160 to 200g obtained from the animal house of the Department of Physiology, University of Calabar, Nigeria were employed for this study for 6 weeks. The animals were allowed free access to their feed and drinking water. The rats were weighed before commencement of the feeding experiment and thereafter were weighed daily. Ethical approval was obtained from the Ethics Committee of the Faculty of Basic Medical Sciences, University of Calabar, Nigeria. They were nursed under control of environmental conditions in accordance with international standard [24].

2.2. Experimental plant

Fresh leaves of *Moringa oleifera* Lam (gene code number JX091931: Encycl. were purchased from the Botanical Garden of Calabar Municipality, Cross River State, Nigeria during the rainy season and were identified as authenticated by a botanist (Mr. Frank Adepoju) in the Department of Biological Sciences, University of Calabar, Calabar, where a voucher specimen was deposited with voucher number ERU/2011/345.

2.3. Preparation of plant extract

Fresh leaves of *M. oleifera* first washed free of sand and debris. Wash water was blotted off and the leaves ground to paste. A quantity of the ground sample (50 g) was weighed and Soxhlet extracted with 150 mL distilled water at 100°C for 9 h. Where larger ground samples were used, extraction was done under reflux with an appropriate volume of distilled water. The extract was slowly evaporated to dryness in vacuo at 40°C using a rotary evaporator. A total yield of 31% was obtained. Weighed samples of the extract were then used to prepare the stock solution [25].

2.4. Preparation of high salt diet

High salt diet containing 8% of sodium chlo-

ride was prepared using a standard diet containing 0.3% sodium chloride after the method of Obiefuna and Obiefuna [26].

2.5. Experimental protocol

The forty-eight (48) male albino Wistar rats were divided into 2 batches of 24 rats each. Batch 1 was used for gastric acid secretion and mucus secretion studies, while batch 2 was used for the ulcer study. Each batch was further sub-divided into 4 groups of 6 rats each. They were fed as follows: The group 1 (served as control) was fed on normal rat pellet + drinking water. The group 2 (NT) was fed on normal rat pellet + drinking water + 600 mg/kg b.wt. of *M. oleifera* extract orally once daily. The group 3 (SF) was placed on high salt diet (8% sodium chloride) + 1% sodium chloride drinking water. The group 4 (ST) received same as the third group + *M. oleifera* extract (600 mg/kg b.wt.) orally once daily. The feeding regimens lasted for six weeks. The animals were weighed daily.

2.6. Measurement of gastric acid secretion

Measurement of gastric acid secretion was done by the continuous perfusion method of Ghosh and Schild [27], modified by Osim et al. [28]. Rats from the control and test groups were fasted for 18-24 hours before the start of the experiment. The rats were anaesthetized with 0.6 ml/100 g body weight of 25% (wt/v) solution of urethane (Sigma, UK) given intraperitoneally. The trachea was exposed and cannulated. An infusion tube 75 cm length and 3mm diameter connected to 60 ml syringe carried by a pump was passed to the stomach through mouth and oesophagus. A ligature to stop back flow was made around the oesophagus in the neck. The abdomen was opened along the *linea alba* to minimise bleeding. The small intestine was reached and a semi-transection of 1-2 cm away from the pylorus was made and a fistula 8 cm long passed gently into the stomach through the pyloric sphincter and knotted.

Normal saline solution pH 7.00 placed in the pump was perfused through the stomach at 1 ml/minute via a perfusor. After an initial wash, the perfusate collected every 10 minutes interval and was titrated with 0.01 N NaOH solution in a 25 ml burette using phenolphthalein as indicator with pink coloration indicating the end point.

The pH of the saline was maintained by passing the perfusion tube through a water bath maintained at temperature of 37°C. Also a low wattage bulb was placed above the animal to warm it and the body temperature monitored. A rectal thermometer was inserted via the anus to ensure that the body temperature was at 37°C, care had to be taken not to ligate the vagus nerve or other blood vessels. To each 10 minute perfusate was added 2 drops of phenolphthalein indicator before titration against 0.01 N NaOH (Analar BOH, England) to determine total acidity.

2.7. Effect of histamine and cimetidine on gastric acid secretion

Upon collection of the basal gastric acid output using the normal saline for one hour (i.e. 6 aliquots were collections at 10 minutes interval), histamine (100 mg/kg) was thereafter injected into the rats subcutaneously, and the perfusate collected for another one hour. Thereafter, cimetidine (11.3 mg/kg) was injected intramuscularly, followed immediately with histamine (100 mg/kg). and the perfusate collected for one hour.

A total of 18 aliquots were collected at 10 minutes each, the time for each collections in 10 minutes was converted into 1 hour (by multiplying 10 minutes by 6).

2.8. Analysis of gastric acid

Gastric acid output was measured by titrimetric analysis. The calculation of acid in millimole per litre per hour (Mol/L/hr) follows the principle that states that a gram equivalent of acid balances a gram equivalent of the base at neutralization point. This means that:

Normality (N) of Acid x Volume (V) of Acid = Normality of Base x Volume of Base

i.e. $N_A \times V_A = N_B \times V_B$

From the above equation since Normality (N) of base is known i.e. 0.01 N and the volume of base needed for neutralization is known, the gram equivalent can be calculated thus: $N_B \times V_B$. This at the end points to the gram equivalent of the acid. If the volume is in mls, the acidity end point is in

milli-equivalent of acid. For a small animal like the rat milliequivalent will be too small and is always converted to μeq or μmol.

2.9. Ulcer studies

Gastric ulceration was induced in rats as described by Tekeuchi et al. [29], by oral instillation of 1 ml of 0.1 N HCl + 70% ethanol through intubation after an over night fast. One hour later, the animals were sacrificed using over dose of diethyl ether/chloroform and the stomachs were removed and opened along the greater curvature. Haemorrhagic lesions were examined microscopically using a hand lens (x18) and scored with a Vanier calliper as described by Elegbe [30].

Ulcer scoring:

Score	Description
0	Plan
0.5	0-6 mm
1	2-3 mm
2	>3 mm

2.10. Determination mucous secretion

The adherent gastric mucous was determined by the method described by Ettarh and Okwari [31]. The stomach was removed and washed in normal saline and then opened along the greater curvature. It was again rinsed in saline and pinned to a cork board with dissecting pins. Mucus was extracted using a spatula from the spread stomach into a known weight of beaker containing 4 ml of water. The weight of mucus was derived from the difference in the initial and final weights of beaker + 4 ml of water as follows:
Wt of beaker + 4 ml of water = x
Wt of beaker + 4 ml of water + mucus = y
Weight of Mucus = (y-x) g
This procedure has also been described by Tanet al. [32].

2.11. Statistical Analysis

Data are presented as mean ± SEM. Data were analysed using a one way analysis of variance (ANOVA) then followed with post hoc test (Least Significant Difference). P value of less than 0.05, 0.01 or 0.001 were declared as significant statistically.

3. RESULTS

3.1. Comparison of mean basal gastric acid output in control and tests groups

The mean basal acid output (BAO) in the control (group 1) was 7.27 ± 0.16 mmol/L/hr. The BAO was significantly ($p<0.001$) increased in salt fed untreated (group 3) and salt treated group (group 4) with basal acid output of 9.03 ± 0.17 mmol/L/hr and 10.10 ± 0.27 mmol/L/hr respectively compared with control and normal + extract (group 2). The BAO in salt treated was in turn significantly ($p<0.001$) higher compared with salt fed untreated group (Figures 1 and 2).

3.2. Comparison of the effect of histamine on gastric acid secretion in control and tests groups

Administration of histamine in the control group increased mean BAO significantly ($p<0.01$) from 7.27 ± 0.16 mmol/L/hr to 11.4 ± 1.44 mmol/L/hr (producing about 58.66% increase in gastric acid output). In normal + extract group the increase was 47.66%. In groups 3 and 4, their mean gastric output changed from basal levels of 9.03 ± 0.17 mmol/L/hr to 9.27 ± 0.88 mmol/L/hr (2.12% increase) and from 9.87 ± 1.00 mmol/L/hr to 10.10 ± 0.27 mmol/L/hr (2.52% decrease) respectively following histamine administration. Showing significant ($p<0.01$) decreases in groups 3 and 4 compared to control and normal + extract treated groups (Figures 1 and 2).

3.3. Comparison of the effect of cimetidine on histamine-induced gastric acid secretion in control and tests groups

Administration of cimetidine attenuated the effects of histamine in all the groups. But the attenuation was marked in salt fed groups compared with the normal rats. In the control and normal + extract groups, administration of histamine + cimetidine decreased mean gastric output by 16.35% and 14.48% respectively. While in the salt fed untreated and treated groups the reductions

were 43.13% and 46.80% respectively. Showing significant (p<0.001) reductions in the salt groups compared with the normal rats (Figures 2 and 3).
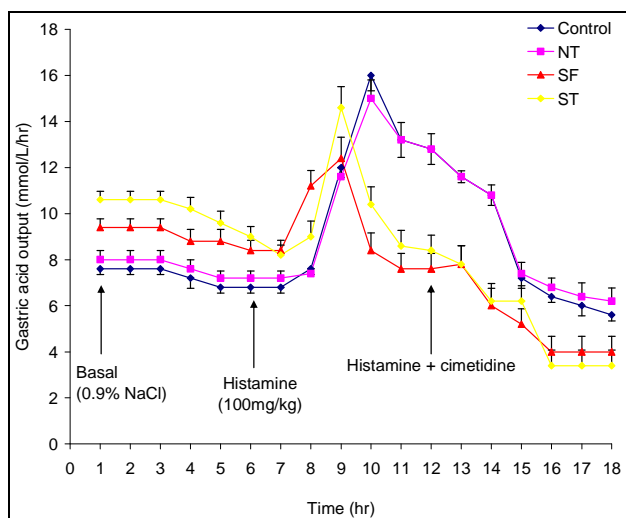

Figure 1. Basal gastric acid output and induced secretion to histamine and cimetidine in the different experimental groups. Values are expressed as mean ± SEM, n = 6.
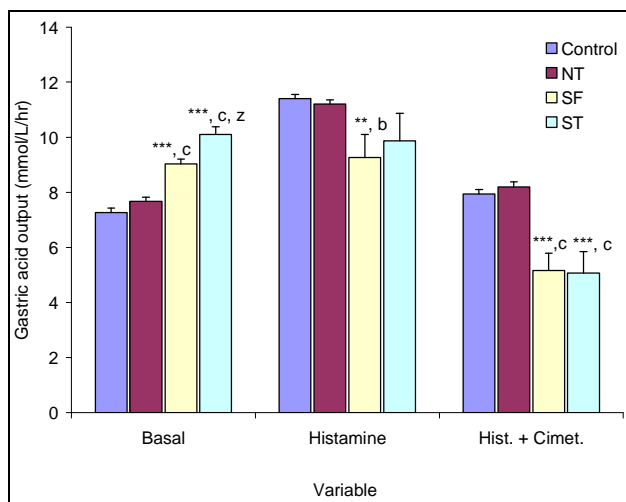

Figure 2. Comparison of basal, histamine and histamine + cimetidine induced gastric acid secretion in the different groups.

Values are mean ± SEM, n = 6. ** = p<0.01, *** = p<0.001 vs. control; b = p<0.01; c = p<0.001 vs. NT; z = p<0.001 vs. SF.

3.4. Comparison of mean gastric mucus levels in control and tests groups

The salt fed untreated group had significant (p<0.001) increase in mean gastric mucus output compared with other groups. The mean gastric mucus output for the different experimental groups were 0.13 ± 0.02 g in the control group, 0.12 ± 0.01 g for normal treated group, 0.25 ± 0.004 g in the salt fed untreated group and 0.16 ± 0.003 g in the salt treated group (Fig. 3).

3.5. Comparison of ulcer scores in control and tests groups

As shown in Fig. 4, the mean gastric ulcers in the salt fed untreated group (10.83 ± 0.70) was significantly (p<0.001) higher compared with the control (6.42 ± 0.48) and normal + extract (5.83 ± 0.48) and (7.92 ± 0.88) groups.
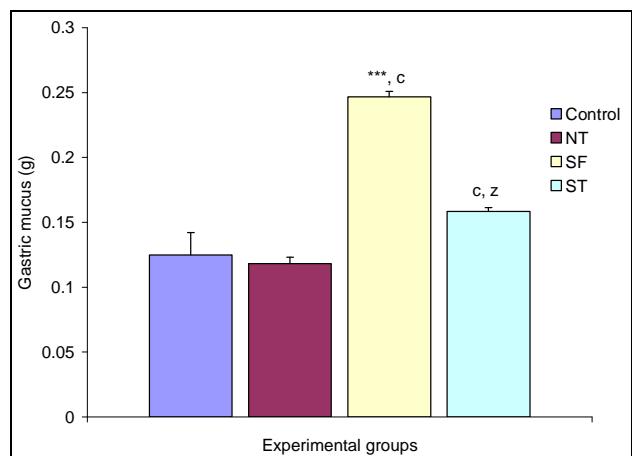

Figure 3. Comparison of mean gastric mucus in the different experimental groups.

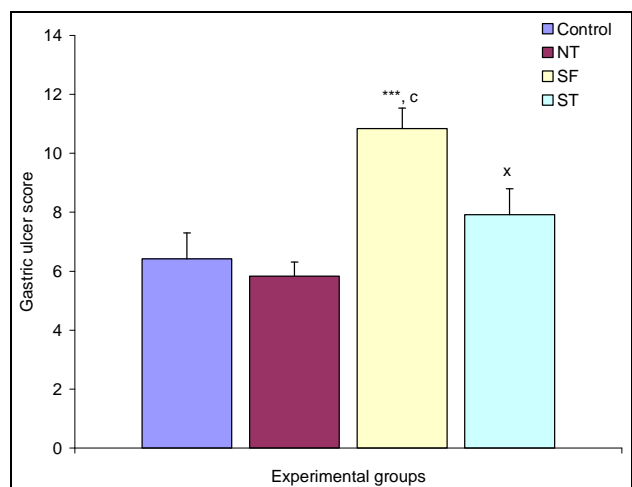Values are mean ± SEM, n = 6. *** = p<0.001 vs. control; c = p<0.001 vs. NT; z = p<0.001 vs. SF.

Figure 4. Comparison of mean gastric ulcer score in the intestinum in the different experimental groups.

Values are mean ± SEM, n = 6. *** = p<0.001 vs. control; c = p<0.001 vs. NT; z = p<0.001 vs. SF.

4. DISCUSSION

The effect of aqueous extract of *Moringa oleifera* Lam leaf on some gastrointestinal function in high salt loaded rats was investigated in this study. Gastrointestinal function indices studied included changes in gastric acid secretion, gastric mucus secretion and gastric ulcers. The results obtained from this study has strong indication that the aqueous leaf extract of *M. oleifera* leaf has tremendous effect on the gastrointestinal function following high salt loading.

Gastric acid output is an eminent parameter for assessing gastrointestinal function. This study revealed significant increase in basal gastric acid output of rats placed on high salt diet. The direct effect of high salt on the parietal cells could be a possible explanation for this elevated gastric acid output in high salt fed rats. Because one of the mechanisms earlier postulated for gastric acid secretion is the combination of $NaCl + H_2O + CO_2$ in the parietal cells, with a forward reaction of $HCl + NaHCO_3$ being produced. Hence, the increase availability of NaCl, the ultimate source of chloride ions for HCl formation, can step up the speed of reaction and increase gastric acid secretion [33], while salt withdrawal has been shown to depress gastric acidity, solely due to alteration in the rate of ionic transfer in the parietal cells.

This effect was not possibly via the histaminergic H_2 receptors, since gastric acid output in the salt fed rats was depressed following histamine administration. High salt loading is directly correlated with the incidence of *Helicobacter pylori*. *H. pylori* is responsible for the many gastric acidity, ulcerations and cancers in people [34, 35]. *H. pylori* gastritis, which is confined to the antrum and unaccompanied by atrophy, results in hyper secretion of acid. The increased acid secretion in subjects with antral predominant non-atrophic gastritis is mainly due to the *H. pylori* gastritis stimulating increased release of the hormone gastrin which circulates and stimulates the body of the stomach to secrete acid. Subjects with *H. pylori* antral gastritis have increased basal, meal stimulated and gastrin releasing peptide (GRP) stimulated serum gastrin concentrations [36-38]. The increased circulating gastrin associated with *H. pylori* is mainly due to an increase in gastrin-17 [39]. This

form of gastrin originates mainly from the antral mucosa and increase after meals. The increase in gastric acidity observed in the high salt loading in this present study could probably be due to increase in *H. pylori*.

The above explanation suffices for the increase in gastric ulcerations evidenced in high salt loaded rats recorded in this study. Besides solubilisation of mucus constituents could be a possible reason, as earlier noted by Glavin and Szabo et al. [40]. *M. oleifera* extract was effective in reducing the ulcers but not the gastric acidity in this study, possibly by enhancing the protective mechanism of the stomach in the presence of gastric acidity. Gastric ulceration involves breaking the mucosal barrier and exposing the underlying tissue of the stomach or duodenal lining to corrosive action of acid and pepsin or gastrin [40]. Among the factor proposed for the pathogenesis of peptic ulceration are increase gastric acidity and pepsin secretion, decreased in mucosal resistance and mucosal blood flow and increase in free radical generation and inhibition of somatostatin, some of these may be acquired during life, while some are predetermined [5]. *M. oleifera* is rich in anti-oxidant activity due to the present of phytochemicals like flavonoids, tannins, vitamins A, E and C in it, these are known protective chemicals to the stomach, thereby reducing gastric lesion and ulcers [41].

In the high salt loaded rats, gastric mucus concentration was elevated compared to other groups. Earlier report endorses high mucus secretion following high salt loading due to release of prostaglandins which stimulates mucus secretion and that the mucus can be degraded by proteases originating from enteric parasite [41]. However, one would have expected that the increase in mucus secretion in the salt fed untreated group would protect the gastric mucosal from injury and ulcerations. Previous reports has shown that, in-spite of raised gastric mucosa, the presence of a high concentration of sodium chloride damages to the gastric mucosa, leading to cell death and consequent regenerative cell proliferation, while in the longer term high NaCl concentration leads to inflammation and diffuse erosion of gastric mucosa [42]. Also, it has been observed from previous study that gastric mucus consists of two histo-chemically different kinds of mucin, surface mucous cell mucin (SMCM)

and gland mucous cell mucin (GMCM), salt loading shift gastric mucosa from the glandular to cell surface, where *H. pylori* strive most, leading to ulcerations [43, 44]. It is possible that the loosely adherent mucus that can be easily excised was produced following high salt loading and not the firmly adherent mucus that anchor firmly to the epithelium thereby preventing erosion by gastric acidity [45]. Research shows that the layer of mucus closest to the epithelium (firmly adherent mucus) is responsible for maintaining the integrity of the gastric mucosa [46].

5. CONCLUSION

High salt loading increases gastric acidity and ulcerations in rats despite the elevated gastric mucus following high salt loading. *Moringa oleifera* Lam extract prevented the ulcerogenic effect of high salt loading, and enhanced the sensitivity of the histaminergic receptors blunted in high salt loaded rats.

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AUTHORS' CONTRIBUTION

EEI wrote the initial draft of the manuscript; OEO designed the study and did the statistical analysis; AAN proof read, and edited the word. All authors were involved in the execution of the research plan. The final manuscript has been read and approved by all authors.

TRANSPARENCY DECLARATION

The authors declare no conflicts of interest.

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