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Anti-ulcerogenic and gastric anti-secretory effects of *Nauclea latifolia* extract in male albino rats

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ABSTRACT

Anti-ulcerogenic and gastric anti-secretory effects of methanol extract of Nauclea latifolia was investigated in indomethacin-induced gastric ulceration in rats. Sixty (60) male albino rats were divided into two experimental studies of thirty (30) rats each. Each of the experimental studies was further divided into groups according to study design. The extract was administered orally at the doses of 200, 400 and 800 mg/kg body weight for the experimental groups while the control and reference groups received distilled water (2ml/kg, p.o) and cimetidine (100 mg/kg, p.o) respectively. In the second study, gastric acid output was measured by the continuous perfusion of rat's stomach under anesthesia with normal saline at the rate of 1 ml/min. Gastric acid, mucous secretion and ulcer index were determined according to standard procedures. The phytochemical screening confirmed the presence of saponins, tannins, flavonoids, terpenoids, anthraquinones and cardiac glycosides. The extract (200, 400 and 800 mg/kg) exhibited significant ($P<0.05$), and dose-dependent inhibition of indomethacin-induced gastric ulceration that seems to be stronger than cimetidine (100 mg/kg). A significant decrease in gastric acid secretion with concomitant increase in intragastric mucous secretion was produced by the extract at all doses studied. The results suggest that the extract possesses a significant gastro protective effect in indomethacin-induced gastric lesions.

Keywords: *Nauclea latifolia*; gastric ulcer; cimetidine; phytochemical screening; gastric acid; H_2-receptors.

INTRODUCTION

Peptic ulcer is one of the most common gastrointestinal diseases, which causes a high rate of morbidity particularly in the population of non-industrialized countries [1]. Pathophysiology of ulcer is due to an imbalance between aggressive factors (acid, pepsin, *Helicobacter pylori*, non steroidal anti-inflammatory drugs etc.) and local mucosal defensive factors (mucus, bicarbonate, blood flow, prostaglandins etc.). Gastro duodenal mucosa integrity is maintained through a homeostatic balance between these aggressive and defensive factors [2]. Previous researches have indicated a correlation between increased gastric acid secretion and predisposition to peptic ulcer [3, 4].

Factors that cause a decrease in basal and maximal gastric acid output will therefore have protective capabilities on the gastric mucosa, acting as a defense against ulcerogenesis, a characteristic that is used in the treatment of ulcers [5]. In the recent years, a widespread search has been launched to identify new anti-ulcer drugs from natural sources. *Nauclea latifolia* (smith) belongs to the family Rubiaceae. It is commonly known as pin cushion tree being a straggling shrub or small tree, native to the tropical Africa and Asia [6]. It bears an interesting flower, large red ball fruit with long projecting stamens. It grows up to an altitude of 200 meters. It is widespread in the humid tropical rainforest zones or in the savannah wood land of West and Central Africa [7]. *N. latifolia* is commonly known as "Ubulu inu" among the Igbo in the Eastern part of Nigeria; as "Tafashiya" among the Hausas in the Northern part of Nigeria; as "Egbesi" among the Yoruba in the Western part of Nigeria and as "Itu" among the Itsekiri [8]. *N. latifolia* herbal remedies have been commonly seen in various cultures throughout recorded history and still serve as the main means of therapeutic medical treatment. It is used in the treatment of fever, diarrhea and even as an anti-parasitic drug [9]. The sticks are used as chewing stick and a remedy against tuberculosis [7, 10]. Experimental studies have established hypolipidemic and hypoglycemic effects like most other plants extracts [11-14]. The anti-hypertensive activity of this herb has also been documented [13]. Decoctions from the stem bark of the leaves of *N. latifolia* are used for treatment of stomach pain and constipation (Eno and Owo, 1999). Abbiw [15] stated that root infusion of *N. latifolia* is used in Sudan for the treatment of gonorrhea, its roots and leaves are used in Ghana for treating sores. In Nigerian folklores the fruit are sometimes used in the treatment of piles and dysentery [16]. In addition, the plant is used in the treatment of sleeping sickness and to prolong menstrual blood flow [17]. Gidado *et al*. [6] reported anti-diabetic properties for the root and leaf extracts while Taiwe *et al*. [18] reported the anti-depressant and anti-anxiety effects of the root extract of the plant. Flavonoids in the plants are among the cytoprotective materials for which anti-ulcerogenic efficacy have been extensively confirmed [19-21]. We had reported in our previous study the anti-ulcer activity of aqueous leaf extract of *N.latifolia* against indomethacin-induced ulcers in rats [22]; however, there is a dearth of information in the literature concerning the anti-ulcer and anti-secretory effects of methanol extract from leaves of *N. latifolia* on gastrointestinal tract in animal model. Therefore, the current study was undertaken to investigate the anti-ulcer and gastric anti-secretory effects of the methanolic leaf extract of *N. latifolia* in indomethacin-induced gastric ulcer in rats.

MATERIALS AND METHODS

Chemicals and drugs

All chemicals and drugs used in this investigation were of analytical grade and were obtained from Sigma, Saint Louis, USA. Cimetidine (H_2-receptor antagonist) was used as the reference anti-ulcer drug. In this study, cimetidine was administered orally to reference control group of rats in a dose of 100 mg/kg suspended in distilled water (2ml/kg) [23].

Experimental animals

Male albino rats of Wistar strain weighing between (200 to 240) g were obtained from the Central Animal House, Faculty of Medicine, Pre-Clinical Unit, Ebonyi State University, Abakaliki, Nigeria. The animals were housed in cross ventilated room in cages at (22 ± 2.5°C) with 12 h dark/12 h light cycles and were feed with standard growers mash feeds (Pfizer Feeds LTD, Enugu, Nigeria) and tap water *ad libitum*. Animals were acclimatized for one week and fasted overnight, with free access to water, prior to experiments. The experimental procedures and techniques used in the study were in accordance with accepted principles for laboratory animal use and care by National Institute of Health [24]. This study was approved by Animal Ethics Committee of the Faculty of Medicine, Ebonyi State University with reference number (EBSU/REC/BM14/021).

Plant material and preparation of methanol extract

The fresh leaves of *N. latifolia* were collected within the campus of the Ebonyi State University, Abakaliki, Nigeria, identified and authenticated by Mr. P.O. Ugwuozo in the herbarium of the Plant Science and Biotechnology Department of University of Nigeria, Nsukka, with deposition of authenticated voucher specimen (UNH-303i). The leaves were air-dried and blended to fine powder. Hot extraction of this powder (20 g) in a Soxhlet apparatus using 100 ml of methanol was carried out. The collected extracts were concentrated and dried *in vacuo* and the percentage yield of the extract was 6.54%. The concentrate was dissolved in 2–3 drops of tween-80 and diluted to desire concentrations [25]. The solutions were prepared fresh on the day of experiments prior to the administration.

Preliminary phytochemical screening
The methanolic leaf extract of the plant was subjected to various qualitative phytochemical tests, to identify the secondary metabolites; saponins, tannins, terpenes, steroids, flavonoids, anthraquinones and cardiac glycosides present in the leaves. The methods of analysis employed were those described by Trease and Evans [26] and Sofowora [27].

Experimental design
A total of sixty (60) rats were used for the study. The rats were divided into two groups, each of thirty (30) rats, each group being for a different study. The first study involved rats that underwent experimental Indomethacin-induced gastric ulceration. These were used to assess the degree of ulceration, total gastric acid content and mucous secretion in control and pretreatment test groups. Rats in the second study were assessed for both basal and maximal (histamine-induced) gastric acid secretion.

Gastric ulceration
This was carried out as described by Ukwe and Nwafor [28]. Food was withdrawn 24 hours and water one hour before drug treatment. Thirty (30) male albino rats were randomly divided into 5 groups (n=6) rats each. Animals in groups 1 and 2 received distilled water and cimetidine, respectively, while those in group 3, 4 and 5 were pre-treated with 200, 400 and 800 mg/kg of the extract respectively. After one hour, indomethacin 30 mg/kg (dissolved in 5% sodium bicarbonate solution) was administered orally to all the rats. Seven hours later the rats were killed by cervical dislocation. The rats' stomachs were removed and each opened along the greater curvature. After fixing the tissues by immersing in 10% formalin for 24 hours, it was rinsed under a stream of water and examined for ulcers. The ulcers were counted by the aid of a hand lens (X- magnification) and ulcer score was calculated for each animals according to the arbitrary scale used by Singh *et al*. [29], where 0 = no lesion, 1 = hyperemia, 2 = one or two slight lesions, 3 = very severe and 4 = mucosal full of lesion.

Ulcer index was calculated as mean ulcer scores [30].

Determination of gastric acid content
Before scoring the ulcer, the gastric content was drained into a centrifuge tube and 8 ml of freshly prepared normal saline was added and centrifuged at 3000 rpm for 10 min. The total gastric acidity was determined by titrating 5ml of the supernatant against M/400 NaOH to an end-point using 1–2 drops of phenolphthalein as an indicator according to Lai [31].

Determination of gastric mucous secretion
The adherent gastric mucous was determined by the method described by Ettarh and Okwari [32]. 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. Mucous was extracted using a spatula from the spread stomach into a known weight of beaker containing 4ml of water. The weight of mucous was derived from the difference in the initial and final weights of beaker + 4ml of water as follows:

Wt of beaker + 4ml of water = x
Wt of beaker + 4ml of water + mucous = y
Weight of Mucous = (y-x) gm

The procedure has also been described by Tan *et al*. [33].

Determination of gastric acid secretion
The effects of *N. latifolia* (400mg/kg) on basal and histamine-induced gastric acid secretion in albino rats were studied as described by Ghosh and Schild [34], modified by Amure and Ginsburg [35]. Thirty (30) male albino rats were randomly divided into 6 groups (n=5) rats each. Adult male rats (180–250 g) fasted for 24h were anaesthetized with an i.p injection of 0.6 ml/100 g of 25% urethane (ethyl carbamate). The femoral vein, esophagus and pyloro-duodenal junction were cannulated. The stomach was perfused with normal saline (37°C) and gastric effluent was collected at a constant rate of 10 ml/10 min. The effluent was titrated against M/400 (NaOH) solution with phenolphthalein as indicator. The effects of *N. latifolia* extract (400 mg/kg) alone and in combination with histamine and/or cimetidine, on gastric acid secretion were studied. Titrable acidity was expressed in μEq/L/10mins. The

histamine-induced gastric acid was collected 30 minutes post-surgery at which time a steady (basal) acid secretion had been obtained.

Statistical analysis

Results were expressed as mean ± S.E.M. The data were statistically evaluated by one way ANOVA. Comparison between treatment and control group were made by Student's t- test then followed with Fisher's exact. Significance of difference was accepted at $P < 0.05$ using Graph-Pad Prism version 5.00 for Windows (Graph Pad Software, San Diego, California, USA).

RESULTS AND DISCUSSION

Phytochemical Screening

Phytochemical analysis revealed that the extract contains saponins, tannins, flavonoids, terpenoids, anthraquinones and cardiac glycosides.

Gross evaluation of gastric lesions

Indomethacin induced ulcers in 100% of the animals in the negative control (distilled water; 2ml/kg) group (Table 1). The ulcer index was 4.08±0.75, which was characterized with severe disruption of surface epithelium of gastric mucosa. Pre-treatment with cimetidine significantly ($P< 0.05$) reduced the severity of indomethacin-induced ulcers compared to rats pre-treated with distilled water (ulcer control). The extract was also shown to exert cytoprotective effects in a dose-dependent manner (Table 1).

Gastric content acidity

The extract produced a significant ($p<0.05$) and dose dependent decrease in mean total gastric acidity in indomethacin induced gastric ulcers in pre-treatment groups compared to control (Table 2). Mean total gastric acidity decreases with increasing doses of the extract. Cimetidine produced a lower gastric acidity ($P < 0.05$) than any of the doses of the extract studied (Table 2). In order to determine the probable mechanism by which *N. latifolia* extract reduced total gastric acidity, the effect of 400mg/kg body weight of the extract separately and in combination with histamine and/or cimetidine on acid secretion *in situ* was studied. The extract produced a significant decrease in basal and histamine induced gastric acid secretion in rats (Table 3). Moreover, the extract appears to augment cimetidine inhibition of gastric acid secretion.

Gastric mucous studies

The extract produced a significant ($p<0.05$) and dose dependent increase in gastric mucous production in indomethacin induced gastric ulcers in rats compared to control (Table 1). The effect of the methanol extract on gastric mucous secretion was more pronounced in pretreatment tested groups when compared to rats pre-treated with cimetidine (Table 1).

Table 1. Effects *Nauclea latifolia* on gastric ulceration and mucous secretion induced by indomethacin

Group	Pre-treatment	Dosage (p.o)	Mean Ulcer Index ± SEM	Percentage Protection	Mucous content (g)
1	Distilled water	2 ml/kg	4.08 ± 0.75	0.00	0.31 ± 0.01
2	Cimetidine	100 mg/kg	1.96 ± 0.28*	51.96	0.52 ± 0.03*
3	Extract	200 mg/kg	1.42 ± 0.36*	65.20	0.56 ± 0.01*
4	Extract	400 mg/kg	0.69 ± 0.14*	83.09	0.67± 0.07*
5	Extract	800 mg/kg	0.54± 0.56*	86.76	0.72± 0.05*

**Significant. All values are expressed as mean ± SEM, n=6 in each group.*P<0.05 as compared with the negative control animal.*

Percentage inhibition to ulcer formation in rats by the extract was calculated as follows:

% Inhibition of Ulceration= [(Ulcer index $_{Control}$ - Ulcer index $_{Test}$) / Ulcer index $_{Control}$] x 100%

Table 2. Effect of *Nauclea latifolia* on total gastric acid content induced by indomethacin

Group	Pre-treatment	Dosage (p.o)	Total gastric acid content (*μ*Eq HCl/100g B.W)
1	Distilled water	2 ml/kg	12.92 ± 0.18
2	Cimetidine	100 mg/kg	5.56 ± 0.15*
3	Extract	200 mg/kg	10.62 ± 0.07*
4	Extract	400 mg/kg	9.80 ± 0.13*
5	Extract	800 mg/kg	7.20 ± 0.17*

**Significant. All values are expressed as mean ±SEM, n=6 in each group.*P<0.05 as compared with the negative control animal.*

Table 3. Effects of *Nauclea latifolia* extract on gastric acid secretion in rats

Group	Pre-treatment	Basal acid Output (μEq/L/10mins)	Gastric acid secretion (*μ*Eq/L/10mins)
1	Normal saline (1 ml/kg)	1.45 ± 0.01	1.55 ± 0.15
2	Extract (400 mg/kg)	1.52 ± 0.03	1.15 ± 0.24*
3	Histamine (100 mg/kg)	1.50 ± 0.01	6.40 ± 0.08*
4	Histamine + Extract	1.67 ± 0.07	2.25 ± 0.17*
5	Cimetidine (100 mg/kg)	1.55 ± 0.02	1.05 ± 0.13*
6	Cimetidine + Extract	1.54 ± 0.03	0.98 ± 0.75*

**Significant. All values are expressed as mean ±SEM, n=5 in each group.*P<0.05 as compared with the negative control animal.*

DISCUSSION

The present study was designed to investigate the anti-ulcer and gastric anti-secretory activities of methanolic leaf extract of *N. latifolia* against indomethacin-induced gastric ulceration in albino rats. The results of this study demonstrated that methanol extract of *N. latifolia* leaves significantly protected against mucosal damage induced by indomethacin and curative ratios of plant extracts 200, 400 and 800 mg/kg body weight were 65.20%, 83.09% and 86.76% respectively. The effect of the extract compared favorable to cimetidine 100 mg/kg (positive control). As shown in Table 1, cimetidine produced a weaker anti-ulcer effect than any of the doses of extract. Research has shown that indomethacin is an ulcerogenic agent especially when administered on an empty stomach [36]. The ulcerogenic activity of indomethacin and other non-steroidal anti-inflammatory agents as postulated might be due to their ability to inhibit prostaglandin synthesis [37]. Several lines of evidence suggest that prostaglandins inhibit gastric secretion and are important to normal gastric physiology and mucosal integrity [38-40]. Some of the mechanisms suggested for their effect include tightening of the gastric mucosal barrier [41] and stimulation of the gastric sodium pump [42]. The protective effect of the extract on indomethacin induced-ulcers in rats might be related to any of the mechanism suggested.

Phytochemical analysis identifies saponins, tannins, flavonoids, cardiac glycosides and terpenoids as the major components; these results support the findings of Akinloye and Olaniyi [25]. Flavonoids are among the cytoprotective materials for which anti-ulcerogenic efficacy have been extensively confirmed [19-21]. As flavonoids have already been identified in this plant [25], we believe strongly that the anti-ulcer activity of this extract is probably due to the antioxidant activity of the extract. Antioxidant activities of flavonoids have been well documented in the literature. Moreover, flavonoids have been reported for their anti-ulcerogenic activity and gastric protection [43, 44]. It is suggested that, these active compounds would be able to stimulate mucous, bicarbonate, and the prostaglandin secretion and counteract with the deteriorating effects of reactive oxidants in gastrointestinal lumen [44- 46].

The significant reduction in total gastric acidity observed in this study strongly suggests that *N. latifolia* may act by inhibiting gastric acid secretion. Moreover, this extract inhibited basal and histamine-induced acid secretion and seems to augment the inhibitory action of cimetidine (an H_2-receptor blocker) on gastric acid secretion. These findings indicate that the extract probably acts by inhibiting H_2-receptor leading to blockade of histamine release whose stimulatory action on gastric acid secretion via H_2-receptor, has been well reported [47-49]. There is, however, the possibility of the involvement of other receptors, which are yet to be investigated.

It is well-known that gastric mucous provides an important barrier against the corrosive action of hydrochloric acid on the gastric mucosa. Thus, this observation provides another gastric cytoprotective mechanism of the extract and supports the work of Menguy [50], who reported that irrigating canine antral pouches with 0.1 M HCl stimulated mucous secretion, providing an adaptive protection against mucosa lesions. However, the mechanism of mucous secreting activity of *N. latifolia* is not understood, further experiments to investigate its mucous secreting activity may be interesting.

CONCLUSION

The results of this study have shown that *N. latifolia* extract exhibits a significant gastro protective and anti-ulcerative effect on the stomach. The observed significant reduction in mean ulcer count in this study may likely be explained by the anti-secretory effect of *N. latifolia*, which significantly reduces the formation of ulcers. The anti-ulcer properties probably act via a reduction in gastric acid secretion and an increase in the intragastric mucous secretion. However, efforts are ongoing to characterize and explore the biological activity of the contributory compounds present in the extract.

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