Gastric acid anti-secretory effects of aqueous leaf extract of *Nauclea latifolia* (Rubiaceae) in rats

**Morufu E. Balogun**, **Daniel Nwachukwu**, **Peter E. Onwe**, **Moshood A. Folawiyo**

**Abstract**

The aqueous leaf extract of *Nauclea latifolia* has been shown to have anti-ulcer effect. **Objectives:** The present study was undertaken to investigate the effects of aqueous leaf extract of *N. latifolia* on gastric acid secretion as the possible mechanism of its anti-ulcer actions in male albino rats. **Materials and Methods:** A total of 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 100, 200 and 400 mg/kg for the experimental groups while the control and reference groups received distilled water (2 ml/kg, p.o) and cimetidine (32 mg/kg, p.o) respectively, 60 minutes prior to oral administration of indomethacin (30 mg/kg) to induce gastric mucosal injury. 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. **Results:** Phytochemical analysis revealed the presence of saponins, tannins, flavonoids, alkaloids and cardiac glycosides. Acute toxicity studies showed there were no deaths within 24 h after the application of the extract up to 1600 mg/kg B.W (i.p). Rats pre-treated with *N. latifolia* exhibited significant (*P < 0.05*), and dose-dependent inhibition of indomethacin-induced gastric ulceration. A significant decrease in gastric acid secretion was produced by the extract at all doses studied. **Conclusion:** Findings of this study suggest that, aqueous extract of *N. latifolia* significantly reduced gastric acid secretion in indomethacin-induced gastric ulceration by inhibiting histamine-stimulated gastric acid secretion probably by occupying H₂-receptors in rats.

**Keywords:** *Nauclea latifolia* leaf, Mucosal injury, Cimetidine, Indomethacin, Gastric acid secretion, Albino rats.

**Introduction**

*Nauclea latifolia* (smith) belongs to the family Rubiaceae. It is commonly known as a pin cushion tree being a straggling shrub or small tree, native to the tropical Africa and Asia.¹ 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 woodland of West and Central Africa.² *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 “Iwu” among the Isekiiri.³ *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. ⁴ The sticks are used as chewing sticks and a remedy against tuberculosis.⁵⁻⁷ Scientific studies have established hypolipidemic and hypoglycemic effects like most other plant extracts.⁶⁻⁹ In West and South Africa, infusions and decoctions of the stem bark and leaves of *N. latifolia* are used for treatment of stomach pain, fever, diarrhea and constipation.⁹ Abibw¹⁰ stated that root infusion of *N. latifolia* uses in Sudan for the treatment of gonorrhea, its roots and leaves are used in Ghana for treating sores. In Nigerian folklores the fruit is sometimes used in the treatment of piles and dysentery.¹¹ In addition, the plant is used in the treatment of sleeping sickness and to prolong menstrual blood flow.¹２ Gidado et al.¹⁰ reported anti-diabetic properties for the root and leaf extracts while Taiwe et al.¹¹ 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.¹⁴⁻¹⁶ We had earlier reported in our previous study that the aqueous leaf extract of
N. latifolia exhibits a dose-dependent anti-ulcer activity against indomethacin-induced ulcers in rats. Nevertheless, the effect of N. latifolia on gastric acid secretion as a probable mechanism by which its anti-ulcer property occurs was neither examined nor explored. Therefore, the aim of the present study is to investigate the effects of aqueous extract of N. latifolia on gastric acid secretion, and the possible mechanism(s) of action by which the extract produced its anti-ulcer effects in male albino rats. This is with a view of providing a scientific justification for the ethnomedical uses of the plants leaf in the management, control and treatment of gastric lesions.

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 (H₂-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 32 mg/kg suspended in distilled water (2 ml/kg).18

Experimental animals

Male albino rats of Wistar strain weighing between 180 to 240 g were obtained from the Central Animal House, Faculty of Medicine, Preclinical Section, Ebonyi State University, Abakaliki, Nigeria. They were housed in netted cages under standard laboratory conditions and were fed with standard rat pellets (Pfizer Feeds LTD, Enugu, Nigeria) and tap water was given ad-libitum. Excess feeds and water was removed and replaced daily. The rats were allowed to stabilize for 2 weeks before commencement of the experiment. The experimental procedures and techniques used in the study were in accordance with accepted principles for laboratory animal use and care by the National Institute of Health (NIH, 1985); all protocols and procedure were approved by Animal Ethics Committee of the University with reference number (EBSU/REC/BM14/021).

Plant material and preparation of aqueous 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 a fine powder. To 200 g of the powdered leaves in a container with lid, 1 liter of boiling water was added and covered. It was allowed to stand for 24 hours with intermittent shaking. The mixture was then filtered with NO.1 Whatman qualitative filter paper to obtain a pure filtrate. The collected extracts were concentrated and dried in vacuo and the percentage yield of the extract was 23.1%. The concentrate was later reconstituted in sterile distilled water to give the required doses of 100, 200 and 400 mg/kg/ body weight in 2 ml of the vehicle respectively. It was stored in the refrigerator throughout the period of the experiment to preserve the prepared extract.

Preliminary phytochemical screening

The aqueous leaf extract of the plant was subjected to various qualitative phytochemical tests, to identify the secondary metabolites; saponins, tannins, terpenoids, steroids, alkaloids, flavonoids, cardiac glycosides and anthraquinones present in the leaves. The methods of analysis employed were those described by Trease and Evans19 and Sofowora20.

Acute toxicity study

The lethal dose (LD₅₀) of the aqueous extract of N. latifolia was determined by the method of Lorkë21 and Sandow,22 using thirteen rats. In the first phase rats were divided into three groups of three (3) rats each and were treated with the aqueous leaf extract of N. latifolia at doses of 10, 100 and 1000 mg/kg body weight intraperitoneally. They were observed for 24h for signs of toxicity. In the second phase four rats were divided into four (4) groups of one rat each and were also treated with the aqueous extract N. latifolia at doses of 1000, 1600, 2900 and 5000 mg/kg body weight (i.p.). The median lethal dose (LD₅₀) was calculated using the second phase.

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 in a different study. The first study involved rats that underwent experimental Indomethacin-induced gastric ulceration. These were used to assess the degree of ulceration and total gastric acid content 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.23 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 100, 200 and 400mg/kg of the extract respectively. After one hour, indomethacin 30 mg/kg/2 ml were 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 animal according to the arbitrary scale used by Singh et al.24, where 0 = no lesion, 1 = hyperemia, 2 = one or two slight lesions, 3 = very severe and 4 = mucosal full of the lesion.

Ulcer index was calculated as mean ulcer scores.25

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 5 ml of the supernatant against M/400 NaOH to an endpoint using 1–2 drops of phenolphthalein as an indicator according to Lai.26

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**Gastric acid secretion in situ**

The effects of *N. latifolia* (200 mg/kg) on basal and histamine-induced gastric acid secretion in albino rats were studied as described by Ghosh and Schild, modified by Amure and Ginsburg. This was used together with the titration method described by Olowokorun. 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 anesthetized 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 (200 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 by 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).

**Table 1:** Effects *Nauclea latifolia* on gastric ulceration induced by indomethacin

<table>
<thead>
<tr>
<th>Groups</th>
<th>Pre-treatment</th>
<th>Dosage (p.o)</th>
<th>Mean Ulcer Index ± SEM</th>
<th>Percentage Protection</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Distilled water</td>
<td>2 ml/kg</td>
<td>4.87 ± 0.25</td>
<td>0.00</td>
</tr>
<tr>
<td>2</td>
<td>Cimetidine</td>
<td>32 mg/kg</td>
<td>1.56 ± 0.28</td>
<td>67.97</td>
</tr>
<tr>
<td>3</td>
<td>Extract</td>
<td>100 mg/kg</td>
<td>1.92 ± 0.36</td>
<td>60.57</td>
</tr>
<tr>
<td>4</td>
<td>Extract</td>
<td>200 mg/kg</td>
<td>1.50 ± 0.14</td>
<td>69.20</td>
</tr>
<tr>
<td>5</td>
<td>Extract</td>
<td>400 mg/kg</td>
<td>0.54 ± 0.32</td>
<td>88.91</td>
</tr>
</tbody>
</table>

*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:

\[
\text{% Inhibition of Ulceration} = \left[ \frac{\text{Ulcer index}_\text{Control} - \text{Ulcer index}_\text{Treat}}{\text{Ulcer index}_\text{Control}} \right] \times 100%
\]

**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 200 mg/kg B.W of the extract separately and in combination with histamine and/or cimetidine on acid secretion *in situ* was studied. As shown in Table 3, this extract produced a significant decrease in basal and histamine induced gastric acid secretion in rats. Moreover, the extract appears to augment cimetidine inhibition of gastric acid secretion.

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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Pre-treatment</th>
<th>Dosage (p.o)</th>
<th>Total gastric acid content (μEq HCl/100g B.W)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Distilled water</td>
<td>2 ml/kg</td>
<td>10.92 ± 1.18</td>
</tr>
<tr>
<td>2</td>
<td>Cimetidine</td>
<td>32 mg/kg</td>
<td>5.52 ± 0.15*</td>
</tr>
<tr>
<td>3</td>
<td>Extract</td>
<td>100 mg/kg</td>
<td>7.25 ± 0.17*</td>
</tr>
<tr>
<td>4</td>
<td>Extract</td>
<td>200 mg/kg</td>
<td>6.85 ± 0.23*</td>
</tr>
<tr>
<td>5</td>
<td>Extract</td>
<td>400 mg/kg</td>
<td>6.14 ± 0.05*</td>
</tr>
</tbody>
</table>

*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

<table>
<thead>
<tr>
<th>Groups</th>
<th>Pre-treatment</th>
<th>Basal acid Output (µEq/L/10 mins)</th>
<th>Gastric acid secretion (µEq/L/10 mins)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal saline (1 ml/kg)</td>
<td>1.42 ± 0.01</td>
<td>1.45 ± 0.15</td>
</tr>
<tr>
<td>2</td>
<td>Extract (200 mg/kg)</td>
<td>1.54 ± 0.03</td>
<td>1.15 ± 0.04*</td>
</tr>
<tr>
<td>3</td>
<td>Histamine (100 mg/kg)</td>
<td>1.50 ± 0.0</td>
<td>5.60 ± 0.28*</td>
</tr>
<tr>
<td>4</td>
<td>Histamine + Extract</td>
<td>1.55 ± 0.07</td>
<td>2.05 ± 2.52*</td>
</tr>
<tr>
<td>5</td>
<td>Cimetidine (32 mg/kg)</td>
<td>1.45 ± 0.02</td>
<td>1.05 ± 0.10*</td>
</tr>
<tr>
<td>6</td>
<td>Cimetidine + Extract</td>
<td>1.40 ± 0.03</td>
<td>0.86 ± 0.25*</td>
</tr>
</tbody>
</table>

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

**Discussion**

This research work was designed to investigate the gastric acid anti-secretory effects of aqueous leaf extract of *N. latifolia* against indomethacin-induced gastric ulceration in albino rats. The results of the toxicity study suggest that the extract has a wide margin of safety and thus administration as done in folk medicine may not have any immediate deleterious effects. The findings of the present study demonstrated that aqueous extract of *N. latifolia* leaves significantly protected against mucosal damage induced by indomethacin and curative ratios of plant extracts 100, 200 and 400 mg/kg were 60.57%, 69.20% and 88.91% respectively. It is remarkable that the leaf extract at the tested doses of 200 and 400 mg/kg produced a greater protection than cimetidine (32 mg/kg) against the indomethacin. The effect of the extract compared favorably to cimetidine 32 mg/kg (positive control). As shown in Table 1 cimetidine produced a weaker anti-ulcer effect than the extract at the tested doses of 200 and 400 mg/kg body weight. It is not known which of the components of *N. latifolia* extract possesses this property. It has been established that indomethacin is an ulcerogenic agent especially when administered on an empty stomach. The ulcerogenic activity of indomethacin and other non-steroidal anti-inflammatory agents as postulated might be due to their ability to inhibit prostaglandin synthesis. Several lines of evidence suggest that prostaglandins inhibit gastric secretion and are important to normal gastric physiology and mucosal integrity. Some of the mechanisms suggested for their effect include tightening of the gastric mucosal barrier and stimulation of the gastric sodium pump. 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, alkaloids and cardiac glycosides as the major components. It has also been reported that, the flavonoids like flavones, glycosides, tannins and isoflavonoid (Indicanine B and Indicanine C) have been isolated from its leaves. Flavonoids are among the cytoprotective materials for which anti-ulcerogenic efficacy have been extensively confirmed. It is suggested that, these active compounds would be able to stimulate mucus, bicarbonate, and the prostaglandin secretion and counteract with the deteriorating effects of reactive oxidants in gastrointestinal lumen.

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 H₂-receptor blocker) on gastric acid secretion. These findings indicate that the extract probably acts by inhibiting H₂-receptor leading to blockade of histamine release whose stimulatory action on gastric acid secretion via H₂-receptor, has been well reported. There is, however, the possibility of the involvement of other receptors, which are yet to be investigated.

**Conclusion**

The present study showed that pre-treatment with the leaf extract of *N. latifolia* caused a beneficial effect on indomethacin-induced gastric lesions in rats as evidenced by the reduction in the ulcer index and gastric acid secretion. The gastric acid anti-secretory effect of the extract is dose-dependent and this may justify its use as an anti-ulcerogenic agent. However, further studies are necessary to isolate the responsible active compound(s) and elucidate its mechanism of action especially with respect to mechanism of gastric mucous secreting activity and prostaglandin synthesis. If these findings are extrapolated to man, aqueous extract of *N. latifolia* may therefore be beneficial to peptic ulcer-prone individuals. Since the extract inhibits gastric acid released by histamine a potent secretagogue that acts via H₂ receptor.

**Acknowledgements**

The authors are grateful to Dr. (Mrs.) Besong Elizabeth of Physiology Department of Ebony University, Abakaliki for the technical assistance received.

**Ethical approval**

All authors hereby declare that "Principles of laboratory animal care" (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments have been examined and approved by the appropriate ethics committee.

**Declaration of interests**

Authors have declared that no competing interests exist.
The Journal of Phytopharmacology

References


