INVESTIGATION INTO THE ANTI-ULCER ACTIVITY OF THE METHANOL EXTRACT OF STEMONOCOLEUS MICRANTHUS HARMES, BARK (FAM: LEGUMINOSEAE)

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ABSTRACT

The objective of this study was to investigate the anti-ulcer activity of methanolic extract of Stemonocoleus micranthus Harms, Bark (Leguminoseae) in rats. Fresh dried barks of Stemonocoleus micranthus were extracted by cold maceration technique with methanol as the solvent and the yield was concentrated on a rotary evaporator. Anti-ulcer effects of the methanolic extract at 25, 50 and 100 mg/kg were evaluated in rats using indomethacin induced ulcer model. Phytochemical analysis and lethality tests (LD$_{50}$) were carried out using standard methods. Results showed that the methanolic extract exhibited significant (P<0.05) and dose-dependent anti-ulcer activity in the model used. Percentage ulcer inhibitions of the methanolic extract at 25, 50 and 100 mg/kg for indomethacin induced ulcers were 30.80, 52.70 and 70.50 % respectively. Ulcer protections in the model used by the methanolic extract is dose – dependent and the ulcer inhibitory effect of the extract was comparable to cimetidine at 100 mg/kg. Oral LD$_{50}$ value greater than 5000 mg/kg was obtained indicating the safety of the plant for consumption. Phytochemical analysis showed the presence of tannins, saponins, glycosides, carbohydrates, reducing sugars, proteins, terpenoids, steroids and flavonoids. Therefore, results of our study suggest that the methanolic extract of Stemonocoleus micranthus possesses anti-ulcer activity as claimed by its traditional use.
KEYWORDS: Anti-ulcer activity, S. micranthus stem bark, phytochemical analysis.

INTRODUCTION
Peptic ulcer is a mucosal break, 3 mm or more in size with depth, that can involve the stomach or duodenum. Ulcers are produced when any factor causes an imbalance between the protective factors (mucus and bicarbonate) and aggressive factors (acid and pepsin) through endogenous defense mechanisms in the stomach [1, 2]. Such factors could range from natural causes (gastric cancer), infections (*H. pylori*), lifestyle (drugs-non-steroidal anti-inflammatory agents, alcohols, stress and cigarette smoking) [3, 4]. Oxygen derived free radicals have been implicated in the pathogenesis of gastric damage caused by physical, chemical and psychological factors that leads to gastric ulceration in human and experimental animals [5]. To regain the balance, different therapeutic agents including plant extracts are used [6, 7]. Herbal medicine is fast emerging as an alternative treatment to available synthetic drugs for treatment of ulcers possibly due to their lower costs, availability, fewer adverse effects and perceived effectiveness [8]. The current world wide trend towards utilization of plant – derived natural remedies has, therefore, created a dire need for accurate and up to date information on the properties and uses, efficacy, safety and quality of medicinal plant products [9]. Many tropical herbs have been scientifically reported to possess potent anti-ulcer activities [10 - 13].

*Stemonocoleus micranthus* (Nre in Igbo, Erhanebeni in Edo, Ahianana in Ivory Coast) is a very large tree up to 45 m high, with a large, straight, cylindrical trunk to 1.5 m diameter and buttressed to 6 m, scattered, and not common. The plant is distributed in the primary forest of ivory coast, Southern Nigeria, Eastern Cameroun, Gabon and Congo, sap-wood is white and hard [14]. In literature, no experimentally work has been reported on this plant. In the Central African Republic, the bark of this plant is claimed to be effective in the treatment of rheumatism, infertility in women, diarrhoea and dysentery, ulcers, hypertension, pyretic, emetic, helmintics and mental illnesses especially when pounded in water and applied as a paste or as an aqueous decoction [15]. This study was designed to evaluate the anti-ulcer activity of the plant and to identify phytochemically the constituents of the extract responsible for the observed activity.

MATERIALS AND METHODS
Plant Material: The barks of *Stemonocoleus micranthus* were collected in large quantities from Orba, in Udenu- Local Government Area of Enugu State, Nigeria in August, 2010 and
were identified by Mr. A.O. Ozioko of the Bioresources Development and Conservation Project (BDCP) in Nsukka. A voucher specimen has been deposited in the herbarium of the Department of Pharmacognosy and Environmental Medicine, University of Nigeria, Nsukka for future reference.

**Preparation of the Methanolic Extract**: 400 g of dried powdered bark of *Stemonocoleus micranthus* was macerated in methanol (Sigma-Aldrich, Germany) for 48 hours. The mixture was filtered and the solution was concentrated on a rotary evaporator (Fulton NYC R205 D, Shenshun). The concentrate of the methanolic extract was packaged in an amber coloured bottle and was stored in a refrigerator till its use.

**Phytochemical Screening**: The methanolic extract was tested for the presence or absence of secondary metabolites using standard phytochemical procedures [16].

**Animals**: Mice of both sex (20-30 g) and rats of both sex (150 – 250 g) supplied by the staff of the Department of Zoology of the University of Nigeria, Nsukka were used. They were housed in steel cages, placed on standard pellet feed (Niger feed, Nigeria) and were given free access to clean water. They were kept in well ventilated rooms with a 12/12 h light/dark conditions and ambient room temperature. Animals were procured two weeks before the experiments to acclimatize with the laboratory environment. Animal experiments were done in compliance with the National Institute of Health Guide for care and use of laboratory animals (Pub. No. 85-23, revised 1985).

**Acute – Toxicity and Lethality Tests**: Acute toxicity and lethality test of the methanolic extract of *Stemonocoleus micranthus* was ascertained using standard procedure [17]. Three groups of 3 mice each were administered 10, 100 and 1000 mg/kg of the methanolic extract orally. The mice were observed for 24 hours for effects of toxicity and the number dying in each group within the period noted. When no deaths were recorded, another tree groups of 3 mice each were administered 1600, 2900 and 5000 mg/kg of the extract orally. The animals were observed for 48 hours for effects of toxicity and the number dying in each group within the period was recorded.

**Anti-ulcer Activity**: The model, indomethacin with effective induction of ulcer experimentally in rats was employed to evaluate the anti – ulcer activity of the methanolic
extract of *Stemonocoleus micranthus*. All the rats used were fasted for eighteen hours but were given water freely till the start of the experiment.

**Indomethacin – induced Ulcer:** Animals (five group of six rats each) in groups A, B, C, D and E received distilled water 2 ml/kg p.o (negative control), cimetidine 100 mg/kg (CIMETAG®), Hovid) p.o (positive control), 25, 50 and 100 mg/kg p.o of methanolic extract respectively. After 30 min, indomethacin 40 mg/kg (METHACIN®, Hovid) p.o was administered to each rat. After 8 h of drug treatment [18], the rats were sacrificed with chloroform (Sigma – Aldrich, Germany) anaesthesia. The stomachs were isolated, washed gently under clean flowing water and cut open along the greater curvature. The stomachs were then fixed in 10% formalin and craters observed and ulcer scores were recorded using standard method [13].

**Statistical Analysis:** Ulcer indices were shown as the Mean ± SEM (standard error of mean) and level of ulcer protection presented as percentage inhibition. The significance of the differences in mean ulcer indices between extract and negative control was calculated at 95% confidence interval using student’s t-test.

**RESULTS**

Phytochemical screening showed that the methanolic extract contains saponins, tannins, glycosides, carbohydrates, reducing sugars, proteins, steroids, terpenoids and flavonoids. Acute toxicity results showed that the LD₅₀ was greater than 5000 mg/kg.

**Indomethacin – induced Ulcer:** The methanolic extract of *Stemonocoleus micranthus* protected the rats from experimentally induced ulcers at all dose levels with the lesions produced being noticeably severe. Ulcer protection in the model used by the methanolic extract is dose – dependent and the ulcer inhibitory effect of the extract was comparable to cimetidine at 100 mg/kg. Percentage ulcer inhibition of the methanolic extract at 25, 50 and 100 mg/kg for indomethacin – induced ulcers were 30.80, 52-70 and 70.50 % respectively. The doses of 50 and 100 mg/kg of the extract proved to be the doses with the statistically significant protection (52.70 and 70.50 %, P< 0.05).

Effects of methanolic extract of *S. micranthus* bark on indomethacin-induced ulcers in rats (n=6).
### Table

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose mg/kg P.O</th>
<th>Quantal Ulcer incidence</th>
<th>Ulcer index</th>
<th>Ulcer inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Distilled water</td>
<td>2 ml/kg</td>
<td>6/6</td>
<td>3.34 ± 0.40</td>
<td></td>
</tr>
<tr>
<td>Cimetidine</td>
<td>100</td>
<td>6/6</td>
<td>0.50 ± 0.09</td>
<td>75.30</td>
</tr>
<tr>
<td>Extract</td>
<td>25</td>
<td>6/6</td>
<td>3.32 ± 0.23</td>
<td>30.80</td>
</tr>
<tr>
<td>Extract</td>
<td>50</td>
<td>6/6</td>
<td>1.69 ± 0.10*</td>
<td>52.70</td>
</tr>
<tr>
<td>Extract</td>
<td>100</td>
<td>6/6</td>
<td>1.33 ± 0.15*</td>
<td>70.50</td>
</tr>
</tbody>
</table>

Values are Mean ± SEM, n = number of animals in each group. *P<0.05 Vs negative control (student t-test).

### DISCUSSION

The anti-ulcer activity of the methanolic extract of *S. micranthus* bark was established in this study. Results of acute toxicity showed that the plant is safe.

The extract protected the stomach against indomethacin’s necrotic damage and its effect at 100 mg/kg was comparable to cimetidine at 100 mg/kg, a cytoprotective agent. The protection by the extract of this type may suggest a possible cytoprotective mechanism of action.

However, an anti-secretory effect might be indicated as the extract protected the stomach mucosa from NSAID (indomethacin) induced damage. This damage is elicited by the inhibition of prostaglandin synthesis which is essential for mucosal integrity and regeneration [19]. This results to a sustained reduction in mucosal blood flow and a subsequent generation of ulcer.

The presence of saponins, tannins, glycosides, steroids, flavonoids, terpenoids, proteins, carbohydrates and reducing sugars has been shown in this study and the ulcer protection of the extract may be attributed to any of these phytochemical constituents as flavonoids, tannins and saponins have been shown to produce anti-ulcerogenic and anti-gastric activity [20, 13]. However, until specific constituents are isolated and characterized, exact mechanism of action cannot be ascertained.

We have demonstrated in this study that the methanolic extract of *S. micranthus* bark has an ulcer healing property against experimentally induced ulcers in rats and this study confirms the ethnomedicinal claims of the benefits of *S. micranthus* bark in the treatment of ulcer.
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