Inhibition of Acetylcholine and Histamine Receptors may be the Possible Mechanisms of Anti-ulcer Activity of Solenostemon monostachyus P. Beauv

Oghenevware Onome Francisca 1, Ilodigwe Emmanuel Emeka 1, Okonta Jemefuna Matthew 2
Erhirhie Earnest Oghenesuwe 1 and Ajaghaku Daniel Lotanna 1

1Department of Pharmacology and Toxicology, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria
2Department of Clinical Pharmacy, University of Nigeria, Nsukka, Enugu State, Nigeria.

*Corresponding Author: Erhirhie Earnest Oghenesuwe, Department of Pharmacology and Toxicology, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria

ABSTRACT
This study evaluated the possible mechanism of anti-ulcer activity of Solenostemon monostachyus P. Beauv. (Lamiaceae). Acute toxicity test, phytochemistry, preventive and curative anti-ulcer activities as well as the ability of the extract to modulate acetylcholine and histamine induced muscle contraction in isolated guinea pig ileum were carried out. LD 50 of 2449 mg/kg and above 5000 mg/kg were recorded in mice and rats respectively. Phytochemical screening on the extract and fractions revealed the presence of alkaloids, saponins, tannins, flavonoids, steroids, terpenoids, cardiac glycosides and proteins. In all the ulcer models, the extract and n – hexane fraction showed remarkable dose dependent significant (p ≤ 0.05) inhibitions against all ulcerogens (acetic acid, ethanol and indomethacin). The extract also dose dependently inhibited acetylcholine and histamine induced contraction of guinea pig ileum. This study validated the anti-ulcer ethno-medicinal use of Solenostemon monostachyus, whose mechanism may be due to inhibition of acetylcholine and histamine receptors.

Keywords: Solenostemon monostachyus, Peptic ulcer, Acetylcholine receptors, Histamine receptors, ulcer models

INTRODUCTION
Peptic ulcer disease (PUD) is a spectrum of diseases that occurs when the endogenous defense mechanisms of the protective mucosal barrier have failed to sufficiently counteract the aggressive factors (hydrochloric acid, pepsin, and Helicobacter pylori) [1,2]. PUDs are recurrent and most clinical studies had shown that approximately 50% of all ulcer patients will have recurrence within one year of diagnosis [3]. Some signs and symptoms associated with peptic ulcer are gnawing pain, burning discomfort, and tenderness in the epigastric area [4].

Several orthodox pharmaceutical drugs such as anti-cholinergic drugs, H 2-receptor antagonists, antacids, and more recently, proton-pump inhibitors have been employed in the management of peptic ulcers, but they provoke adverse effects [5].

In recent years, there has been growing interest by about 80 percent of Africa population in alternative therapies (especially from plant sources) in the management of peptic ulcer due to accessibility, affordability and perceived lower side effects of medicinal plants [6]. To corroborate these facts, ethno-medicinal claims of numerous medicinal plants had been scientifically validated to possess anti-ulcer properties [5,7].

Solenostemon monostachyus (S. monostachyus) an annual herb belonging to the family, Lamiaceae is widespread in West and Central Africa regions. It is slightly succulent, aromatic and grows up to 100 cm tall [8]. The leaves are eaten as a pot herb, used locally to treat ulcer, dysmenorrhea, haematuria, female sterility, rheumatism, diabetes, kidney problems, food infections, convulsion, fever, headache, cough and snake bites [9]. Its anti-inflammatory and anti-nociceptive activities [10], acute toxicity and anti-oxidant activity [11] and anti-microbial properties [12] as well as screening of its
Inhibition of Acetylcholine and Histamine Receptors may be the Possible Mechanisms of Anti-ulcer Activity of Solenostemon monostachyus P. Beauv

phytochemical constituents (including; alkaloids, saponins, tannins, flavonoids, steroids, terpenoids, cardiac glycosides, proteins coumarin, polyphenol, diterpenoids, lipids, calcium, phosphate and β-pinene, oct-1-en-3-ol, β-caryophyllene, octan-3-ol and (E, E)-α-farnesene) [8,9] had been documented in literatures.

Following previously reported antiulcer activity of S. monostachyus, the present study therefore revalidated its anti-ulcer activity and also further investigated the possible mechanisms by which it elicits its anti-ulcer activity using isolated guinea pig ileum.

MATERIALS AND METHODS

Plant Materials

Fresh leaves of S. monostachyus were collected from Enugu-Ezike, Enugu State in August 2014. The plant was identified and authenticated by Mr. Alfred Ozioko, a taxonomist at the International Centre for Ethnomedicine and Drug Development (INTEREDD/ Bio-resources Development and conservative program (BDCP) Nsukka, Nigeria. The leaf sample was deposited in the herbarium and was assigned a voucher specimen number of “INTERCEDD/ 055”.

Drugs and Chemicals

Acetylcholine chloride (Kemlight Laboratories, Mumbia India), Methanol (BDH, England), ethanol (BDH, England), n-hexane (BDH, England) and other chemicals used in the study were of analytical grades.

Animals

Adult albino mice (20 - 25 g), Wistar rats (150 - 180 g), and guinea pigs (350 -400 g) of either sex, were procured from the animal house of the Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka. The animals were acclimatized for 14 days and housed under standard conditions of room temperature. Animals were fed with standard livestock pellets diet (Guinea Feed Nigeria Limited) while the guinea pigs were fed with local grass. All the animals were allowed free access to clean water through-out the experimental periods. Approval for the use of the experimental animals was obtained from the Ethical committee of the Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka.

Extraction and Fractionation

Following the method of Okonko et al. [9], one kilogram (1 kg) of the powdered leaves of S. monostachyus was cold macerated in four (4) litres of methanol. The mixture was allowed to stand with occasional agitation for 48 hours. After filtration, the filtrate was concentrated in a rotary evaporator in vacuo at 40°C to obtain the methanol extract. The extract was further subjected to liquid-liquid partitioning with n-hexane, ethyl acetate and methanol solvents. The solvent fractions were separately concentrated using rotary evaporator at 40°C.

Phytochemical Analysis

The crude extract and fractions were subjected to phytochemical screening according to the method of Evans and Trease (1989) and Harborne (1973) as described by Yadav and Agarwala [13].

Acute Toxicity Studies

The acute toxicity study on S. monostachyus crude extract was carried out in mice and rats, using the modified Lorke’s method and the median lethal dose (LD₅₀) was determined [14].

Anti-Ulcer Tests

Animals were divided into groups (n =6) as follow; negative control, (vehicle, 5 mL/kg of 5% tween-80), Misoprostol (200 µg/kg, positive controls for prophylaxis study), Omeprazole (20 mg/kg, positive controls for curative study), and crude extract (100, 200 and 400 mg/kg), and n-hexane fraction (200 and 400 mg/kg).

For prophylaxis model, animals were pretreated for 14 days before inducing ulcer with; 1.5 mL of 4% acetic acid, 5 mL/kg of 95% ethanol and 40 mg/kg of indomethacin on the 15th day. For curative model, animals were induced ulcer (using 1.5 mL of 4% acetic acid, 5 mL/kg of 95% ethanol and 30 mg/kg of indomethacin) and treatment followed after 24-hours for 14 days. At the end of 14-days, animals were sacrificed and their stomachs were removed and opened along the greater curvature. They were rinsed under a running tap water, pinned on corkboard and observed with a hand lens (x10) and erosions formed on the glandular portions of the stomach were counted and a severity rating on a 0-3 scale based on the diameter of ulcer (i.e 0.0 - normal, 0.5 - puncture or pin-point, 1.0 - two or more hemorrhagic ulcers, 2.0 - ulcers greater than 3 mm in diameter mm², and 3.0 - several ulcers) was recorded [15-16]. Average
total ulcer score for each group was used as ulcer index (U.I). The healing effect and level of protection was calculated as a percentage of the negative control [17].

**In vitro Studies**

Segments of the ileum, 2-3 cm long, were suspended in 20 ml organ baths filled with Tyrode solution containing the following salts per litre: NaCl-8.00 g, KCl-0.20 g, CaCl₂-0.20 g, NaHCO₃-1.00 g, MgCl₂-0.10 g, Na₂HPO₄-1.00 g, and glucose-2.00 g. The mounted tissue was maintained at 37.0 ± 1.0 °C and aerated with air (oxygen). The preparations were allowed to equilibrate for 30 minutes during which the bathing fluid was changed every 10 minutes. At the end of equilibration period, the extracts of *S. monostachyus* was tested for spasmogenic or spasmolytic activity by adding increasing concentrations of each of these extracts (100-12800 μg/ml) into the organ bath containing guinea pig ileum preparation. The effects of the extracts on sub-maximal responses of Acetylcholine and Histamine were also determined and the percentage inhibition was calculated for each treatment.

The contact time for the activity of each extract was 120 s while the standard spasmogen acted for 30 seconds in a 3 min time cycle. Responses were recorded using Ugo Basile four chamber isolated organ bath (Model 4400, serial no: 0608c13, Italy).

**Statistical Analysis**

Data were expressed as mean ± Standard error of mean (SEM), and further analyzed using one-way analysis of variance (ANOVA), followed by post hoc turkey’s test using graph pad prism version 5. Mean differences with p ≤ 0.05 were taken to be statistically significant. Inhibitory concentration (IC50) values were calculated using regression equation.

**RESULTS**

Table 1 showed the qualitative abundances of phytocompounds present in the extract and fractions of *S. monostachyus*. Tannins, steroids and glycosides were abundant in the n – hexane fraction while terpenoids and cardiac glycosides were more in the ethyl acetate fraction.

Oral acute toxicity test showed no obvious signs of toxicity in rats. However, in mice, death was recorded at higher doses from 3000 to 5000 mg/kg. The LD₅₀ of the extracts was estimated to be above 5000 mg/kg for rats and 2449 mg/kg for mice respectively.

**Table 1. Phytochemical Screening of Solenostemon monostachyus**

<table>
<thead>
<tr>
<th>Phytochemical</th>
<th>Crude Extracts</th>
<th>Methanolic Extracts</th>
<th>N-Hexane Fraction</th>
<th>Ethyl Acetate Fraction</th>
<th>Methanolic Fraction</th>
<th>Aqueous Fraction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>Tannins</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>++</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Steroids</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Terpenoids</td>
<td>++</td>
<td>++</td>
<td>+++</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Cardiac glycosides</td>
<td>+</td>
<td>+++</td>
<td>+++</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Proteins</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td>++</td>
</tr>
</tbody>
</table>

**Key:** +++ = abundantly present; ++ = moderately present; + = present in trace amount - = no activity present

Results of the prophylactic model in table 2 showed that the crude extract and n-hexane fraction dose dependently protected against acetic acid, ethanol and indomethacin induced ulcer models. Although, their activities were not comparable to that of the reference group (Omeprazole, 20 mg/kg), except high dose (400 mg/kg) of n-hexane that elicited a better activity than Omeprazole in ethanol induced gastric lesion model.

**Table 2. Prophylactic effect of S. monostachyus extracts on ulcers induced by different ulcerogens.**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>Ulcer indices for the different ulcerogens</th>
<th>Acetic acid (1.5 ml of 5%, p.o)</th>
<th>Ethanol ulcer (1ml of 96%, p.o)</th>
<th>Indomethacin ulcer (30 mg/kg, p.o)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>10m/kg</td>
<td>200 mg/kg</td>
<td>400 mg/kg</td>
</tr>
<tr>
<td>Tween -80</td>
<td>10m/kg</td>
<td>13.83 ± 2.06 (0%)</td>
<td>7.66 ± 1.08 (0%)</td>
<td>9.50 ± 1.76 (0%)</td>
<td></td>
</tr>
<tr>
<td>Misoprostol</td>
<td>(20 μg/kg)</td>
<td>0.66 ± 0.42 (95%)*</td>
<td>0.00 ± 0.00 (100%)*</td>
<td>3.50 ± 1.56 (64%)*</td>
<td></td>
</tr>
<tr>
<td>Crude extract</td>
<td>200 mg/kg</td>
<td>1.08 ± 0.68 (92%)*</td>
<td>1.41 ± 0.66 (85%)*</td>
<td>4.50 ± 1.66 (54%)*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>400 mg/kg</td>
<td>0.83 ± 0.00 (94%)*</td>
<td>1.00 ± 0.00 (89%)*</td>
<td>4.30 ± 1.60 (56%)*</td>
<td></td>
</tr>
<tr>
<td>n-Hexane fraction</td>
<td>200 mg/kg</td>
<td>1.17 ± 0.16 (97%)*</td>
<td>0.76 ± 0.37 (90%)*</td>
<td>3.04 ± 1.58 (60%)*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>400 mg/kg</td>
<td>0.00 ± 0.00 (100%)*</td>
<td>0.00 ± 0.00 (100%)*</td>
<td>3.33 ± 1.54 (66%)*</td>
<td></td>
</tr>
</tbody>
</table>

Mean (±SEM), n=6. *p < 0.05: Significantly different from 5% tween- 80.

Results of curative model in table 3 below, revealed dose dependent protective effect of the crude extract and n-hexane fraction against
Inhibition of Acetylcholine and Histamine Receptors may be the Possible Mechanisms of Anti-ulcer Activity of Solenostemon monostachyus P. Beauv

Acetic acid, ethanol and indomethacin induced ulcer models. The n-hexane fraction (400 mg/kg) showed a better activity than the reference group (Misoprostol, 20 ug/kg) in acetic acid model and a comparable activity in ethanol induced gastric lesion.

Table 3. Curative effect of S. monostachyus extracts on ulcers induced by different ulcerogens.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose</th>
<th>Ulcer indices for the different ulcerogens</th>
<th>Acetic acid (1.5 ml of 5%, p. o)</th>
<th>Ethanol ulcer (1ml of 96%, p. o)</th>
<th>Indomethacin ulcer (40 mg/kg p.o)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tween -80</td>
<td>10mg/kg</td>
<td>10.01 ± 1.36 (0%)</td>
<td>5.58 ± 1.62 (0%)</td>
<td>5.41 ± 1.11 (0%)</td>
<td></td>
</tr>
<tr>
<td>Omeprazole</td>
<td>(20 mg/kg)</td>
<td>1.08 ± 0.49 (89%)*</td>
<td>0.5 ± 0.34 (91%)*</td>
<td>0.66 ± 0.33 (88%)*</td>
<td></td>
</tr>
<tr>
<td>Crude extract</td>
<td>200 mg/kg</td>
<td>1.41 ± 0.58 (86%)*</td>
<td>1.00 ± 0.63 (82%)*</td>
<td>1.66 ± 0.55 (69%)*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>400 mg/kg</td>
<td>1.16 ± 0.49 (88%)*</td>
<td>0.58 ± 0.37 (90%)*</td>
<td>1.08 ± 0.53 (80%)*</td>
<td></td>
</tr>
<tr>
<td>n-Hexane fraction</td>
<td>200 mg/kg</td>
<td>1.09 ± 0.18 (89%)*</td>
<td>1.00 ± 0.00 (89%)*</td>
<td>1.12 ± 0.12 (79%)*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>400 mg/kg</td>
<td>0.33 ± 0.16 (97%)*</td>
<td>0.00 ± 0.00 (100%)*</td>
<td>1.06 ± 0.83 (81%)*</td>
<td></td>
</tr>
</tbody>
</table>

Mean (±SEM), n=6, *p < 0.05: Significantly different from 5% tween-80.

From the preliminary dose response study of acetylcholine and histamine, Ach elicited 81.40% response at a sub-maximal dose of 64 ug while histamine elicited a response of 92.32% at a sub-maximal dose of 32 ug. These fixed sub-maximal doses were used to determine the effect of varying concentrations of the crude extract on the contractile responses to Ach and histamine.

From figure 1, S. monostachyus extract caused dose dependent inhibition on acetylcholine (Ach) induced muscle contraction of guinea pig ileum, with an IC50 value of 22.80 mg/ml.

From figure 2, Solenostemon monostachyus extract caused dose dependent inhibition on histamine induced muscle contraction of guinea pig ileum, with an IC50 value of 9.53 mg/ml.

**Dissection**

Peptic ulcer is a worldwide problem [18]. Attempt in searching for better alternative therapies from natural source in the management of peptic ulcer had motivated us to revalidate the anti-ulcer activity as well as also ascertain the possible mechanism behind the already established anti-ulcerogenic potential of Solenostemon monostachyus, a popular medicinal plants used in the management of peptic ulcer in Nigeria.

The estimated median lethal dose (LD50) above 5000 mg/kg for rats and 2449 mg/kg recorded for mice suggests that the extract may not likely be tolerable at higher doses. Previous studies by Kokon and co-workers reported a lower LD50 value of 766.35±37.56 mg/kg in mice using intraperitoneal route. Excitation, paw licking, increased respiratory rate, decreased motor activity, gasping and coma have also been reported from previous acute toxicity study on Solenostemon monostachyus [10]. Variation in LD50 value may be due differences in specie (rats and mice) response to the crude extract.

Various models of ulcer induction had been reported [17]. The use of ethanol as an ulcerogenic agent could be attributed to its ability to cause generation of free radicals, capillary necrosis, direct toxic effect, depletion of bicarbonate production and wearing off of the gastric mucosa [1,17]. Indomethacin, a non-steroidal anti-inflammatory drug (NSAID) has also been a popular ulcer inducing agents in experimental model. It had been reported to suppress prostaglandin synthesis, thereby causing gastroduodenal ulceration [17,19]. Acetic acid induced gastric ulceration model
Inhibition of Acetylcholine and Histamine Receptors may be the Possible Mechanisms of Anti-ulcer Activity of Solenostemon monostachyus P. Beauv

resembles human ulcers in terms of both pathological features and healing mechanisms. Acetic acid is known to generate free radicals, depletion in mucosal integrity and secretion [20].

In this present study, reduction in ulcer index elicited by crude extract and n-hexane fraction against ulcerogens (acetic acid, ethanol and indomethacin) in both prophylactics and curative models (tables 2 and 3) lend credence and supported recent study by Amazu et al [19] where aqueous and chloroform fractions of S. monostachyus (75-225 mg/kg) showed significant inhibition against ethanol, indomethacin and histamine induced ulcer models and whose activities were also comparable to that of the standard drugs (Cimetidine, 100 mg/kg).

Dose dependent inhibition of acetylcholine and histamine contraction by various concentrations (1, 2, 4, 8, 16 and 32 mg) of crude extract (figures 1 and 2) also suggests that the anti-ulcer activity posed by S. monostachyus could be related to its antihistaminic and antimuscarinic effects [21] mediated by inhibition of cAMP mediate protein kinase A, an enzyme which facilitate gastric acid secretion [22]. Also, interference of the extract with acetylcholine mediated gastric secretion in the gastric parietal cells [23] could also be a possible mechanism. Lesser IC$_{50}$ value of 9.53 mg/ml elicited by the extract against histamine induced ileum contraction, compared to an IC$_{50}$ value of 19.953 ug against acetylcholine induced ileum contraction, suggests that, the anti-secretory mechanism of the extract is more mediated via histamine receptors.

Phytochemicals play major roles in biological activities elicited by medicinal plants [13], flavonoids and tannins and other phytochemicals had been reported to work in synergy as anti-spasmodic against isolated tissues (guinea pig ileum, among others) [24-26].

The active phyto-constituents (including: alkaloids, saponins, tannins, flavonoids, steroids, terpenoids, cardiac glycosides, proteins) demonstrated in this present study (Table 1), as well as other phytochemicals including: coumarin, polyphenol, diterpenoids, lipids, calcium, phosphate and β-pinene, oct-1 -en-3-ol, β-caryophyllene, octan-3-ol and (E, E)-α-farnesene, previously reported on S. monostachyus, [11, 15] could be responsible to its anti-ulcer activity and anti-spasmodic activity.

CONCLUSION

The present study revealed that S. monostachyus leaves possess anti-ulcer and anti-spasmodic activities which may be due to inhibition of both muscarinic and histaminic receptors. This justifies its Nigerian folkloric claim in the treatment of peptic ulcer. Hence, isolation and structural elucidation of the active compound(s) in the leaf extract is recommended for future investigations.

REFERENCES

[12] Ekundayo EO, Ezegou LI. Evaluation of antimicrobial activities of extracts of five plants


