ANTI-ULCER ACTIVITY OF THE AQUEOUS EXTRACT OF ANCHOMANES DIFFORMIS

*1Okpo, S. O., 2Ayinde B. A., 1Ugwa, Z. I., 3Ching, F. P., 4Alonge, P. O. and 1Udi, O. O.

1Department of Pharmacology & Toxicology, Faculty of Pharmacy, University of Benin, P.M.B. 1154, Benin City 300001, Nigeria
2Department of Pharmacognosy, Faculty of Pharmacy, University of Benin, P.M.B. 1154, Benin City 300001, Nigeria
3Department of Pharmacology, Faculty of Basic Medical Sciences, Niger Delta University, Wilberforce Island, Nigeria
4Department of Pharmaceutical Chemistry, Faculty of Pharmacy, University of Benin, P.M.B. 1154, Benin City 300001, Nigeria

Author for correspondence: steveokpo@uniben.edu or stevolee@yahoo.com
Phone: +234 806 402 8832, +234 802 380 2411

ABSTRACT
Anchomanes difformis (Blume) Engler (Araceae) is a tuber used ethno-medically in Nigeria, especially in the South West, for the treatment of dysentery, cough, constipation, trypanosomiasis and peptic ulcer disease. The effect of the aqueous-methanolic extract of Anchomanes difformis on ulcer was evaluated using pylorus ligation-, ethanol- and indomethacin-induced ulcer models in rats. The extract (250-1000mg/kg) given one hour before ethanol (preventive) produced a dose-dependent and significant (p<0.05) protection from ulceration. This was similar to the effect of the highest dose of the extract (1000 mg/kg) administered 15 mins (curative) after ethanol. Administration of the extract (125-500mg/kg) orally one hour before induction of ulcer with indomethacin followed the same trend as in ethanol-induced ulcer. However, it showed a greater protection curatively than preventively but this was not significant. A high dose of the extract (500mg/kg) caused a dose-dependent and significant (p<0.001) reduction in total acid output and severity of ulceration in the pylorus ligation model. The aqueous extract caused little or no variation in pH which shows that it may not be acting through the neutralization of gastric acid. The results obtained suggest that the anti-ulcer effects of the extract may be mediated via the production of prostaglandins and free radical scavengers which protect the gastric mucosa.

Keywords: Anchomanes difformis, Antiulcer, indomethacin, ethanol, ranitidine

INTRODUCTION
Peptic ulcer disease is a chronic inflammatory condition involving a group of disorders characterized by ulceration in regions of the upper gastrointestinal tract where parietal cells secrete pepsin and hydrochloric acid (Henderson and Lander, 1996). It results from an imbalance between aggressive factors and the maintenance of the mucosal integrity through the endogenous defense mechanism (Piper and Stiel, 1986; Hoogerwerf and Pasricha, 2001). To regain and retain the balance,
different therapeutic agents including synthetic drugs, and in some cases medicinal plants, are used to inhibit gastric acid secretion or to boost the mucosal defense mechanism by increasing mucus production. *Anchomanes difformis* (Blume) Engler (Araceae) is a self-supporting rhizomatous herb with a long stem that can reach two meters in height. The stem is green in colour with a diffuse white colour at the base close to the soil level (Dalziel, 1937). The plant grows in tropical African forests in moist and shady places.

The tuber called “Isu igo”, in the South West of Nigeria, is used to prepare a decoction used in cough treatment and in ulcer. The peeled tuber soaked in water is used in treating cases of dysentery while the tuber powder given with honey is used as diuretic and purgative (Gills, 1992). Except for a few studies on the anti-trypanosomal (Atawodi *et al.*, 1992) and anti-microbial effects (Chukwura and Ajali, 2000; Oyetayo, 2007), no literature exists on the scientific evaluation of the anti-ulcer properties of this plant.

The present study is, therefore aimed at evaluating the possible anti-ulcer activity of *Anchomanes difformis* in peptic ulcer disease, using different experimental models.

**MATERIALS AND METHODS**

**Plant collection and identification**

Fresh rhizomes of *Anchomanes difformis* were collected in the month of October from Ifon in Owan Local Government Area Edo State. The plant material was identified and authenticated by Mr. S. Olufemi of the Forestry Research Institute of Nigeria, Ibadan, Oyo State, Nigeria where a voucher specimen (No. FHI 109585) has been deposited for future reference.

**Plant preparation and extraction**

The rhizomes were washed, peeled and sliced thinly to aid drying. The sliced rhizomes were oven-dried for one week, at 60°C (Sofowora, 1984) after which they were blended into powdery form. Extraction was performed by macerating 500 g of the sample in 2.5 litres of water/methanol mixture (3:1) for 48 hours. The resultant mixture was filtered and concentrated into a semi-solid form using a rotary evaporator. Stock solution of the extract (500mg/ml) was prepared from which other concentrations were made as required.

**Phytochemical Screening**

Phytochemical screening of the plant material for the presence or otherwise of alkaloids, saponins, tannins, flavonoids, anthracene derivatives, cardiac and cyanogenic glycosides were carried out using standard procedures (Sofowora, 1993; Trease and Evans, 1978).

**Animals**

Sprague-Dawley rats (150±20g) of both sexes were obtained from the Laboratory Animal Centers of Ambrose Allii University, Ekpoma and Department of Animal and Environmental Biology, University of Benin, Benin City, Edo State, Nigeria. The animals maintained on standard diet (Bendel Feeds Ltd. Ewu-Edo State, Nigeria) and water *ad libitum*, were acclimatized for two weeks and fasted overnight, with free access to water prior to experiments.

Approval for the use of animals in the experiments was obtained from the Ethical Committee on the Use of Laboratory Animals, Faculty of Pharmacy, University of Benin, Benin City Nigeria. Animals were handled according to the protocol outlined in “*Principles of Laboratory Animal Care*” (National Institute of Health Guide for Care and Use of Laboratory Animals, Pub No. 85 – 23, revised 1985).
Ethanol-induced ulcers
The rats were randomly divided into 5 groups of 5 animals each and starved for 24 hours but had free access to water. Water was however withdrawn 2 hours before experiments.

Group 1 served as the control and received normal saline (5 ml/kg), orally. Group 2 received ranitidine (100 mg/kg) orally, while groups 3, 4 and 5 received 250 mg/kg, 500 mg/kg and 1000 mg/kg of the extract, respectively, by oral intubation (Robert, 1979).

One hour later, 1ml of absolute ethanol was administered by intragastric intubation to all the groups. One hour following ethanol administration, the animals were sacrificed by overdose of anaesthetic ether. The stomach was isolated, opened along the greater curvature and washed with tap water.

The effect of the extract (1000 mg/kg) administered orally 15mins after induction of ulcer with ethanol (curative effect) was investigated according to the procedure already described above.

The stomachs of the animals in all the groups were examined macroscopically and the ulcer lesions counted using a magnifying glass. The diameter of the ulcers was measured using a vernier caliper and scored (Martin, 1988).

Indomethacin-induced ulcers
Rats were randomly allotted to 5 groups of 5 animals each and fasted for 24 hours with access to drinking water ad libitum. Water was however withdrawn 2 hours prior to the experiment.

Group 1 served as the control and received distilled water (5 ml/kg) orally. Group 2 received ranitidine (100 mg/kg) while groups 3, 4 and 5 were administered by oral intubation the extract at doses of 125 mg/kg, 250 mg/kg and 500 mg/kg, respectively (Robert 1979; Franzone et al, 1988). Indomethacin (20 mg/kg) was administered, by oral intubation, to all the groups one hour after the administration of extract or indomethacin.

Six hours later, each rat was sacrificed by ether anaesthesia and the stomach removed. Formalin saline (2% v/v) was injected into the totally ligated stomach for overnight storage, at room temperature. The next day, the stomach was opened along the greater curvature and washed in warm water.

In another set of experiments (curative study), the effect of the extract (500 mg/kg) administered orally 2 hours after induction of ulcer with indomethacin (20 mg/kg) was investigated as already described above.

Macroscopic examination of the stomachs of the animals in all the groups was done. The presence of ulcers was noted and scoring of the ulceration was done according to the method of Martin (1988).

Pylorus ligation-induced ulcers
A modified method of Shay et al. (1954) was adopted. Rats were divided into 3 groups of 7 animals each and fasted for 30 hours with access to drinking water ad libitum. Group 1 received distilled water (5 ml/kg), group 2 received the aqueous extract (500mg/kg), while the last group was administered ranitidine (100mg/kg) one hour prior to pyloric ligation.

The abdomen was opened by a midline incision, under light ether anaesthesia, and pyloric ligation was done without causing any damage to its blood supply. The stomach was replaced carefully and the abdomen was closed with interrupted sutures. Eighteen hours later, the animals were sacrificed with overdose of anaesthetic ether. The stomach was isolated and the gastric juice collected into a centrifuge tube and spun at 5000 rpm for 20 minutes. The volume of the supernatant was recorded and
The total acid output was determined using 0.1N NaOH for titration and phenolphthalein as indicator.

The stomach was then opened along the greater curvature and washed. Ulcer lesions were counted using a magnifying glass while the diameter of the ulcers was measured using a vernier caliper and scored on a scale of 0-10 (Martin, 1988).

Neutralizing effect of Anchomanes difformis extract
The ability of the extract (100 mg/kg) to resist change in pH on addition of known volumes of 0.1N HCl or 0.1N NaOH was evaluated accordingly (Shay et al, 1945; Trucheau et al, 1975).

Statistical analysis
Statistical analysis was done using student’s t-test for unpaired data and significance of difference was accepted at p<0.05. Data are presented as mean ± standard error of mean (SEM).

RESULTS

Ethanol-induced ulceration
Intense and widespread thickened gastric lesions of the mucosa were evident in control rats that received 1ml of absolute ethanol. Pre-treatment with Anchomanes difformis extract caused a dose related reduction in both the number and severity of the gastric lesions. However, only the highest doses of the extract (1000mg/kg, preventive and curative) showed significant (p< 0.01) reductions in the ulcer index, but these effects were significantly (p< 0.01) lower than the effect of ranitidine (Table 1).

Table 1: Effect of A. difformis on ethanol-induced ulceration

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg)</th>
<th>Ulcer index (UI)</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>19.00 ± 1.10</td>
<td>-</td>
</tr>
<tr>
<td>A. difformis</td>
<td>250</td>
<td>18.80 ± 1.07</td>
<td>1.05</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>16.00 ± 1.10</td>
<td>15.80</td>
</tr>
<tr>
<td></td>
<td>1000</td>
<td>12.20 ± 1.43&lt;sub&gt;ab&lt;/sub&gt;</td>
<td>35.80</td>
</tr>
<tr>
<td></td>
<td>1000 (15mins after ethanol)</td>
<td>12.60 ± 1.44&lt;sub&gt;ab&lt;/sub&gt;</td>
<td>33.70</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>100</td>
<td>5.00 ± 0.63&lt;sup&gt;a&lt;/sup&gt;</td>
<td>73.70</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. <sup>a</sup>p<0.01, significantly different from control; <sup>b</sup>p<0.01 significantly different from ranitidine; Student’s t-test (n= 5-6 animals)

Indomethacin-induced ulceration

Table 2 shows the effect of the extract on indomethacin-induced ulceration. Pre-treatment with the extract produced a dose-dependent reduction in both the number and severity of ulcers. All the dose levels of the extract caused significant (p<0.01) reduction in the ulcer index. The extract (500mg/kg) administered two hours after indomethacin produced a significant (p<0.01) reduction in the ulcer index, reduction similar to the effects of ranitidine (Table 2).
Table 2: Effect of *A. difformis* on indomethacin-induced ulceration

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Ulcer index (UI)</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>34.30 ± 5.06</td>
<td>-</td>
</tr>
<tr>
<td><em>A. difformis</em></td>
<td>125</td>
<td>23.40 ± 2.77&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>31.8</td>
</tr>
<tr>
<td></td>
<td>250</td>
<td>20.00 ± 2.66&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>41.7</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>17.20 ± 2.48&lt;sup&gt;a&lt;/sup&gt;</td>
<td>49.9</td>
</tr>
<tr>
<td></td>
<td>500</td>
<td>12.20 ± 3.01&lt;sup&gt;a&lt;/sup&gt;</td>
<td>64.4</td>
</tr>
<tr>
<td></td>
<td>(2hr after indomethacin)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ranitidine</td>
<td>100</td>
<td>12.40 ± 3.56&lt;sup&gt;a&lt;/sup&gt;</td>
<td>63.8</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. <sup>a</sup>p<0.01, significantly different from control; <sup>b</sup>p<0.05, significantly different from ranitidine; Student’s t-test (n= 5-6 animals).

**Pylorus ligation-induced ulceration**

Pylorus ligation caused the accumulation of gastric secretions and hence, intense lesions in the ruminal/antral portion of the stomach in control rats. Pretreatment with the extract (500mg/kg) caused no alterations in the volume of gastric secretions but significantly affected the total acid output and reduced the number and intensity of the lesions when compared to the control. Ranitidine produced the same effect as the extract in a reduction of ulcer index (Table 3).

Table 3: Effect of *A. difformis* on pylorus ligation in rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>Vol. of acid (ml/100g rat)</th>
<th>Total acid output (μEq/L)</th>
<th>Ulcer index</th>
<th>Ulcer inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>6.57 ± 1.07</td>
<td>956.22 ± 48.20</td>
<td>14.17 ± 0.40</td>
<td>-</td>
</tr>
<tr>
<td><em>A. difformis</em></td>
<td>500</td>
<td>5.42 ± 1.65</td>
<td>409.13 ± 20.98&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.80 ± 0.99&lt;sup&gt;a&lt;/sup&gt;</td>
<td>59.07</td>
</tr>
<tr>
<td>Ranitidine</td>
<td>100</td>
<td>6.13 ± 1.67</td>
<td>663.17±36.19&lt;sup&gt;a&lt;/sup&gt;</td>
<td>5.12 ± 0.54&lt;sup&gt;a&lt;/sup&gt;</td>
<td>63.87</td>
</tr>
</tbody>
</table>

Values are mean±SEM of experiments from at least 5 animals, <sup>a</sup>p<0.001 vs. control (Student’s t-test)

**Neutralizing effect**

The extract did not exhibit any buffering effect since the addition of 5ml of either 0.1N HCl or 0.1N NaOH produced a pH change in 1ml of the extract (equivalent to 100mg of the dried extract) (Table 3).
Table 4: Buffer capacity of *Anchomanes difformis*

<table>
<thead>
<tr>
<th>Protocol</th>
<th>pH after 15 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 ml extract alone</td>
<td>5.61 ± 0.01</td>
</tr>
<tr>
<td>1 ml distilled water + 5 ml 0.1N HCl</td>
<td>3.72 ± 0.02</td>
</tr>
<tr>
<td>1 ml extract + 5 ml 0.1N HCl</td>
<td>4.02 ± 0.02</td>
</tr>
<tr>
<td>1 ml distilled water + 5 ml 0.1N NaOH</td>
<td>8.26 ± 0.02</td>
</tr>
<tr>
<td>1 ml extract + 5 ml 0.1N NaOH</td>
<td>8.07 ± 0.03</td>
</tr>
</tbody>
</table>

Values are mean ± SEM (n = 5).

DISCUSSION

The anti-ulcer activity of the aqueous extract of *Anchomanes difformis* was evaluated against gastric lesions induced by ethanol and indomethacin, two potent ulcerogens. The results obtained show a dose dependent gastro-protective effect in both models of ulcer.

In ethanol-induced ulceration, the highest dose of 1000mg/kg exerted a significant (p<0.01) protection when administered before (preventive) or after (curative) the ulcerogen. However, this effect was significantly (p<0.05) lower than that of ranitidine. The mechanism by which the extract produces its antiulcer effect is not clear. Stimulation of oxygen-derived free radicals (Oates and Hakkinem, 1988) stimulation of histamine and serotonin release from mast cells (Alarcon de la Lastra and Martin, 1997) and hyperoxidation of lipids (Reshma *et al.*, 2000) have been implicated in ethanol-induced ulceration. It is probable that the cytoprotective effect of the extract resides in its ability to produce protective antioxidants that scavenge reactive oxygen species as well as the ability to inhibit mast cell secretory products.

The extract showed a better and significant (p<0.01) protection in the indomethacin model at all the tested doses compared to that of the ethanol-induced model. The extract (500mg/kg) administered after the ulcerogen produced a significant reduction in ulcer index which is similar to the effect caused by ranitidine (100mg/kg).

Since prostaglandins are known to protect gastric mucosal cells against injury caused by indomethacin (Robert, 1975; Whittle, 1977), it is also possible that the extract stimulates the production of endogenous prostaglandins, which provide the protection.

The better protective and curative effect observed in indomethacin-induced as against ethanol-induced ulceration suggests that the extract may act mainly by stimulating the synthesis of endogenous prostaglandins.

Reduction in ulcer index (in both models) following administration of the extract before and after the ulcerogen demonstrates the ability of the extract to protect the gastric mucosa against ulcer as well as the ability to suppress already established ulcer respectively.

Based on the effect of the extract on the other two models of ulcer, it was further evaluated on pylorus ligated rats - a widely used model for studying the effect of drugs on gastric secretion. Ligation of the pyloric end of the stomach causes accumulation of
acidic gastric juice in the stomach, leading to the development of gastric ulcers (Khare et al., 2008). Since high gastric acidity is an important factor in peptic ulcer, the ability of the extract to effectively attenuate gastric acid secretion in the Shay rat suggests that it can also protect ulceration by causing a decrease and/or inhibiting the aggressive factors (e.g. acid). Reactive oxygen species have also been implicated in the pathogenesis of pyloric ligation-induced gastric mucosal injury (Rastogi et al., 1998).

The lack of buffering activity, on either acid or alkali, by the extract suggests that it may not be acting through the neutralization of stomach acidity like the antacids. It also suggests that the extract has no dilution effect on the ulcerogen in the gastrointestinal tract and, thus, does not act as a physical protectant. This, probably, explains the pronounced curative effect of the extract which was even more obvious with the indomethacin-induced ulcers.

Phytochemical screening of the tuber of *Anchomanes difformis* revealed the presence of alkaloids, saponins and cardiac glycosides. Adegoke et al. (1968) had reported the presence of strong alkaloids and saponins in *Anchomanes difformis*, while Oyetayo (2007) found tannins in addition to alkaloids and saponins. Saponins (Marhuenda et al., 1993), flavonoids, glycosides and terpenoids (La Casa et al., 2000) have been reported to possess anti-ulcerogenic activity. Hence, alkaloids and saponins could be the phytochemicals responsible for the anti-ulcer effect of the extract. However, further studies, preferably with the active principles of the extract, will be necessary to confirm this.

On the basis of the present study, it is concluded that the aqueous extract of *Anchomanes difformis* possesses gastroprotective effects which could probably be mediated via the production of gastrointestinal prostaglandins and, possibly, free radical scavengers which protect the gastric mucosa. More detailed studies are needed to elucidate the exact mechanism by which it produces its effects. The study also revealed that the extract contains alkaloids, cardiac glycosides and saponins. Exhaustive fractionation of the extract will, therefore, be necessary to isolate the active principles responsible for the observed activity.

REFERENCES


