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<td>AGUWA, C. N.</td>
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PHARMACOLOGIC EFFECTS OF AN AQUEOUS EXTRACT OF RHIGOCARYA RACEMIFERA

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(Accepted October 23, 1985)

Summary
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C. NIZE AGUWA

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Summary

Several pharmacologic studies were carried out with an aqueous extract of Rhigiocarya racemifera after phytochemical tests revealed the main constituent to be glycosides, saponins and tannins. Studies on intact mice showed that the extract has an intraperitoneal LD₅₀ of 142 mg/kg and that 50 mg/kg of the extract reduced gastrointestinal motility comparable to 40 mg/kg of atropine. In rats, 20 mg/kg of extract showed significant anti-ulcer activity against indomethacin-induced ulcer and this effect was equivalent to 100 mg/kg of cimetidine. Studies on isolated tissue revealed that it may have musculotrophic antispasmodic effects. These preliminary investigations seem to support its use by herbalists to treat various gastrointestinal disorders.

Introduction

Rhigiocarya racemifera (Menispermaceae) is a woody climber which grows wild in the tropical part of Southern Nigeria especially around the towns of Mbaise and Ochia in Imo State where it is called Igba (Igbo). Its botanical characteristics have been described by Hutchinson (1939) and Treae and Evans (1978). Morphologically, the features are in agreement with the descriptions of Hutchinson and Daniel (1966). The leaves of this plant are used locally by herbalists for the treatment of acute gastrointestinal pain, bloody diarrhoea, painful menstruation and/or spasmodic dysmenorrhea.

Preliminary work in our laboratory has shown that it has no antibacterial activity, but may have significant anti-ulcer effects. The purpose of this work, therefore, was to investigate this latter aspect and perhaps confirm the pharmacologic basis for its successful application in the treatment of various gastrointestinal disorders by traditional medical practitioners in Nigeria.

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Materials and methods

Identification

The plant was collected from Myrtle town and was identified by the staff of the Department of Pharmacognosy. A specimen has been preserved in the herbarium of the Department for future reference.

Preparation of extract

The leaves were collected in the month of October with the help of a herbarist and dried under shade. They were ground to a coarse powder, macerated over 24 h and a cold water extract prepared in such a manner that 1 ml of the water extract represented 0.5 g of dried leaves. The extract was stored in a refrigerator to prevent deterioration and dilutions prepared fresh each day for the experiments. All doses in this study are expressed in terms of the dried material.

Animals

Inbred albino mice of both sexes were used in the whole animal experiments. In the isolated tissue experiments, rat uterus, guinea pig ileum and rabbit jejunum were used where applicable.

Drugs and chemicals

Sources are as follows: di-propranolol HCl, dihydroergotamine and pento-barbital sodium (Sigma); seerotonin creatine sulfate, acetylsalicylic HCl, carbamazepine and nicotinic HCl (British Drug House); L-adrenaline bitartarate (Koch-Light Lab.); L-phenylamine, histamine dihydrochloride and activated charcoal (Merc); stilbestrol dipropionate and tragacanth (May and Baker); carbamazepine (Ciba-Geigy); heparin (Evans Medical Ltd.); cimetidine (Smith, Kline and French); atropine (Burroughs and Wellcome); oxytocin (G. Richter).

Phytochemical studies

The freshly prepared aqueous extract was chemically tested for the presence of alkaloids, glycosides, saponins tannins and reducing or oxidizing agents.

(a) Test for alkaloids (Sim 1968)

Using belladonna leaf powder as a positive reference sample, 5 ml of the extract was added dropwise to 1 ml each of Mayer's reagent (potassium mercuric iodide solution) and Wagner's reagent (iodine in potassium iodide solution) precipitate noted.

(b) Test for glycosides (Sim, 1968)

Five milliliters of the extract was boiled with 5 ml of dilute sulfuric acid
in a water bath for 15 min, cooled and neutralised with 20% potassium hydroxide. Five milliliters of this neutralised solution was taken and boiled with 1 ml of FeHling's solution. A control containing the extract and FeHling's solution was also run.

(c) Test for saponins (Sim, 1968)
Five milliliters of the extract was diluted with water and shaken vigorously.

(d) Test for tannins (Trease and Evans, 1969)
To 5 ml of the extract a few drops of ferric chloride were added and c-barreraed.

(e) Test for oxidizing agents (Clarke, 1975)
Two procedures were used: the diphenylamine test where to 2 drops of the extract was added 1 drop of 0.1% diphenylamine in sulphuric acid, and the benzidine test where a few drops of 2% benzidine in 10% acetic acid were added to 1 ml of the extract made slightly acidic with dilute sulphuric acid.

Pharmacological experiments

(a) acute toxicity test
Thirty albino mice of either sex weighing (24–28 g) were divided into five groups of six mice each. They were fasted overnight and different doses of the extract were administered i.p. to each group. The maximum volume administered per animal was 0.5 ml. The number of deaths were recorded for each group after 24 h. The doses administered were selected after preliminary experiments so that the lowest caused no death while the highest resulted in 100% death. The percentage dead were converted into probits and the LD50 calculated by the method of Miller and Tainter (1944). Autopsy of the dead mice was performed to determine the cause of death and possible damage to any organ.

(b) Gastrointestinal motility
Forty albino mice of either sex (24–28 g) were randomly divided into four equal groups. All were starved for 24 h but allowed free access to water. The mice in group A received i.p. 50 mg/kg of aqueous extract; group B, 1 mg/kg of carbahex; group C, 10 mg/kg of atropine while control group D received 50 ml/kg of normal saline. Ten minutes after the injection, 0.5 ml of 5% v/v charcoal in tragacanth mucilage was orally administered. Twenty minutes after the charcoal meal, the mice were killed with an overdose of pentobarbital and the abdomen opened. The intestines were carefully brought out and the length of movement of the charcoal meal towards the caecum from the stomach was measured and expressed as percentage of the total length of the small intestine.
(c) Anti-ulcer activity

Thirty intraduodenal saline doses of either sex (150–200 g) were divided into groups of 10 each. Group A rats served as control and were administered i.p. with 5 ml/kg of normal saline. Group B received the extracts 25 mg/kg i.p. in the same volume and group C was given cimetidine 100 mg/kg i.p., a 2% suspension in normal saline.

Ulcers were induced by the method described by Urushidani et al. (1979). The rats were fasted for 24 h with free access to water allowed. Thirty minutes after treatment with either saline, extract or cimetidine as described above, indomethacin was injected s.c. 20 mg/kg as a suspension in 1% carboxymethylcellulose with a trace of Tween-80. Seven hours after indomethacin administration, the animals were killed by a blow on the head and examined for ulcers by the method of Main and Whittle (1975).

(d) Isolated tissue experiments

Guinea-pig ileum: Several guinea pigs of either sex (300–320 g) were used. The experiments were set up by the method described by staff of the Department of Pharmacology, University of Edinburgh (1976) using a 50-ml organ bath containing Tyrode's solution with the following composition: calcium chloride 0.2 g, glucose 1.0 g, magnesium chloride 0.1 g, potassium chloride 0.2 g, sodium bicarbonate 1.0 g, sodium chloride 8.0 g, sodium hydrogen phosphate 0.05 g and deionized water to make a 1:4 solution. The bath was kept at constant temperature of 32 °C and well aerated with air. Contact time for each drug was 30 s and a 3-min cycle was used. The contractions were recorded on a slow moving smoked drum by means of a frontal writing lever (5X magnification).

Rabbit jejunum: Six rabbits of either sex (1.5–2 kg) were used. The preparation was set up in the same manner as for guinea-pig ileum.

Non-gravid rat uterus Five non-pregnant female rats (150–350 g) were used. They were treated with 0.1 mg/kg of stilbestrol s.c. 24 h before the uterus was used for experiment. The set-up consisted of constant temperature and continuous aeration in De Jaeger's solution with the following composition: sodium chloride 9 g, potassium chloride 0.4 g, calcium chloride 0.06 g, sodium bicarbonate 0.5 g, glucose 0.5 g and deionized water to make a 1:4 solution. The methodology was as described by Turner (1965) and Anika and Shetty (1982).

Results

Phytochemical studies

The chemical tests carried out revealed the presence of glycosides, saponins and tannins. The test for reducing sugars (Fehling's test) was positive while the tests for alkaloids were negative (Table 1).
<table>
<thead>
<tr>
<th>Constituent</th>
<th>Test</th>
<th>Observation</th>
<th>Inference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alkaloids</td>
<td>Mayer's reagent</td>
<td>No color change</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Wagner's reagent</td>
<td>No color change</td>
<td>—</td>
</tr>
<tr>
<td>Glycosides</td>
<td>Hydrolysis by mineral</td>
<td>Heavy reddish-brown</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>acids and a yield of</td>
<td>precipitate</td>
<td></td>
</tr>
<tr>
<td></td>
<td>reducing sugar</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Saponins</td>
<td>Shaking of dilute aqueous</td>
<td>Frothing</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>extract</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tannins</td>
<td>Ferric chloride test</td>
<td>Bluish-black precipitate</td>
<td>+</td>
</tr>
<tr>
<td>Oxidizing agents</td>
<td>Diphenyleneamine test</td>
<td>No color change</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Benzidine test</td>
<td>No color change</td>
<td>—</td>
</tr>
</tbody>
</table>

**Acute toxicity test**

The LD<sub>50</sub> was calculated to be 141.5 mg/kg. Autopsy revealed that most of the mice died of massive cerebral haemorrhage. No gross abnormalities of liver or other organs were apparent.

**Gastrointestinal motility**

As can be seen from Table 2, the aqueous extract produced a very highly significant decrease in the gastrointestinal motility of mice.

The extract appeared to be as inhibitory as atropine when the distances travelled by the charcoal meal were compared with the control and carbachol responses.

**Antulcer activity**

Indomethacin induced ulcers in 90% of the control animals and the mean ulcer index was 2.12. Only 40% and 50% of the animals developed ulcers after the administration of extract and cinetidine, respectively, and the ulcer indexes were very highly significantly reduced (P < 0.1).

**Isolated guinea pig ileum**

Both the aqueous and alcoholic extract greatly reduced the contractions induced by acetylcholine. The addition of 28 mg of aqueous extract to the 50-ml bath reduced the contraction induced by 2 µg of acetylcholine from 9 cm to 1 cm. At a dose of 5.6 mg, the aqueous extract abolished histamine-induced contractions (10 µg) and those of nicotine (200 µg). Because of the lack of specificity, the effects may be due to direct muscular relaxation instead of specific receptor blockade.
TABLE 2

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment (dose)</th>
<th>% of total length travelled by charcoal meal</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Extract (50 mg/kg)</td>
<td>1.25 ± 0.35*</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>B</td>
<td>Carbocchio (1 mg/kg)</td>
<td>62.0 ± 0.5</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>C</td>
<td>Atropine (10 mg/kg)</td>
<td>0.90 ± 0.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>D</td>
<td>Normal saline (50 mg/kg)</td>
<td>36.0 ± 7.5</td>
<td>—</td>
</tr>
</tbody>
</table>

* Mean of 10 determinations ± 1 S.E.M.

Isolated rabbit jejunum

The extract did not appear to have adrenergic actions as its effect could not be blocked by propranolol or dihydroergotamine. The extract produced a rather marked muscular relaxation.

Isolated non-gravid rat uterus

The extract (22.4 mg) reduced the sensitivity of the uterus to the contractile effects of oxytocin (0.04 I.U.) and significantly reduced the contractile effect of serotonin (2 [micro]g).

Discussion

The aqueous extract of the leaves of Rhigicarya racemifera appears to have a definite anti-ulcer effect since premedication with the extract (25 mg/kg i.p.) in rats reduced indomethacin-induced gastric ulcers with an effect comparable to that of 100 mg/kg of cimetidine. At a dose of 50 mg/kg i.p.,

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Quantal ulcer</th>
<th>Mean ulcer index ± S.E.</th>
<th>Degree of Significance (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Saline</td>
<td>5 ml/kg</td>
<td>9/10</td>
<td>2.12 ± 0.20</td>
<td>—</td>
</tr>
<tr>
<td>Extract</td>
<td>28</td>
<td>4/10</td>
<td>0.12 ± 0.04</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>100</td>
<td>5/10</td>
<td>0.20 ± 0.20</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
it decreased gastrointestinal motility in the rat. This could be due to anti-
cholinergic and/or direct muscle relaxant effects, however, the in vitro
evidence seems to support a direct smooth muscle antispasmodic effect.

These pharmacological effects may explain its effective use in traditional
medicine in the treatment of various gastrointestinal and gynecological
diseases. Saponins, tannins and glycosides are present and their specific roles
have not been studied. Saponins may be responsible for the smooth muscle
relaxant effect since saponins are known to have anti-ulcer effects (Tresse
and Evans, 1978).

This preliminary pharmacological study, raises several questions. There is
a need to find out the specific saponin, glycoside or tannin responsible for the
observed pharmacological activities and its mechanism of action. Detailed
studies including chronic toxicity are in progress in our laboratory.

Acknowledgement

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