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PHARMACOLOGIC STUDIES ON THE ACTIVE PRINCIPLES OF CALLIANDRA PORTORICENSIS LEAF EXTRACTS

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(Accepted September 18, 1987)
PHARMACOLOGIC STUDIES ON THE ACTIVE PRINCIPLES OF CALLANDRA PORTORICENSA LEAF EXTRACTS

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Summary

The constituents of ethanolic and aqueous extracts of Callandra portoricenса leaves were identified to be saponins, tannins, flavonoids and glycosides. The intraperitoneal LD₅₀ of the ethanolic and aqueous leaf extracts in mice were 120.2 and 78.4 mg/kg, respectively. Both extracts inhibited the ulcerogenic effects of pyruvic ligation and stress (cold restraint) in rats at a dose of 50 mg/kg i.p. The anti-ulcer effects of the aqueous extract were always more significant than that of the ethanolic extract. This indicates that the higher content of the saponins and/or tannins of the leaf extract may be responsible for the anti-ulcer effects. The leaf extracts also had antimicrobial effects against Escherichia coli, Staphylococcus aureus and Staphylococcus faecalis. Preliminary screening using isolated smooth muscle indicated some anticholinergic potential.

Introduction

Aqueous and alcoholic leaf extracts of Callandra portoricenса Benth (Mimosaceae) (Tunde: Yoruba; Igbo: Igbo) have long been the drug of choice for gastrointestinal disorders among herbalists in Southern Nigeria.

The morphological characteristics of the plant have been described by Porter (1969). The herbalists who helped in procuring the plant sample for the present study claimed that the aqueous-alcoholic plant extract possesses anticonvulsant, antispasmodic and antidiarrheal properties. The plant was also alleged to have been used successfully in the treatment of patients with gastrointestinal disorders associated with melena. The aqueous-methanolic root extract of C. portoricenса has been reported to possess muscletecl (Adewumi and Marquis, 1990; Koos and McCullough, 1990), antimicrobial, analgesic and moderate anticonvulsant effects (Adesina and Akinnusi, 1984).

In the present study, parameters such as ulcer index and the volume and total solid content of gastric secretion in experimental ulcer models were
used to evaluate the ulcer-protective and antisecretory activity of leaf extracts of C. portoricensis in an attempt to justify the ethnomedicinal use of the plant extract. The study also attempted to identify the chemical mechanism(s) by which the plant extracts evoke their anti-ulcer activity.

**Materials and methods**

**Identification of plant sample**

The leaves of *C. portoricensis* were collected in the month of November 1985 and subsequently identified by the staff of the Pharmacognosy Department, University of Nigeria. The plant sample was dried under shade.

**Preparation of extracts**

About 250 g of the dry powdered leaves were extracted successively in a soxhlet apparatus with petroleum ether (60–80°C), chloroform and ethanol (Trease and Evans, 1985). The aqueous extract was obtained by macerating the dry marc obtained after the ethanol extraction with distilled water for 24 h. The ethanol extract was concentrated in a rotary evaporator while the aqueous extract was filtered and used as such. The solid content of the extracts were determined and yields for the ethanol and aqueous extracts were 0.004% and 0.002% percent of the starting material, respectively. Both extracts were kept in the refrigerator for future use throughout the study.

**Drugs and chemicals**

All the substances used were of analytical grade and included: carboxymethylcellulose (CMC) (Sigma), chloroform (Vickera), 95% ethanol (Vickera), anaesthetic ether (Vickera), indomethacin (Duexia), petroleum ether, 60–80°C (Ktedol-de-Haan), glutone (Merck), sodium chloride (Fisher), serotonin (Sigma), histamine (Merck), stropeine (Abbott) and nicotine (Sigma).

**Phytochemical tests**

Standard phytochemical procedures were used in confirming the presence of saponins, tannins, flavonoids and glycosides in the leaf extracts (British Pharmacopoeia, 1996; Trease and Evans, 1985).

**Acute toxicity tests**

Preliminary toxicity testing allowed for the selection of a suitable dose of the extracts that could be used in the anti-ulcer screening tests. Forty-five inbred albino mice (20–25 g) were divided into five groups of nine animals
each. Each group of mice was given increasing doses (10, 20, 40, 80) and 160 mg/kg (p.s.) of the plant extracts. The number of mice dying in each group within 24 h was noted. Computation of the LD₅₀ was done by the use of the graphical methods of Miller and Tainter (1944).

Induction of experimental ulcers

The methodology for indomethacin-induced ulcers was essentially that of Okabe et al. (1974a, b). Main and Whittle (1975) and Agwu (1984). Twenty-four albino rats (150–200 g) were fasted for 24 h but allowed free access to water. The water was withdrawn 1 h before the experiment. The animals were divided into three equal groups and coded to avoid bias. The groups were given the vehicle 5 ml/kg of 1% CMC, 50 mg/kg of ethanolic extract and 50 mg/kg of aqueous extract, i.p., respectively. One hour later, indomethacin 20 mg/kg i.p. was administered to the rats. Seven hours after indomethacin administration, the animals were killed and exsanguinated. The stomachs were resected, opened along the greater curvature, rinsed under a stream of water and examined for ulcers. The ulcers were counted with the aid of a hand lens (x10 magnification) and each was given a severity rating as follows: <1 mm = 1; 1–2 mm = 2; and >2 mm = 3. The summation of the scores was divided by a factor of 10 to derive the ulcer index for each animal.

The methodology for pyloric ligation-induced ulcers followed the procedures of Shy et al. (1945). Twenty-four albino rats (150–200 g) of both sexes were fasted for 48 h but allowed free access to water containing 8% dextrose and 0.09% sodium chloride. The water was withdrawn 1 h before pyloric ligation. The animals were divided into three groups of eight rats each and coded to avoid bias. The rats were anesthetized lightly with ether and a midline incision (2–3 cm) made through the abdominal wall. Each stomach was retrieved, the pylorus ligated and the abdomen sutured. Immediately, the vehicle 5 ml/kg of 1% CMC, 50 mg/kg ethanolic extract and 50 mg/kg aqueous extract were administered i.p. to the three groups of rats, respectively. After 18 h, the rats were killed and the stomachs retrieved. The volume, pH and total acidity of the gastric content were assayed and the ulcer indices computed as described above.

Stress-induced (cold-extractant) ulcers were produced following the procedures of Long et al. (1983). Twenty-four indomethacin rats (150–200 g) of both sexes were fasted for 28 h and deprived of water 18 h before the experiment. The animals were divided into three groups and coded. Thirty minutes after the intraperitoneal dosing of 1% CMC, ethanolic extract and aqueous extract to the three respective groups, ulceration was induced by immobilization of the animals in a perforated cylindrical vessel with exposure to cold (8°C) for 2 h. After the exposure, the animals were killed and examined for ulcers in the usual manner.
Smooth muscle screening for activity

Segments of the guinea pig ileum from freshly killed animals (200—300 g) were suspended in organ baths (50 ml) maintained at 32°C and containing aerated Tyrode's solution. Agonists, antagonists and extracts were introduced into the bath with the aid of L-m syringes. Tissue responses were recorded using a smoked drum (Staff, 1970). Other tissue preparations namely, the rat fundus strip, gravid rat uterus and rabbit jejunum were set up in a similar manner. However, DeJalon's solution was used as the physiologic solution in experiments on gravid rat uterus. The rabbit jejunum was maintained at 37°C.

Antimicrobial testing

Screening was undertaken to determine whether the extracts could be active against pathogenic microorganisms present in the gastrointestinal tract.

Pure cultures of Escherichia coli, Staphylococcus aureus and S. faecalis obtained from the Department of Microbiology were seeded aseptically on nutrient agar plates. Using a sterile syringe, different dilutions of the extract were poured into cups seated on the agar medium. The agar plates were incubated at 37°C for 24 h after which the plates were examined for growth and inhibition zones.

Statistical analysis

Data were expressed as mean ± S.E.M. The means for the population samples were compared at the 95% confidence interval using the Student's t-test.

Results

Phytochemical constituents

The ethanolic and aqueous extracts of C. portoricensis leaves contained tannins, saponins, flavonoids and glycosides (Table 1). The aqueous extract appeared to contain higher levels than the ethanolic extracts. Both extracts gave negative reactions to the Lieberman-Burchard test. This implies that the saponin content of the extracts may be triterpenoidal in nature. The tests revealed the absence of alkaloids in the extracts.

Acute toxicity

The LD₅₀ values for the aqueous and ethanolic extracts were found to be 79.4 mg/kg (95% confidence limits = 45.3—101.6 mg/kg) and 120.2 mg/kg (95% confidence limits 88.5—153.8 mg/kg), respectively.
TABLE 1

PHYTOCHEMICAL-CONSITITUENTS OF C. PORTORICENSIS LEAF EXTRACTS

<table>
<thead>
<tr>
<th>Plant constituents</th>
<th>Ethanolic extract</th>
<th>Aqueous extract</th>
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<tbody>
<tr>
<td>Alkaloids</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Glycoflavones</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Tannins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Saponins</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Pata and Ota</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ , copiously present; + , moderately present; - , absent.

Inhibition of induced ulcers

As shown in Table 2, the aqueous extract at a dose of 50 mg/kg significantly reduced the ulcerogenic effects of indomethacin in rats as recorded by a reduction in ulcer index. The incidence of gastric lesions was also reduced.

Gastric lesions were induced by pylorus ligation in all the control rats (Table 3), however, the incidence and indices of the gastric lesions were reduced by prior administration of the ethanolic and aqueous extracts. The extracts also significantly reduced the volume, pH and total acidity of gastric contents in rats.

The ulcer indices of animals subjected to cold-restraint ulcers were significantly reduced by both the ethanolic and aqueous extracts (Table 4).

It is pertinent to note that in the three experimental ulcer models used, the protective effect of the aqueous extract was more significant (P < 0.001) than that of the ethanolic extract, except for ulcer index and total acid content in the Shay rat (Table 5).

TABLE 2

EFFECTS OF C. PORTORICENSIS LEAF EXTRACTS ON INDOMETHACIN-INDUCED ULCERS IN RATS

<table>
<thead>
<tr>
<th>Drug (p.n.)</th>
<th>Dose (mg/kg)</th>
<th>Quantal ulcer response</th>
<th>Mean ulcer index ± S.E.M.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% GNC</td>
<td>50</td>
<td>8/8</td>
<td>3.78 ± 0.90</td>
<td>&gt;0.05</td>
</tr>
<tr>
<td>Ethanolic extract</td>
<td>50</td>
<td>8/8</td>
<td>1.83 ± 0.48</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Aqueous extract</td>
<td>50</td>
<td>6/8</td>
<td>0.82 ± 0.42</td>
<td>&lt;0.01</td>
</tr>
</tbody>
</table>
Effects on smooth muscle preparations

The ethanolic and aqueous extracts at doses of 1.2 mg/ml and 0.6 mg/ml, respectively, reduced the response of the guinea pig ileum to acetylcholine but did not modify the responses of the tissue to histamine or nicotine. The responses of the rat fundus strip to serotonin were not modified by either the ethanolic (2.40 mg/ml) or aqueous (0.2 mg/ml) extracts. The aqueous and ethanolic extracts inhibited the normal rhythmic movement of the rabbit jejunum at a bath concentration of 0.6 mg/ml. The leaf extracts did not stimulate the gravid rat uterus or modify the tissue responses to oxytocin at the maximum concentration of 1.2 mg/ml. The anticholinergic effects may be ulcer protective in reducing gastric secretions.

Antimicrobial tests

The ethanolic and aqueous extracts inhibited the growth of *Escherichia coli* *Staphylococcus aureus* and *S. faecalis* at a concentration of 0.3 to 0.5 mg/ml.

### Table 3

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Quotient ulcer response</th>
<th>Gastric acid pH</th>
<th>Volume of gastric contents (ml)</th>
<th>Total acid (mEq/1)</th>
</tr>
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<tbody>
<tr>
<td>1% CMC</td>
<td>8.8</td>
<td>2.22 ± 0.55</td>
<td>1.24 ± 0.03</td>
<td>8.30 ± 0.92</td>
<td>67.6 ± 2.60</td>
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<tr>
<td>Ethanol extract 50</td>
<td>7.8</td>
<td>0.95 ± 0.48**</td>
<td>3.94 ± 0.31***</td>
<td>5.25 ± 0.74*</td>
<td>28.00 ± 6.21**</td>
</tr>
<tr>
<td>Aqueous extract 50</td>
<td>4.8</td>
<td>0.32 ± 0.10**</td>
<td>6.02 ± 0.14**</td>
<td>2.94 ± 0.24**</td>
<td>18.70 ± 2.17**</td>
</tr>
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Significant from the vehicle control group: *P* < 0.05; **P** < 0.001

### Table 4

<table>
<thead>
<tr>
<th>Drug (p.p.)</th>
<th>Dose (mg/kg)</th>
<th>Quotient ulcer response</th>
<th>Mean ulcer index ± S.E.M.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>1% CMC</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>20</td>
<td>0.88</td>
<td>2.14 ± 0.07</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Aqueous</td>
<td>50</td>
<td>0.88</td>
<td>0.38 ± 0.13</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
Discussion

This study has confirmed the alleged therapeutic effects of aqueous and alcoholic extracts of Caltandra portoricensis leaves in experimental ulcer models. At a dose of 50 mg/kg p.o., the aqueous and ethanolic leaf extracts reduced both the incidence and severity of pylorus ligation and stress-induced ulcers in rats. At this dosage, only the aqueous extract was effective in reducing endomethecin-induced ulcers.

Phytochemical studies of the leaf extracts revealed the presence of tannins, flavonoids, saponins and glycosides. Saponins, tannins and glycosides were present more abundantly in the aqueous extract than the ethanolic extract and the aqueous extract was clearly more effective in rats with endomethecin, stress- and pylorus ligation-induced gastric lesions than the ethanolic extract. This allows for the speculation that the active anti-ulcer principle in the leaf extract may be correlated with either the saponins or tannin content.

Saponins, especially those of the triterpene type, have been implicated as antiluer agents which mediate their action through the formation of a protective mucus on the gastric mucosa (Lipkin, 1971) or by inhibition of prostaglandin degrading enzymes (Peskay et al., 1976). Since saponins of the triterpene type have been shown to be present in the leaf extract of C. portoricensis, it is logical to conclude that these constituents may account for the antiluer activity.

Tannins could also account for the anti-ulcer activity. Tannins when applied to the gastric mucosa in low concentrations render the outermost layer less permeable and more resistant to chemical and mechanical injury or irritation. Tannins also induce local vasconstriction of small mucosal blood vessels and consequently reduce the amount of gastric acid secreted by the mucosa (Rasmad, 1959).

The precise mechanisms of action of C. portoricensis leaf extracts in protecting rats against induced gastric lesions is (are) unknown. Several researchers (Vane, 1971; Bennett et al., 1978; Miller et al., 1984; Okabe et al., 1974) have reviewed the possible mechanisms of action by which therapeutic agents mediate their anti-ulcer action. The ulcerogenic action of endomethecin has been proposed to be due to its ability to inhibit prostaglandin synthetase (Vane, 1971). This inhibition would reduce the pharmacological action of gastric prostaglandins leading to: (i) tightening of the gastric mucosal barrier and stimulation of mucus and bicarbonate secretion (Bennett et al., 1978; Garner and Heylings, 1979); (ii) inhibition of the formation of cyclic adenosine monophosphate (cAMP) by the gastric mucosa which would reduce the secretory rate of the parietal cells of the stomach (Miller et al., 1984); and (iii) enhancement of gastric mucosal blood flow which might render the gastric mucosa less susceptible to numerous agents (Miller et al., 1980). Apart from prostaglandin synthetase inhibition, endomethecin can cause back diffusion of hydrogen ions and decrease cellular resistance to damage (Mach et al., 1982).
The formation of gastric ulcers by pyliar ligation and stress (cold-restraint) is dependent on the degree of acidity of the gastric juice (Olabe et al., 1974b). This implies that the nornisine and/or tannins of C. porterocerassei leaf extracts may mediate their action by reducing total acid secretion from the parietal cells of the stomach. Preliminary work with smooth muscle preparations has revealed that the anti-ulcer effects of the leaf extract of C. porterocerassei may be due to an ability to block gastric mucosal receptors.

Antimicrobial tests revealed that the aqueous and alcoholic extracts of C. porterocerassei inhibit the growth of E. coli, S. aureus and S. fecalis. These effects are similar to those of an aqueous-alkaline root extract reported by Adesina and Akinwusi (1964). Such results indicate that the extracts may be an effective therapeutic agents in managing gastrointestinal disorders caused by a susceptible microorganism.

The results obtained in this study help to justify the ethnomedicinal use of the plant for various gastrointestinal disorders.

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