Antispasmodic Effects of *Bauhinia microstachya* on Isolated Smooth Muscle

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Abstract

*Bauhinia microstachya* (Raddi) Macbr. (Leguminosae) is a Brazilian medicinal plant used in folk medicine to treat several ailments, including kidney disorders. This study reports on the evaluation of a methanol extract and ethyl acetate fraction from the leaves of this plant against two isolated smooth muscles, guinea-pig ileum and rat uterus, precontracted by acetylcholine, bradykinin, oxytocin, and calcium chloride. The results indicated that both caused noncompetitive antagonism and concentration-dependent relaxation against all the neurotransmitters tested without selectivity. The ethyl acetate fraction demonstrated the most pronounced antispasmodic effect, suggesting that the phenol compounds are the main active components of this plant. These results support and justify the popular use of this plant for the treatment of kidney problems.

Keywords: Antispasmodic effects, *Bauhinia microstachya*, phenol compounds, smooth muscles.

Introduction

The genus *Bauhinia* (Leguminosae), which is mainly found in the tropical areas of the planet, comprises more than 300 species and is used in folk medicine around the world for the treatment of several ailments. Phytochemical investigations of the genus have revealed the presence of a number of compounds including steroids glycosides, triterpenes, lactones, and flavonoids (Da Silva & Cechinel Filho, 2002). Biological studies have confirmed that these plants exert several medicinal properties, especially antinociceptive, antimicrobial, and antidiabetic effects (Da Silva & Cechinel Filho, 2002; Meyre-Silva, 2004).

Previous investigations carried out in our laboratories with the plants belonging to the genus *Bauhinia* have demonstrated the following biological and chemical results: *Bauhinia splendens* exhibits antibacterial and antinociceptive activities related to the presence of steroids or flavonoids (Cechinel Filho et al., 1995; Savi et al., 1997; Willain Filho et al., 1997). *Bauhinia forficata* revealed the presence of β-sitosterol and kaempferitrin, and the pharmacognostic investigation indicated that the latter might be useful for suitable quality control of phytotherapeutics (Da Silva et al., 2000).

*Bauhinia microstachya* (Raddi) Macbr. popularly known as *escada-de-macaco* or *pata-de-vaca*, is widely distributed in the southern region of Brazil. The leaves and bark, like the other plants of the genus, are employed in folk medicine for the treatment of several diseases, such as infections, inflammation, diabetes, kidney (Pio Correia, 1984; Cirilo, 1993) and respiratory diseases, and dolorous processes, among others (Franco & Fontana, 2001).

Previous investigations carried out by our research group enables us to demonstrate that *B. microstachya* has a potent analgesic action in mice, related to the presence of phenol compounds, especially flavonoids (Meyre-Silva et al., 2001). In the current study, we have extended our pharmacological experiments and evaluated the possible antispasmodic activity *in vitro* of the...
methanol extract (ME) and ethyl acetate (EA) fractions of *Bauhinia microstachya* on smooth muscle preparations.

Materials and Methods

Plant material and preparation of methanol extract and ethyl acetate fraction

Leaves of *Bauhinia microstachya* were collected in January 2000 in Urussanga, in the State of Santa Catarina in southern Brazil, and were classified by Dr. Ademir Reis (Department of Botany, Federal University of Santa Catarina). Vouchers were deposited at the Barbosa Rodrigues Herbarium (Itajaí) under number VC Filho 021. Air-dried leaves were cut into small pieces and extracted with methanol at room temperature for approximately 2 weeks. Afterward, the solvent was evaporated, the extract concentrated and successively partitioned with n-hexane, dichloromethane, ethyl acetate, and butanol, as described previously (Cechinel Filho & Yunes, 1998). Considering the more suitable chromatographic profile and the best activity of ethyl acetate fraction (results not shown), it was used in the current study. The ME and EA fractions were dissolved in dimethylsulfoxide (DMSO) solution at the desired concentration just before use.

Animals

Hartley guinea-pigs (250–350 g) and female Wistar rats (200–300 g) were kept in automatically controlled temperature conditions (22 ± 2°C), in 12-h light-dark cycles and with food and water ad libitum. Each experimental group was matched with a parallel control group treated only with vehicle.

Evaluation of pharmacological activity

Guinea-pig ileum

Guinea-pigs of both sexes were sacrificed by cervical dislocation, and the ileum was removed from the exposed abdominal chamber. Ileal strips of about 10–20 mm in length were taken from the portion situated 15 cm proximal to the ileum-cecal junction (Schlemper et al., 1996). The intestinal content was removed by washing with Tyrode solution, and the mesenteric residues were eliminated. Preparations were set up for recording isotonic contractions in 5-ml jacketed organ baths containing Tyrode solution at 37°C continuously bubbled with air under 1 g of load. The Tyrode solution had the following composition (mM): NaCl 153.8, KCl 6.17, CaCl2 0.54, NaHCO3 5.95, and glucose 2.77. After an equilibrium period of at least 45 min, cumulative concentration-response curves for acetylcholine (10 nM to 100 μM), oxytocin (10^-7 to 10^-1 IU/ml), bradykinin (0.1 μM to 1 μM), and calcium chloride (1 μM to 1 M) were obtained for the agonist became stable, different concentrations of ME and EA fraction of *Bauhinia microstachya* (0.10–2.0 mg/ml) were added to the bath and left in contact with the tissue for 25 min. New cumulative concentration-response curves for agonists were then obtained in its presence.

Drugs

The drugs used were acetylcholine iodide, histamine, bradykinin, captopril, calcium chloride, oxytocin, and estradiol benzoate (all from Sigma Chemical Company, St. Louis, MO, USA). All the drugs were stored as 0.1–1 mM stock solutions for up to a week at –4°C and diluted to the desired concentrations in distilled and deionized water or saline 0.9% just before use, except for the estradiol, which was dissolved in peanut oil. Other substances used included glucose, NaCl, KCl, CaCl2(H2O)2, MgCl2(H2O)6, NaHCO3, and...
NaH$_2$PO$_4$ (from Merck KGA, Darmstadt, Germany). The ME and EA fractions were diluted in DMSO.

**Statistical analysis**

The data are shown as mean ± SEM, except for the IC$_{50}$ (concentration of drugs causing half-maximal responses), which are presented as geometric means accompanied by their respective 95% confidence intervals. Statistical analyses were performed by means of the unpaired Student’s $t$-test or by analysis of variance followed by the Tukey’s multiple comparison test when appropriate, and $p < 0.05$ was considered significant.

**Results**

**Effect of ME and EA fractions of *Bauhinia microstachya* on acetylcholine-, histamine-, serotonin-, and bradykinin-induced contractions on guinea-pig ileum**

The incubation of increasing concentrations of ME (0.1–2.0 mg/ml) and EA (0.1–2.0 mg/ml) for 15 min inhibited in a noncompetitive and concentration-dependent manner the contractile response elicited by acetylcholine, histamine, serotonin, and bradykinin on guinea-pig ileum. The respective IC$_{50}$ values (mg/ml) for each experiment are shown in Table 1.

ME and EA in the concentrations described above did not interfere with the basal tension of the preparations, indicating that there was no agonistic effect, with restoration of the contractile response to different agonists after successive washings.

**Effect of ME and EA fractions of *Bauhinia microstachya* on acetylcholine-, bradykinin-, oxytocin-, and calcium chloride-induced contractions on rat uterus**

ME (0.1–2.0 mg/ml) and EA (0.1–2.0 mg/ml), when incubated with the preparations for 30 min, inhibited in a noncompetitive and concentration-dependent manner the contractile response elicited by acetylcholine, bradykinin, serotonin, and oxytocin, as well as the calcium chloride-induced contractions. The results presented here suggest that the action of *B. microstachya* occurs in a receptor-independent manner, as the inhibitory response of both, methanol extract and ethyl acetate fraction, exerted noncompetitive antagonism without selectivity against the neurotransmitters tested (acetylcholine, bradykinin, oxytocin, and calcium chloride). It is interesting to note that the effect induced by *B. microstachya* is not tissue specific-like, suggesting an antispasmodic activity.

**Discussion**

The pharmacological analysis of the crude methanol extract and ethyl acetate fraction of *Bauhinia microstachya* evidenced an effective inhibition of the contraction induced by different agonists in two isolated smooth muscle preparations: guinea-pig ileum and rat uterus. The results presented here suggest that the action of *B. microstachya* occurs in a receptor-independent manner, as the inhibitory response of both, methanol extract and ethyl acetate fraction, exerted noncompetitive antagonism without selectivity against the neurotransmitters tested (acetylcholine, bradykinin, oxytocin, and calcium chloride). It is interesting to note that the effect induced by *B. microstachya* is not tissue specific-like, suggesting an antispasmodic activity.

The higher inhibitory potency of ethyl acetate fraction indicates that the main constituents responsible for the relaxation response observed for *B. microstachya* are present in this fraction, rich in phenol compounds (Meyre-Silva et al., 2001). In contrast, some other plants, including *Artemisia annua* and *Baccharis conferta*, have some advantages for the medical use of extracts as opposed to isolated single entities (Phillipson, 2001; Weimann et al., 2002).

Previous studies with the ethyl acetate fraction of *B. microstachya* revealed the presence of four main compounds, which were identified as methyl gallate, kaempferol 3-O-rhamnosyl, querctin, and myricitrin. The first compound consists of one of the active principles of the genus *Phyllanthus*, showing interesting analgesic action and spasmylytic activity in guinea-pig trachea (Calixto et al., 1998; Paulino et al., 1999), which also helps to explain the antispasmodic activity of this fraction. Although we have not found other studies in the literature showing the relaxant properties of the flavonoids present in *B. microstachya*, several articles have described this pharmacological action to some related

**Table 2.** The mean IC$_{50}$ values of methanolic extract (ME) and ethyl acetate fraction (EA) of *Bauhinia microstachya* in rat isolated uterus strips ($n = 7$).$^a$

<table>
<thead>
<tr>
<th>Agonist</th>
<th>ME (mg/ml)$^b$</th>
<th>EA (mg/ml)$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acetylcholine</td>
<td>1.42 (1.13–1.78)</td>
<td>0.88 (0.85–0.92)</td>
</tr>
<tr>
<td>Bradykinin</td>
<td>0.48 (0.35–0.64)</td>
<td>0.62 (0.57–0.66)</td>
</tr>
<tr>
<td>Oxytocin</td>
<td>0.80 (0.75–0.84)</td>
<td>0.58 (0.54–0.62)</td>
</tr>
<tr>
<td>Calcium chloride</td>
<td>1.22 (0.89–1.55)</td>
<td>0.41 (0.35–0.45)</td>
</tr>
</tbody>
</table>

$^a$Number of experiments.

$^b$Geometric means accompanied by 95% confidence limits.
structures (Gross et al., 1997; Revuelta et al., 2000; Meckes et al., 2002).

To the best of our knowledge, this finding is the first to determine an antispasmodic activity for the genus Bauhinia and strongly suggests an explanation, at least in part, for the potential therapeutic benefit of this plant.

Pharmacological studies are in progress to confirm this hypothesis and to verify the presumed antispasmodic effects of the other phenolic compounds of this plant, as well as to elucidate the mechanism of action of these components.

In summary, our results revealed that B. microstachya exerts antispasmodic properties causing concentration-dependent relaxation in the guinea-pig ileum and rat uterus in vitro precontracted by acetylcholine, bradykinin, oxytocin, and calcium. These results support and justify the popular use of this plant for the treatment of kidney problems.

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References


