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Effects of alkaloids of *Himatanthus lancifolius* (Muell. Arg.) Woodson, Apocynaceae, on smooth muscle responsiveness

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Abstract

Himatanthus lancifolius, popularly known as "agoniada" in Brazil, is largely used in folk medicine against asthma, dysmenorrhea and as an emenagogue and abortive. This study reveals the effects of an alkaloid rich fraction (AlkF) obtained from the bark of *Himatanthus lancifolius* in vascular and non-vascular smooth muscle responsiveness. Incubation of AlkF (3–30 μg/ml) during 15 min generates a concentration-related and fully reversible reduction in maximal contractile responses evoked by acetylcholine and phenylephrine in rat jejune and aorta preparations, respectively. Exposition of endothelium-denuded pre-contracted rat aorta rings to AlkF results in a complete relaxation, with EC_{50} of 22.2 (16.2–28.2 μg/ml). AlkF is also able to induce a concentration-related rightward shift of cumulative concentration curves for calcium in uterus and aorta rings maintained in depolarizing nutritive solution. Moreover, addition of AlkF in calcium-free solution also reduces, in a concentration-dependent manner, the ability of caffeine and phenylephrine to contract aorta rings. This study reveals that the bark of *Himatanthus lancifolius* possesses one or more indole alkaloids able to alter non-vascular and vascular smooth muscle responsiveness, an event that may involve the blocking of calcium entry or changes on intracellular calcium utilization or mobilization.

Keywords: Alkaloids; Smooth muscle; Calcium; *Himatanthus lancifolius*; Apocynaceae; Aorta

1. Introduction

Himatanthus lancifolius (Muell. Arg.) Woodson (Apocynaceae) formerly known as *Plumeria lancifolia*, is popularly called "agoniada" in Brazil. The dried stem bark of this specie is commonly used as a febrifuge, as an emenagogue and as an abortive (Coimbra, 1994). *Himatanthus lancifolius* is an official plant of the Brazilian Pharmacopeia I (1929) and forms part of the composition of commercial products indicated in the treatment of dysmenorrhea and in the amelioration of menopausal symptoms.

Himatanthus lancifolius, like other *Himatanthus* species, has scarcely been mentioned in the scientific literature regarding its biological activities. Among the members of this genus, some species are reported to present moluscicidal activity (e.g. see Perdue and Blomster, 1978), and anti-inflammatory and analgesic properties (de Mi randa et al., 2000). In addition, bioactivity-guided fractionation of the hexane extract of *Himatanthus sucuuba* bark led to the isolation of iridoids plumericin and isoplumericin, described as both potent antimicrobial agents against *Cladosporium sphaerospermum* (Silva et al., 1998)

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and as weakly cytotoxic agents (Abdel-Kader et al., 1997).

Previous Studies had evinced the presence of iridoids, such as glucosylplumeride (Schmid et al., 1952; Halpern and Schmid, 1958), and the alkaloids uleine (Buchi and Warnhoff, 1959; França et al., 2000) and demethoxyaspidospermine (Ferreira et al., 1963; França et al., 2000) in *Himatanthus lancifolius*. Nevertheless, no biological activities were related to any of these known compounds. During this study we have seen the in vitro effects of an indole alkaloid rich fraction (AlkF) obtained from the bark of *Himatanthus lancifolius* on the tonus and responsiveness of both non-vascular and vascular smooth muscles.

2. Materials and methods

2.1. Plant material and extraction of alkaloids

Stem bark from *Himatanthus lancifolius* was commercially acquired in the surroundings areas of São Paulo (SP, Brazil). It was identified according to the description of Brazilian Pharmacopeia I (1929) and by macro and microscopic comparison with an authentic sample at the Laboratory of Pharmacognosy (Department of Pharmacy) of Universidade Federal do Paraná (PR, Brazil), where a voucher specimen is deposited.

Dried and powered stem bark (5 kg) of *Himatanthus lancifolius* previously deslipidified with *n*-hexane in soxhlet was macerated for 48 h in 1% HCl. The extraction was performed until exhaustion (controlled by negative reaction to Dragendorff's reagent). The 1% HCl extract was filtered and basificated (pH 10) with sodium carbonate and successively fractionated with chloroform and then evaporated (vacuum, at room temperature) obtaining 4.02 g of alkaloid fraction base (AlkF; pH 10). This fraction was dissolved in 1% HCl, filtered and concentrated (using a rotavapor). This fraction constituted the total indole alkaloid fraction (AlkF, yield of 0.0804%).

2.2. Characterization of alkaloids: HPLC fingerprint of the alkaloid fraction

LC separations were performed using a Waters 600 (Millford, USA) pump, oven and controller, a Nova-pak C18 (4 μm) (3.9 mm × 150 mm i.d.) column from Waters (Millford, USA), a Waters 2996 photodiode array detector (monitoring 305 nm) and oven fit to 30 °C. A Rheodyne manual injector model 7725i was used for sample injection (Rohnert Park, CA, USA). All reagents used were LC grade, filtered over regenerated cellulose membrane [0.45 um pore diameter (Schleicher & Schuell, Dassel, Germany)], sonicated (15 min) and degassed with a in line degasser AF (Waters). Sample preparation: for the LC analysis all extracts and standards were diluted in MeOH (1:10 (v/v)), filtered over regenerated cellulose membrane [0.45 m pore diameter (Schleicher & Schuell, Dassel, Germany) and injected (20 μl) in triplicategradiente linear de acetonitrile:water (phosphoric acid 0.5%) 10–90 untill 70–30.

2.3. Pharmacologycal procedures

Male and female Wistar rats (3–4 months old) from the colony of the Universidade Federal do Paraná, were maintained under standard laboratory conditions, in a constant 12-h light/dark cycle with controlled temperature (22 ± 2 °C). Standard pellet food and water were available ad libitum. The Institutional Ethics Committee of the Universidade Federal do Paraná approved all the procedures adopted in this study.

2.3.1. Evaluation of AlkF effects on contractile responses of rat jejune

Male rats were killed and the jejune removed and transferred to Petri dishes with Tyrode's solution (mM composition: NaCl 136.9, KCl 2.68, $CaCl_2$ 1.8, $MgCl_2$ 0.53, NaH_2PO_4 0.33, $NaHCO_3$ 11.91, D-glucose 5.6). The intestinal lumen was gently flushed with Tyrode's solution and jejune segments (1–1.5 cm long) were placed in an aerated (95% O_2/5% CO_2) 10 ml organ bath maintained at 37 °C by a thermo-regulated water pump. The segments were hung on the longitudinal axis, attached at one end with tangential levers at a resting tension of 1 g and six-fold amplification for recording isotonic contractions using quimographs (on smoked drums).

After a 30 min equilibration period (including washings at 15 min intervals), the preparations were exposed to cumulative concentrations of acetylcholine (ACh; 1 nM–1 mM). The jejune segments were rinsed three times and equilibrated in fresh Tyrode's solution for a new 30 min interval prior to the reintroduction of ACh (1 nM–1 mM) in the presence of 15 min pre-incubated AF (1, 3 or 30 μg/ml). Control preparations were subjected to the same procedures with incubation of vehicle (nutritive solution) instead of AlkF. Each response was expressed as the percentage of the response to maximal contraction induced by acetylcholine before AlkF incubation.

2.3.2. Evaluation of AlkF effects on calcium-induced contraction in rat uterus and vas deferens

For this, nulliparous female Wistar rats estrogenized by estradiol dihydrobenzoate (100 μg/kg, s.c.) given 24 h before the experiments and adult male Wistar rats were used. The animals were killed by cervical dislocation and uterine horns or *vasa deferentia* were removed, cleaned of surrounding tissues, and had the lumens carefully washed with a nutritive solution lacking calcium (pH 7.4, composition in mM: NaCl 64, KCl 81.7, NaH_2PO_4 0.36, $NaHCO_3$ 15, D-glucose 5.6, EGTA 1 mM). Each organ was suspended in a 10 ml chamber containing continuously aerated (95% O_2/5% CO_2) depolarizing Ca^{2+}-free nutritive solution at 30 °C. Isotonic contractions were