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Effect of *Stryphnodendron adstringens* (barbatimão) bark on animal models of nociception

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The antinociceptive activity of the crude extract and fractions of Stryphnodendron adstringens *(Martius) Coville (*barbatimão*) was evaluated. Three experimental models of pain induction were used: abdominal writhing, formalin, and hot plate. The results demonstrated an antinociceptive effect of barbatimão in the experimental models of writhing induced by acetic acid and pain induced by formalin. However, the extracts did not significantly alter latency time on the hot plate in mice. These results suggest that barbatimão extract has a peripheral antinociceptive effect.*

Uniterms
- *Stryphnodendron adstringens*
- *Barbatimão,*
- Antinociception

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INTRODUCTION

Medicinal plants play an important role in folk medicine, and different plant species have been used in the treatment of many diseases. Detailed studies are necessary to prove their biological activity and provide necessary information about their therapeutic use.

Popularly known as *barbatimão*, *Stryphnodendron adstringens* (Mart.), Coville, Leguminosae, is a medicinal plant abundant in central Brazil and has long been used as a popular herbal medicine. Pharmacological studies with this plant have demonstrated that it possesses anti-inflammatory properties (Bersani-Amado *et al.*, 1996; Lima *et al.*, 1998) and protects gastric mucous membranes (Audi *et al.*, 1999). There also are reports that *barbatimão* extracts have cicatrizing (Lopes *et al.*, 2005; Neves *et al.*, 1992; Panizza *et al.*, 1988), antioxidant (Lopes *et al.*, 2005; Sanches *et al.,* 2005), antiviral (Felipe *et al.,* 2006), antiprotozoa (Holetz *et al.*, 2005), and antimicrobial effects (Audi *et al.*, 1999; Ishida *et al.,* 2006; Sanches *et al.*, 2005).

The purpose of the present study was to evaluate the possible antinociceptive activity of the crude extract and fractions of *barbatimão* in several animals models of nociception.

MATERIAL AND METHODS

Plant material and extract preparation

The stem bark of *Stryphnodendron adstringens* (Martius) Coville, Leguminosae, was collected in São Jerônimo da Serra (longitude: 23º 43' 7.8" S; latitude: 50º 45' 23.5" W; altitude: 926 m) in the state of Paraná, Brazil, in October 1995. A voucher specimen was deposited at the Herbarium of the Biology Department under number HUM-3800. Air dried stem bark (1500 g) was extracted in Ultra-Turax® with Acetone:H_2O (7:3; v/v). The organic

solvent was eliminated by reduced pressure and lyophilised to yield a *barbatimão* crude extract (633 g). The lyophilized *barbatimão* crude extract (500 g) was resuspended in H_2O and then fractionated with ethyl acetate to yield H_2O (F_1; 386 g) and ethyl-acetate (F_2; 98 g) fractions. Both F_1 and F_2 were concentrated under reduced pressure to eliminate the organic solvent, lyophilized, and stored at -20 ºC. The *barbatimão* crude extract and fractions F_1 and F_2 were resuspended in distilled water at the desired concentrations before use.

Animals

Adult male Wistar rats weighing 200-220 g and male Swiss mice weighing 25-35 g were used. They were housed five per cage and maintained in a room with controlled temperature (22 ± 2 ºC) under a 12 h dark-light cycle with free access to standard chow and tap water throughout the experimental period. The protocol for these experiments was approved by the Animal Ethics Committee of University of Maringá under approval number 047/CEEA.

Writhing test

The response to intraperitoneal injection of 0.6% acetic acid (i.e., contraction of the abdominal muscles and elongation of the hind limbs) was induced by the method of Koster *et al.* (1959). Male Swiss mice (25–35 g, $n = 8$) were treated orally with the crude extract and fractions (200, 400, and 800 mg/kg body weight), indomethacin® (Sigma, 5 mg/kg body weight), the reference nonsteroidal anti-inflammatory drug, or saline (control group) 30 min before injection of 0.6% acetic acid (0.1 ml/10 g body weight). The mice were placed in transparent glass cylinders, and the number of abdominal writhes produced in these animals was counted over a period of 20 min. The antinociceptive activity was evaluated in terms of number of writhes.

Formalin test

The formalin test was performed according to the method described previously by Correa and Calixto (1993). The mice were treated orally with the crude extract and fractions (800 mg/kg body weight) or indomethacin (5 mg/kg body weight) 30 min before intraplantar injection of 1% formalin (0.5 ml) into the left hind paw of the animal. The animals were placed in a mirrored cylinder. The reaction to pain then was observed, and the time the animal spent licking or biting its paw was measured with a chronometer during the first phase (0–5 min, neurogenic pain) and during the second phase (15–35 min, tonic pain).

Hot-plate test

The latency time in the hot-plate test was measured by the method of Eddy and Leimback (1953). The animals were divided into groups of eight animals each. The temperature of the plate (Ugo Basile, Varese, Italy) was fixed at 55.0 ± 0.5 °C. The latency time to exhibit a reaction (i.e., jumps, licking one of the hind limbs, or one of the forelimbs, tapping) was measured 30 and 60 min after administration of the crude extract and fractions (800 mg/kg body weight, p.o.), meperidine® (Cristália, 50 mg/kg body weight, i.p.), the reference opioid analgesic drug, or saline (control group). Basal latencies were recorded before treatment. The cutoff time was 30 s to avoid tissue damage.

Statistical analysis

The results are presented as mean ± standard error of the mean (S.E.M.). The data were subjected to analysis of variance (ANOVA) followed by Tukey's *post hoc* test. $P < 0.05$ was considered as the significance level.

RESULTS

The *barbatimão* crude extract and both fractions, F_1 (aqueous fraction) and F_2 (ethyl-acetate fraction), dose-dependently reduced acetic acid-induced writhing when administered orally (400 and 800 mg/kg body weight). Only the F_1 fraction inhibited writhing at a dose of 200 mg/kg body weight. All of the extracts at the 800 mg/kg dose inhibited writhing similarly to indomethacin (5 mg/kg).

In the formalin test, the *barbatimão* crude extract and F_1 fraction (800 mg/kg) caused marked inhibition only in the late phase. The F_2 fraction was not effective in both phases of this model.

The *barbatimão* crude extract (800 mg/kg) and the F_1 fraction (800 mg/kg) pretreatment increased response latency in the hot plate test. This effect, however, was very minimal compared to the effect of meperidine, the analgesic reference drug. Treatment with the F_2 fraction did not alter the response time of the animals. Data are presented in Table I.

DISCUSSION

The results obtained in the present study demonstrated that the *barbatimão* crude extract and fractions exhibited antinociceptive activity in the models tested. This effect was more significant against chemically

TABLE I - Analgesic activity of *barbatimão* extracts on nociception in the writhing, formalin, and hot plate tests.

Treatment (mg/kg body weight)		Acetic acid Number of writhings	Formalin test Early phase	Formalin test Late phase	Hot plate test 30 min	Hot plate test 60 min
Saline		68.4 ± 3.3	51.9 ± 3.9	239.2 ± 20.0	7.2 ± 0.8	8.3 ± 0.8
barbatimão crude extracts	200	54.0 ± 7.0	-	-	-	-
	400	51.1 ± 3.6*	-	-	-	-
	800	24.9 ± 7.2*	49.6 ± 3.1	130.5 ± 7.7*	8.2 ± 2.2	12.6 ± 1.3*
F_1	200	45.2 ± 6.6*	-	-	-	-
	400	30.2 ± 3.8*	-	-	-	-
	800	23.3 ± 4.3*	46.7 ± 7.0	162.8 ± 7.3*	10.8 ± 1.6*	12.5 ± 1.3*
F_2	200	74.5 ± 5.2	-	-	-	-
	400	52.1 ± 6.2*	-	-	-	-
	800	32.8 ± 5.3*	53.1 ± 7.5	264.6 ± 13.7	9.6 ± 0.9	7.4 ± 0.6
Indomethacin	5	23.1 ± 5.3*	39.0 ± 9.0	44.8 ± 11.8*	-	-
Meperidine	50	-	-	-	28.6 ± 0.9*	24.8 ± 1.9*

F_1 = aqueous fraction; F_2 = ethyl-acetate fraction. Values are the mean ± S.E.M for 6-10 animals. Significant differences compared to controls (saline) were evaluated using ANOVA (Tukey-test) *$P < 0.05$.

(acetic acid and formalin tests) than thermally (hot plate) induced nociception.

Acetic acid causes inflammatory pain by inducing capillary permeability (Amico-Roxas *et al.*, 1984) and liberating endogenous substances that excite pain nerve endings (Raj, 1996). Traditional anti-inflammatory drugs (e.g., nonsteroidal anti-inflammatory drugs) inhibit COX in peripheral tissues, thus interfering with production of mediators that stimulate primary afferent nociceptors (Fields, 1987). The formalin test, characterized by two phases, is used to evaluate the mechanism by which an animal responds to moderate, continuous pain generated by the injured tissue (Abbott *et al.*, 1995). The early phase (immediately after formalin injection) seems to be caused by C-fiber activation induced by the peripheral stimulus. The late phase (starting approximately 20 min after injection) appears to depend on the combination of an inflammatory reaction, activation of *N*-methyl-D-aspartate (NMDA) and non-NMDA receptors, and the nitric oxide cascade (Davidson and Carlton, 1998) in the peripheral tissue and functional changes in the dorsal horn of the spinal cord (Abbott *et al.*, 1995).

The extract showed remarkable activity in the writhing test induced by acetic acid and in the late phase of the formalin test, both related to a peripheral inflammatory pain (Ahmadiani *et al.*, 1998; Collier *et al.*, 1968). In contrast, the effects of the extract in the hot plate test, used to evaluate specific central antinociception (Parkhouse *et al.*, 1979), were minimal. Although some doses showed a significant difference compared to the control group, the antinociceptive effect of meperidine (reference drug) was much more evident. In addition, the effect of crude extract and fractions in the early phase of the formalin test was not different from control. The nociception of this phase may be a direct result of stimulation of nociceptive neurons (Coderre, Melzack, 1992). The present study also demonstrated that the F_1 fraction showed greater efficacy than the crude extract and F_2 fraction.

Phytochemical analysis indicates the presence of a proanthocyanidin polymer of molecular weight 2114 Da in the aqueous fraction (F_1) (Ishida *et al.*, 2006) and condensed tannins (flavan-3-ols and prodelphinidins and prorobinetinidins) in F_2 (De Mello *et al.*, 1996a, b, 1999).

From the data of this study we can conclude that the *barbatimão* extract has a peripheral antinociceptive effect. The mechanisms responsible for this effect are not completely understood, but it is possible that the substances responsible for the observed effect are concentrated in the F_1 fraction.

RESUMO

Efeito da casca de *Stryphnodendron adstringens* (barbatimão) em modelos de nocicepção animais

A atividade antinociceptiva do extrato bruto e frações do Stryphnodendron adstringens *(Mart.) Coville (*barbatimão*) foi avaliada. Foram usados três modelos experimentais*

para avaliação da dor: teste de contorção abdominal induzida pelo ácido acético, teste da formalina e teste da placa quente. Os resultados mostraram um efeito antinociceptivo evidente do barbatimão nos modelos experimentais de contorção induzida por ácido acético e dor induzida pela formalina. Por outro lado, o barbatimão não modificou significativamente o tempo de latência dos animais no teste da placa quente. Estes resultados sugerem que o extrato de barbatimão apresenta efeito antinociceptivo por mecanismos periféricos.

UNITERMOS: Stryphnodendron adstringens. *Barbatimão. Antinocicepção.*

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