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.

**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 cicatrizizing (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 animal 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:H2O (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$_2$O and then fractionated with ethyl acetate to yield H$_2$O (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$^\text{®}$ (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).

### 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 body weight). 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.

### 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
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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 F₁ fraction showed greater efficacy than the crude extract and F₂ fraction.

Phytochemical analysis indicates the presence of a proanthocyanidin polymer of molecular weight 2114 Da in the aqueous fraction (F₁) (Ishida et al., 2006) and condensed tannins (flavan-3-ols and prodelphinidins and prorobinetindins) in F₂ (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 F₁ 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.


REFERENCES


