

RESEARCH ARTICLE

Anti-inflammatory and anti-nociceptive effects of *Pterodon emarginatus* stem bark alcohol extract

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Abstract

The tail flick, the acetic acid writhing, the croton oil-induced mouse ear edema, and carrageenan-induced peritonitis tests were used to study the anti-inflammatory effects of the alcohol extract from *Pterodon emarginatus* Vogel. (Leguminosae/Fabaceae) stem bark. The alcohol extract caused inhibition of acetic acid-induced abdominal writhing, reducing the number of leukocytes ($\times 10^7$) per mL of peritoneal wash and ear edema formation. The results suggest that *Pterodon emarginatus* stem bark contains compounds with analgesic and anti-inflammatory actions.

Keywords: *Pterodon emarginatus*; analgesic activity; anti-inflammatory activity; acetic acid-induced abdominal writhing; carrageenan-induced peritonitis

Introduction

The *Pterodon emarginatus* Vogel. (Leguminosae/Fabaceae), known as sucupira branca, is a native species widely distributed over central Brazil (Goiás, Minas Gerais and São Paulo). It is a tree 5–15 m in height, whose wood is used as timber. The decoction of its stem bark and seeds is used in folk medicine against various diseases (Agra et al., 2007; Lima et al., 2006).

Alcohol extracts from the seeds of *Pterodon emarginatus* are used in folk medicine for rheumatism, sore throats, depurative, tonic and analgesic preparations (Belinelo et al., 2002). Moreover, “sucupira branca”, vulgar denomination of the cited species above, is also used for gynecological infections (Almeida & Gottlieb, 1975).

The oil from the fruits and seeds of *Pterodon* species is reputed to inhibit skin penetration of the cercaria of *Schistosoma mansoni* (Demuner et al., 1996) and anti-inflammatory activity (Falcão et al., 2005; Carvalho et al., 1999). The characteristic constituents of this genus are the linear and tetracyclic diterpenoids possessing

either vinhaticane or vouacapane skeletons (Campos et al., 1994). Marques et al. (1998) reported the isolation of isoflavones, *p*-methoxybenzoic acid, lupeol and betulin from sapwood and the heartwood of *Pterodon polygalaeflorus* Benth.

Wood, leaves, roots, stem bark and the seeds of *Pterodon polygalaeflorus*, are used ethnobotanically as anti-rheumatic, in the treatment of throat infections (Agra et al., 2007; Lima et al., 2006).

Based on the reported popular use of *Pterodon emarginatus*, the present study evaluated the analgesic, anti-inflammatory, and other general effects of alcohol extracts of the plant stem bark.

Materials and methods

Botanical material

The stem bark of *Pterodon emarginatus* was collected from plants in their natural habitat in the Cerrado region

of Bela Vista (847 m, 17°02'1,1" S/48°49'0,3" W), Goiás, Brazil (September 2003). Samples were authenticated by José Realino de Paula and a voucher specimen (number UFG-27155) was deposited at the herbarium of the Universidade Federal de Goiás (UFG), Goiás, Brazil. The stem bark was cut into small pieces, dried at 30–40°C for 48 h, and crushed to a powder. The alcohol extract (ExEtOH) was obtained from the powdered botanical material (900 g) by maceration in 4.5 L of cold ethanol (95% V/V), with occasional agitation, for 72 h, followed by filtration. The filtrate was concentrated under reduced pressure and dried in a rotavapor. The extraction procedure gave 270 g of alcohol extract (yield = 30%). At the time of use, the extract was dissolved in distilled water at the required concentrations.

Animals

Male Swiss albino mice weighing approximately 30 g and male Wistar rats weighing 200–250 g, from the Central Animal House of the Universidade Federal de Goiás (UFG), were used in this study. The animals received food and water *ad libitum* and were maintained in a room with light and temperature regulation, in accordance with the Guide for the Care and Use of Laboratory Animals, National Research Council, USA (1996). Animals were acclimatized for 4 days before the beginning of the experiments.

Drugs and dosage

The following drugs were used: 1.2% solution of acetic acid (Merck; 10 mL/kg), 1% solution of carrageenan (Sigma; 0.25 mL/animal), croton oil (Prodome), dexamethasone (Decadron-Prodome; 2 mg/kg), morphine (Roche, Switzerland; 10 mg/kg), indomethacin (Sigma; 20 mg/kg); vehicle (water, 10 mL/kg), crude alcohol extract (ExEtOH; 0.1, 0.3, and 1.0 g/kg). All other chemicals were from Merck (Brazil). All drugs and ExEtOH were dissolved in water.

Effects on general behavior

The effects of the extract on spontaneous behavior of mice and rats were analyzed by the Hippocratic procedure (Malone, 1977). The ExEtOH or vehicle was administered to groups (n = 4) of the animals at doses 0.01 to 2.0 g/kg and 10 mL/kg, respectively (per oral (p.o.), intraperitoneal (i.p.) and subcutaneous (s.c.)). The animals were kept under observation for seven days after drug administration.

Acetic acid-induced abdominal writhing

The response to an intraperitoneal injection of acetic acid solution, i.p., the contractions of the abdominal muscles

and stretching of hind limbs, was studied according to procedures described by Hendershot and Forsaith (1959). Groups of mice (n = 12) were treated subcutaneously with indomethacin (20 mg/kg), ExEtOH (1.0 g/kg), or vehicle (10 mL/kg) and orally with ExEtOH (1.0 g/kg), 45 min before the administration of a 1.2% acetic acid solution (10 mL/kg, i.p.). The number of abdominal writhings produced in each group for the subsequent 30 min was counted and the results were expressed as total number of writhings. A significant reduction in the number of writhing movements in the groups treated with the ExEtOH compared with control was considered to be a positive analgesic response.

Tail flick test

The reaction of mice to thermal stimulation of the tail tip by immersion in water maintained at 56°C was recorded at 60 and 30 min before, and 0, 30, 60, 90, and 120 min after treatment. The mice were divided into five experimental groups (n = 6) consisting of animals treated subcutaneously with vehicle (10 mL/kg), ExEtOH (0.1, 0.3, or 1.0 g/kg) or morphine (10 mg/kg). Analgesia was expressed as mean \pm SEM reaction time(s) relative to time zero, according to the technique of Janssen et al. (1963), as modified by Grotto and Sulman (1967).

Carrageenan-induced peritonitis

Experimental groups (n = 7) were treated subcutaneous with vehicle (10 mL/kg), dexamethasone (2 mg/kg), or ExEtOH (0.1, 0.3, 1.0 g/kg) followed by 1% carrageenan (0.25 mL, i.p.) 60 min later. Four hours after carrageenan administration, mice were killed and 2 mL of modified PBS (heparin, 10 IU/mL) was injected into the peritoneal cavity. Total cell counts in the lavage fluid were performed using a Neubauer chamber as described by Ferrándiz and Alcaraz (1991). The results were expressed as means \pm SEM of the numbers of total leukocytes ($\times 10^7$) per mL of peritoneal wash in relation to the control group.

Croton oil-induced ear edema test

Animals were treated with vehicle (control, 10 mL/kg; s.c.), ExEtOH (0.1, 0.3, or 1.0 g/kg; s.c.) or dexamethasone (2 mg/kg; s.c.) and 60 min later, cutaneous inflammation was induced by applying 25 μ L of a solution of croton oil in acetone (2.5% v/v) to the inner surface of the right ear of mice. The same volume of acetone was applied to the left ear by the method of Zanini et al. (1992). Four hours after treatment, mice were killed by cervical dislocation and a plug (6 mm in diameter) was taken from both treated and untreated ears with a punch. The inflammatory response (edema) was monitored by measuring the differences in weight (mg) between the two plugs.

Statistical analysis

Data were analyzed by one-way ANOVA followed by the Student's *t*-test for unpaired samples (Sokal & Rohlf, 1981). The data were reported as mean \pm SEM. *P* values less than 0.05 ($p < 0.05$) were considered to be significant.

Results

Effects on gross behavior

In the general test of pharmacological activity the animals exhibited analgesia proportionally to the doses applied. The highest dose of ExEtOH i.p. caused death after 1 h of the treatment.

Acetic acid-induced abdominal writhing

The ExEtOH (1.0 g/kg) p.o. or i.p. produced a significant inhibition (48.5 ± 3.5 (p.o.), 26.6 ± 4.4 (s.c.), respectively) of the acetic acid-induced abdominal writhings in relation to the control value (76.3 ± 7.8 , Figure 1).

Tail flick test

The ExEtOH was inactive in the tail flick model of analgesia even at the highest dose tested (1.0 g/kg). Morphine, used as the reference drug, produced an antinociceptive effect at all observation times when compared to control values (Figure 2).

Carrageenan-induced peritonitis

Pretreatment with ExEtOH (0.1, 0.3, and 1.0 g/kg) significantly reduced the total leukocyte migration to the peritoneum induced by carrageenan compared to the control group. ExEtOH administered subcutaneously caused inhibition of total leukocyte migration from (0.70

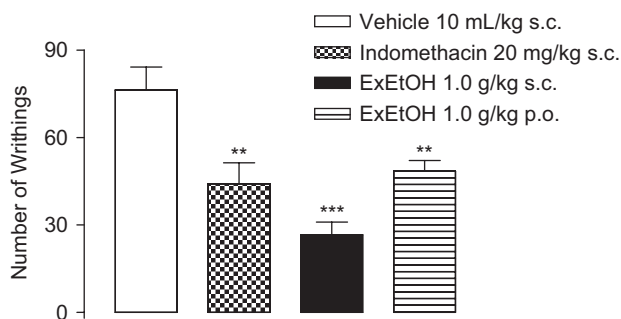


Figure 1. Effect of the alcohol extract of *Pterodon emarginatus* stem bark (ExEtOH 1.0 g/kg, p.o. and s.c.) on the number of acetic acid-induced abdominal writhings. The vertical bars represent the means \pm SEM. Indomethacin (20 mg/kg, s.c.) was used as positive control. ** $p < 0.01$ and *** $p < 0.001$.

± 1.5 , 0.63 ± 0.61 and 0.50 ± 0.37 , respectively) in relation to the control (1.18 ± 1.2 , Figure 3).

Croton oil-induced ear edema test

The ExEtOH (0.1, 0.3, and 1.0 g/kg) produced a significant inhibition the croton oil-induced ear edema (13.1 ± 1.0 , 10.7 ± 1.4 and 8.0 ± 1.9 , respectively) in relation to the control (18.7 ± 1.1 mg, Figure 4).

Discussion and conclusions

The analgesia observed in the general test of pharmacological activity indicated the presence of compounds with analgesic action in ExEtOH. Two different analgesia models have been used to evaluate the antinociceptive effect of the ExEtOH: the tail flick test and the acetic acid-induced writhing test in mice. The ExEtOH had no effect in the tail flick test, but inhibited significantly writhing movements.

The acetic acid introduced by the intraperitoneal route indirectly acts to cause the release of involved endogenous mediators in the modulation of the nociceptive action, in which we can cite bradykinin, serotonin, histamine, PGs, among others, that would be responsible for stimulation of nociceptive neurons and consequently the induced pain (Whittle, 1964; Berkenkopf & Weichman, 1988). The model of acetic acid-induced abdominal writhing is relatively simple, with low specificity but good sensitivity to several drugs that exert analgesic activity, steroidal and non-steroidal anti-inflammatory agents, as well as central and peripheral acting opioid analgesics.

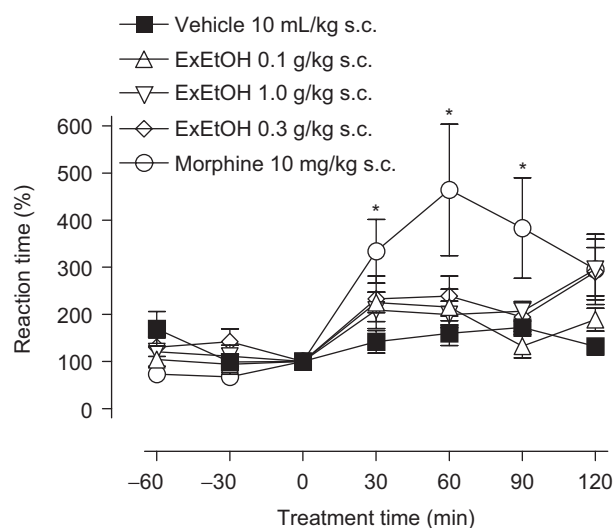


Figure 2. Effect of the previous treatment with alcohol extract of *Pterodon emarginatus* stem bark (ExEtOH 0.1, 0.3, or 1.0 g/kg, s.c.) in the tail flexion. Morphine was used as positive control. The points indicate the means \pm SEM expressed in relative percentage to the zero time. $p < 0.05$.

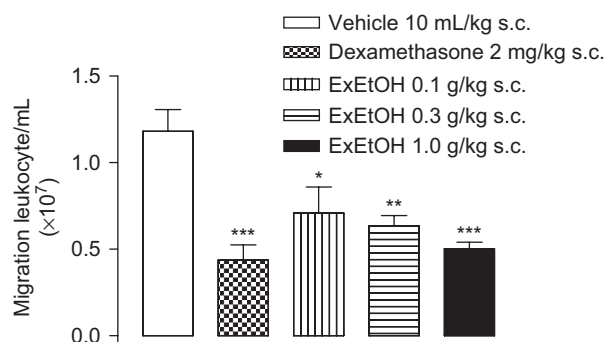


Figure 3. Effect of the previous treatment with alcohol extract of *Pterodon emarginatus* stem bark (ExEtOH 0.1, 0.3, or 1.0 g/kg, s.c.) on total leukocyte migration. The vertical bars represent the means \pm SEM. Dexamethasone (2 mg/kg s.c.) was used as positive control. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

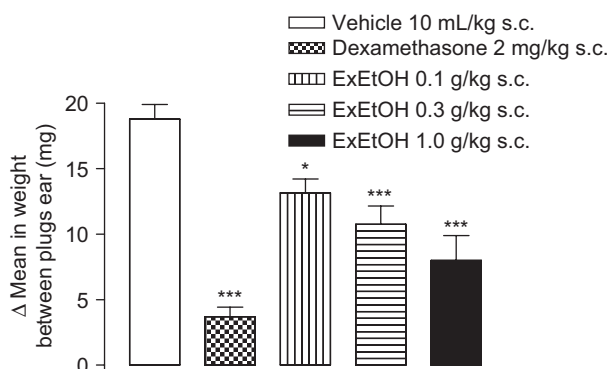


Figure 4. Effect of the previous treatment with alcohol extract of *Pterodon emarginatus* stem bark (ExEtOH 0.1, 0.3, or 1.0 g/kg, s.c.) on croton oil-induced ear edema in mice. The vertical bars indicate the means \pm SEM of differences in weight between right and left ear plugs. Dexamethasone (2.0 mg/kg, s.c.) was used as a positive control. * $p < 0.05$ and *** $p < 0.001$.

The tail flick test is a commonly used method to study the effects of analgesic drugs similar to morphine, as it is for drugs that act on the CNS (Carter, 1991). The results show the morphine produced analgesia. However, the ExEtOH did not have an antinociceptive effect, even at the highest dose tested (1.0 g/kg), suggesting that the ExEtOH does not contain compounds with central analgesic properties.

Although the ExEtOH doesn't contain compounds with central analgesic activity, inhibition of the number of the writhings in mice suggests the extract contains compounds with peripheral analgesic and/or anti-inflammatory activity. In an inflammatory process, a series of cellular and vascular events occur, where some mediators derived from cells and the plasmatic activation produce some signals characteristic of inflammation. Vascular alterations immediately occur after the beginning of the inflammation, prevailing for some hours. They consist of vasodilation, increase of sanguine flow, increase of the vascular permeability and plasmatic

exudation (Williams, 1983). Therefore, we studied the ability of the ExEtOH to inhibit the carrageenan-induced peritonitis test, where the ExEtOH showed anti-inflammatory activity, possibly explained by the interference of chemotactic mechanisms. This pharmacologic model allows the quantification of leukocyte migration to the peritoneal cavity under the action of chemotactic agents, mainly LTs and ILs. The carrageenan induces an acute inflammation involving sequential release of some pro-inflammatory mediators, mainly the histamine, kinins, serotonin, thromboxanes and prostaglandins (Di Rosa et al., 1971; Damas et al., 1990). In the carrageenan-induced peritonitis in rats, the antiexudative effect provoked for the non-steroidal anti-inflammatory could be associated with PGE2 reduction, and reduction of the activity of phospholipase A₂ enzyme, while the anti-inflammatory activity of the steroidal anti-inflammatory would be explained by reduction of leukocyte migration (Mikami & Miyasaka, 1983). In conclusion, this work demonstrated that alcohol extracts from stem bark of *Pterodon emarginatus* develop anti-inflammatory and analgesic activities, which may support the popular use of this plant in analgesic preparations. Further studies are currently underway in our laboratory to isolate and identify the compounds responsible for these activities.

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