Effects of ethanolic extract of leaves of *Lafoensia pacari* A. St.-Hil., Lythraceae (pacari), in pain and inflammation models

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INTRODUCTION

*Lafoensia pacari* A. St.-Hil. is a shrubby plant, of Lythraceae family, natural of Brazilian savannah, ciliary forest and altitude forest, being present since Amapá to Rio Grande do Sul, and also in Paraguay and Bolivia (Santos, 2006). It is used in folk medicine to treat gastric ulcers and inflammation, wounds, itchiness and for slimming (Tonello, 1997); also there are relates of its use as antithermic, scar healing and tonic (Mundo & Duarte, 2007).

The stem bark ethanolic extract has shown an expressive anti-inflammatory activity, through inhibition of eosinophilia in *Toxocara canis*-induced infection, not by an antiparasitary action, but by decreased levels of IL-5, a cytokine involved in differentiation, proliferation and activation of eosinophils (Rogerio et al., 2003). Also it was demonstrated related activity to asthma prevention by reduction of eosinophilia and cytokine expression (IL-4, IL-5 and IL-13) related (Rogerio et al., 2008). Furthermore, an antinociceptive and sedating activity of aqueous extract of pacari’s stem bark was also observed.
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Phytochemical prospection studies of the hydroalcoholic stem bark extract of pacari indicated the presence of tannins, steroids, triterpene and saponins. It was also verified that ellagic acid is the major compound in the ethanolic extract of stem bark, being the principal responsible for anti-scavenging effects (Solon, 2000), gastric anti-secretive and bactericide (Menezes et al., 2006).

Pacari is actually classified as vulnerable, on risk to be ranking on risk of extinction, once it has been indiscriminately explored by its therapeutic properties, besides the destruction of its habitat (Fachim & Guarim, 1995). The stem bark collect for therapeutic uses causes plant annealing, which leads it to death (Tonello, 1997).

The aim of this work was to verify if the ethanolic extract of pacari leaves (EEPL) presents analgesic and anti-inflammatory activity, in order to suggest this part of the plant to be further used on phytopharm or phytotherapic production. Thus, contributes to cerrado’s preservation, which contains this specimen, from the valorization and validation of folk known and the sustainable utilization of this important resource.

MATERIALS AND METHODS

Plant material

The Lafoensia pacari A. St.-Hil., Lythraceae, leaves were collected in a modified cerrado region, in Bela Vista city, Goiás, Brazil (837 m, 16° 58’ 54.2” S, 40° 55’ 45.1” W), on May 2007. Samples were authenticated by Dr. José Realino de Paula (Pharmacy Faculty/UFG) and a voucher specimen was deposited at the Federal University of Goiás herbarium (n° 27031). The leaves were dried at 40°C with forced ventilation and powdered in Willey mill. Three samples of extract were prepared by maceration of leaves powder (15%) in ethanol (70%) for three days, it was filtered, and then, the crude ethanolic extract was concentrated under low pressure. The income was obtained by dry weight method and at time of use, the extract was solubilized in saline solution, at the required concentrations.

Animals

Male albino Swiss mice (25 to 35 g), which were provided by the Central Animal House of Federal University of Goiás (UFG) were used in this study. The animals were maintained under controlled conditions of temperature and light (12 h dark/light), with water and food ad libitum, being acclimatized for 72 h before the beginning of the experiments, in according to SBCAL normatization. All proceedings and experimental models were executed in accordance with the International Guiding Principles for Biomedical Research Involving Animals from Council for International Organizations of Medical Sciences (CIOMS), 1985. The experimental protocols were approved by Research Ethic Council of Federal University of Goiás.

Drugs

Acetone (Isofar, Brazil); Carrageenan (Sigma, USA); Croton-oil (Sigma, USA); Dexamethasone (Hipolabor, Brazil); Ethanol 95% P.A. (Synth, Brazil); Formaldehyde (Synth, Brazil); Glacial acetic acid (Synth, Brazil); Heparin (Hipolabor, Brazil); Indomethacin (Prodome, Brazil); PBS-phosphate solution; Sodium pentobarbital (Cristália, Brazil); Türk solution (Bioshop, Brazil).

Acetic acid-induced abdominal writhings

Groups of eleven mice were treated with vehicle (10 mL/kg, p.o.), or EEPL (1.0 g/kg p.o. or s.c.), or indomethacin (10 mg/kg p.o., used as positive control), according the method described by Hendershot & Forsaith (1959) and Koster et al. (1959).

Formalin test

Groups of eleven mice were treated p.o. with vehicle (10 mL/kg), or EEPL (1.0 g/kg), or indomethacin (10 mg/kg, used as positive control), according the method described by Hunskar & Hole (1987).

Croton oil-induced ear edema test

Groups of nine mice were treated p.o. with vehicle (10 mL/kg), or EEPL (1.0, 0.3 and 0.1 g/kg), or dexamethasone 2 mg/kg (used as positive control), according the method described by Zanini et al. (1992).

Carrageenan-induced peritonitis

Groups of nine mice were treated p.o. with vehicle (10 mL/kg), or EEPL (1.0, 1.5 and 2.0 g/kg), or dexamethasone 2 mg/kg (used as positive control), according the method described by Ferrándiz & Alcaraz (1991).

Central Nervous System Activity

The methods performed to evaluate CNS activity were standardized on Laboratory of Natural Products Pharmacology, using Diazepam as positive control (Oliveira et al., 2008).

Rota rod test

Groups of ten mice were treated p.o. with vehicle.
(10 mL/kg) or EEPL (1.0, 0.3 and 0.1 g/kg) according the method described by Duham & Miya (1957), and was count the standing time on rota rod and the falls.

**Open field test**

This model was performed based on method described by Sielgel (1946) and validated by Archer (1973), with adaptations based on dark-light box proposed by Costall et al. (1989). The animals (n=10) were treated p.o. with vehicle (10 mL/kg), extract (EEPL 1.0, 0.3 and 0.1 g/kg). One hour after the treatments, the mice were conducted, one at time, to an open circular field, divide in thirteen parts with equal area, in dark ambient, with one single lamp above the center of the field. They were observed for 5 min, had being evaluated: exploratory activity (total number of invaded squares and in peripheral and central regions - light and dark, respectively), number of fecal balls, number of rearing and number of grooming.

**Pentobarbital-induced sleep**

Groups of nine mice were treated p.o. with vehicle (10 mL/kg) or EEPL (1.0, 0.3 and 0.1 g/kg) according the method described by Carlini & Burgos (1979).

**Statistical analysis**

The results were demonstrated as mean±SEM. The difference among the groups was verified using ANOVA and Kruskall-Wallis. The degree of significance was verified trough Tukey and Dunn tests. Were considered significant values whose \( p < 0.05 \) (Sokal & Rohlf, 1981).

**RESULTS**

**Extractive process**

With pacari leaves maceration (10%) in ethanol/ water 70% v/v it was obtained 35.7% yield to EEPL, determined by dry weight method.

**Acetic acid-induced abdominal writhings**

The leaves ethanolic extract produced a significant reduction in number of acetic acid-induced writhings. The previous treatments (1 h) with EEPL 1.0 g/kg by oral or subcutaneous ways reduced the number of writhings to 67.7±2.8 and 35.7±6.0, respectively, in relation to control value (vehicle 10 mL/kg) 85.5±3.7 (Figure 1).

**Formalin test**

The previous treatment (1 h) with EEPL 1.0 g/kg p.o. reduced the licking time (time of licking the limb after intraplantar injection of formalin), as in the neurogenic phase: from control value (vehicle 10 mL/kg) 62.9±4.8 s to 47.1±4.3 s, as in the inflammatory phase: of 109.36±8.94 s to 79.1±5.9 s (Figure 2 and 3).

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**Figure 3.** Effects of ethanolic extract of pacari (*Lafoensia pacari*) leaves (EEPL 1.0 g/kg *p.o.* in 1.2% v/v formaline-induced licking time in mice - second phase (15-30 min). Indomethacin (10 mg/kg *p.o.*) was used as positive control. Vertical bars represent mean±SEM of pain reaction time, in seconds. *p*<0.05; **p**<0.01.

**Figure 4.** Effects of ethanolic extract of pacari (*Lafoensia pacari*) leaves (EEPL 1.0, 0.3 and 0.1 g/kg *p.o.*) in croton oil-induced ear edema in mice. Dexamethasone (2.0 mg/kg *p.o.*) was used as positive control. Vertical bars represent mean±SEM of difference between left and right ear plugs, in milligrams. *p*<0.05; **p**<0.01; ***p**<0.001.

**Carrageenan-induced peritonitis**

There was significant reduction on the number of migrated leukocytes/mL to the abdominal cavity, on the two biggest doses of the extract (2.0 and 1.5 g/kg, *p.o.*), in a dose- dependent manner, in relation to control value, from 1.22±0.09 x 10⁷ leukocytes/mL (vehicle 10 mL/kg *v.o.*) to 0.7±0.06 x 10⁷ leukocytes/mL (EEPL 2.0 g/kg *p.o.*) and 0.91±0.06 x 10⁷ leukocytes/mL (EEPL 1.5 g/kg *p.o.*). The group treated with smallest dose (EEPL 1.0 g/kg) does not exhibit significant difference, showing value of 1.18±0.05 x 10⁷ leukocyte/mL (Figure 5).

**Rota rod test**

The previously treated animals, *p.o.*, with extract (EEPL 1.0, 0.3 and 0.1 g/kg, *n*=10) did not showed significant difference on number of falls in rota rod in relation to animals of control group. Also the standing time in the apparatus did not suffer significant difference, from control value (Table 1).

**Open field test**

Table 1 shows that previous treatments with EEPL on used doses did not significantly alter the analyzed parameters, in relation to animals treated with vehicle 10 mL/kg.

**Pentobarbital-induced sleep**

The values, in seconds, observed to sleep induction for vehicle and EEPL (1.0, 0.3 and 0.1 g/kg) showed that there is no significant difference in none of the tested doses, when compared to control group (Table 1). The values for sleep recovery, in minutes, for vehicle and EEPL 1.0, 0.3 and 0.1 g/kg, did not show significant difference too (Table 1).

**DISCUSSION**

The antinociceptive effect of ethanolic extract of pacari leaves (EEPL) was shown in two different analgesia models: the acetic acid-induced writhing test and formalin test, in mice. When tissues and cells suffer a noxious stimulus, chemical mediators are released and stimulate C fibers, causing local pain (Driessen, 2007). Acetic acid itself can cause pain, and also stimulates cytokines release (IL-1β, TNF-α and IL-8) by macrophages and basophiles.
The pacari’s stem bark extract fractionation leads to identification of ellagic acid as the major compound, being the responsible for anti-inflammatory and anti-edematous actions observed in murine model of asthma (Rogerio et al., 2006). Ellagic acid was also identified in Lafoensia pacari leaves trough High Performance Liquid Chromatography (HPLC) (Contin & Solon, 2004).

Anti-inflammatory action was also showed in carrageenan-induced peritonitis, when it was observed a significant reduction on the number of migrated leukocytes to abdominal cavity in the two bigger doses of extract, in a dose-dependent manner. It’s possible that the inhibition of leukocyte migration was due to reduction by ellagic acid in level of some cytokines, especially interleukins, in fact, once demonstrated in other experimental models (Rogerio et al., 2006).

In order to investigate a possible central depressor, sedative or miorelaxant effect of extract in mice (what could interfere in the writhing test), were realized central activity tests: rota rod, open field and pentobarbital-induced sleep tests.

All of them showed no influence of EEPL on central activity on tested doses, what reinforces the safety of its utilization, once does not affect motricity nor causes hypnosis or sedation. Other works have shown the high antioxidant ability of leaves ethanolic extract (Marçal et al., 2004).

The results of this study provide evidences that EEPL maintain analgesic and anti-inflammatory effect observed in stem bark extracts (Matos et al., 2008). The collect of leaves is less damage to preservation of this cerrado’s native species, thus, the results not only confirm the folk utilization of this plant, but also give support for propagation of other kind of utilization - ethanolic extract of leaves. In such a manner, avoid the three anneling and, consequently, its degradation.

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