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Anti-inflammatory and antioxidant properties of hydroalcoholic crude extract from *Casearia sylvestris* Sw. (Salicaceae)

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ABSTRACT

Ethnopharmacological relevance: *Casearia sylvestris* Sw. is widely used in popular medicine to treat inflammatory conditions.
Aim of the study: To investigate the anti-inflammatory and antioxidant properties of hydroalcoholic crude extract (HCE) taken from *Casearia sylvestris* Sw. (Salicaceae).
Methods and results: The effect of the HCE from this plant (3–300 mg/kg) on the reduction of inflammatory response to carrageenan was investigated in pleurisy in rats (intrapleural, 2% in 0.2 mL) or paw edema in mice (intraplantar, 300 μg/20 μL, right hind paw). The plant anti-inflammatory action was assessed by its capability in inhibiting cell migration, enzymatic activity of myeloperoxidase (MPO) and production of nitrite/nitrate or edema. The *in vitro* antioxidant activity of this extract against lipid peroxidation and damage to proteins was assessed as possible pathways to contribute as anti-inflammatory mechanisms. Carrageenan-induced hind paw edema (739.3 ± 11.9 μm) was reduced by HCE (30 mg/kg: 462.8 ± 28.38 μm) to similar extents as dexametasone (365.1 ± 16.7). In pleurisy, treatment of the animals with HCE (100 mg/kg: 0.010 ± 0.001 mU/mg of protein) also reduced MPO activity augmented by carrageenan (0.020 ± 0.001 mU/mg of protein) as well as leukocytes migration (carrageenan: 17.8890 ± 2.3900 leukocytes/mL, HCE 100 mg/kg: 7.0880 ± 9631 leukocytes/mL). Significant effects were also observed in animals treated with different doses of HCE in biochemical tests for oxidative stress analysis.
Conclusion: The anti-inflammatory and antioxidant effects of HCE from *Casearia sylvestris* Sw. suggests a potential therapeutic benefit of this plant in treatment of inflammatory conditions.

1. Introduction

The word “guaçatonga” has its origin in the Tupi-Guarani language and within Brazil it is the most commonly used name for the plant *Casearia sylvestris* Sw. The use of this plant in folk medicine until the present day is closely linked to that employed by different traditional indigenous communities from both Brazil and other Latin American countries, as recently reviewed by Ferreira et al. (2011). This latest work has also recognized the traditional therapeutic uses of different parts of the plant by diverse populations from many countries around the world, showing it is largely employ for treating different disorders of inflammatory origin, such as gastric ulcers and wounds, as well as being used for its analgesic and antipyretic agents.

In Brazil, some authors have tried to relate the common folk uses of the plant with its biological activities. After taxonomic identification of *Casearia sylvestris*, these studies employed *in vitro* or *in vivo* assays and demonstrated antiviral (Simões et al., 1999), antibacterial (Alves et al., 2000), antinociceptive (Mattos et al., 2007) and protective actions against snake venom (Cavalcante et al., 2007). Aqueous extract of the plant has also inhibited the activity of inflammatory enzymes such as phospholipase A_2 (Borges et al., 2000) and metalloproteases (Borges et al., 2001) present in the venom of snakes and bees. In addition to this, the

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antiulcer activity for the same type of extract (Basile et al., 1990) and the antiedematogenic action observed in rats for the essential oils found in the plant's leaves (Esteves et al., 2005) were verified. The hydroalcoholic crude extract (HCE) was also notable for its ability to decrease the nociceptive behavior in models of inflammatory pain (Ruppelt et al., 1991).

The data presented above are consistent with an important anti-inflammatory property for this plant and also consistent with the fact that between the various groups of chemical constituents of the *Casearia sylvestris* Sw., the leaves are rich in flavonoids, a class with notorious anti-inflammatory effects. Besides this, the plant was recently recognized as a member of the Salicaceae family (Ferreira et al., 2011), known to include another specie recognized for its significant anti-inflammatory action (*Salix alba*), from which analogues of salicylic acid are obtained.

Taking the above discussion into account, the present study aimed to investigate the anti-inflammatory activity of HCE of *Casearia sylvestris* Sw. in the animal models of pleurisy and paw edema induced by carrageenan, as well its possible antioxidant activity.

2. Material and methods

2.1. Preparation of the hydroalcoholic crude extract (HCE) of *Casearia sylvestris Sw.*

The plant was collected from the botanical Horto at the campus of University Southern Santa Catarina (UNISUL), in the municipality of Tubarão, Santa Catarina, Brazil (latitude 28°28′00″ south and longitude 49°00′25″ west) during the summer of 2009 and was identified with the assistance of Professor Jasper Zanco (Course of Agronomy, UNISUL), by direct comparison with a voucher specimen (SRS-174) deposited at the herbarium *Laelia Purpurata* (SRS) at this university.

Leaves from the plant were used for HCE preparation. They was dried, minced and standardized through sieves, using particles ranging between 250 μm and 850 μm. The extract was produced at a ratio of 1:3 of plant and solvent with 70% ethanol used as extractor liquid. The plant was engorged in a minimal volume of ethanol and the remaining quantity of extractor liquid was subsequently added to this material. This process of maceration was dynamic, with the extractor liquid remaining in contact with the plant for 10 days under constant agitation in a closed container at room temperature. At the end of this process, the extract was filtered using a vacuum pump.

HCE of *Casearia sylvestris* Sw. was then concentrated in rotavapor under reduced pressure to evaporate the ethanol. This process continued with the extract being deposited in a round bottom container which was placed within a water bath at a temperature of 35 °C to 40 °C until total ethanol evaporation had occurred. The concentrated HCE of *Casearia sylvestris* Sw. was then frozen and lyophilized, and placed in posterior storage in a desiccator until required for use.

2.2. Animals

Male Swiss (25–30 g) mice and Wistar rats (280–300 g) were used in this research. They were maintained under standardized conditions of light (light–dark cycle of 12 h) and temperature (22 ± 2 °C), with free access to food and water. Experiments were conducted from 8:00 a.m. to 5:00 p.m., after acclimatization of the animals to the laboratory. All experiments were approved by the Ethics Committee on Animal Use from the university (register number 09.497.2.10 IV).

2.3. Evaluation of anti-inflammatory activity for HCE of *Casearia sylvestris Sw.*

The possible effects of the plant extract on inflammatory response were investigated on carrageenan-induced hind paw edema in mice or pleurisy in rats, as described below.

2.3.1. Carrageenan-induced hind paw edema in mice

This experiment was performed as previously described by Levy (1969). Edema was induced by intraplantar (i.pl.) λ-carrageenan injection (300 μg/paw, in 30 μL of sterile saline) in the right hind paws of the mice. Prior to this, the thickness of the hind paws were registered (μm) with the use of a digital micrometer and recorded as baseline values.

One hour before this, different groups of animals were orally treated with sterile saline (vehicle, control group), dexamethasone 0.5 mg/kg (in sterile saline) or different doses of HCE of the plant (in sterile saline, doses of 3 mg/kg, 10 mg/kg, 30 mg/kg, 100 mg/kg or 300 mg/kg). After i.pl. injections of the inflammatory agent carrageenan, as described above, individual right hind paw thickness was further measured at 1 h, 2 h, 3 h, 4 h, 5 h and 6 h post injection. Edema was expressed as the difference between the thickness of treated paws after (tested post administration times) and before carrageenan administration (μm) for each animal.

2.3.2. Determination of myeloperoxidase (MPO) enzymatic activity in the hind paw

At the end of the carrageenan-induced hind paw edema analysis (Section 2.3.1), the animals were sacrificed and their right hind paws were removed and analyzed for MPO activity according the method used by Suzuki et al. (1983), with minor modifications. Tissues were homogenated (50 mg/mL) in 0.5% of hexadecyltrimethylammonium bromide and centrifuged at $15{,}000 \times g$ for 40 min. The suspension was sonicated three times for 30 s. An aliquot of the supernatant solution was then mixed with tetramethylbenzidine 1.6 mM and H_2O_2 1 mM. The MPO activity was colorimetrically determined using a spectrophotometer, by analyzing changes in absorbance at 650 nm at a temperature of 37 °C. To compare the effect of carrageenan on MPO activity, the values obtained from samples of the control group were compared to those obtained from the left hind paw of the same animals, which did not received any treatment (naïve group).

2.3.3. Carrageenan-induced pleurisy in rat

The procedure for induction of pleurisy followed that described by Vinegar et al. (1973), with minor modifications. Different groups of rats were anesthetized intraperitoneally (i.p) with a solution of ketamine (10 g/100 mL) and xylazine (2.0 g/100 mL (equal parts) and pleurisy was induced by intrathoracic administration of λ-carrageenan (2% in 0.2 mL) into the pleural cavity, via the intercostal space.

2.3.4. Determination of leukocytes migration to the bronchoalveolar lavage (BAL)

In order to evaluate the potential activity of the plant on leukocytes migration in this model, the animals were orally treated 1 h after the pleurisy induction with sterile saline (vehicle, 0.1 mL/100 g), acetylsalicylic acid (ASA, 100 mg/kg) or different doses of HCE of *Casearia sylvestris* Sw. (30–300 mg/kg). The final group received an intercostal injection of sterile saline (same volume) in the pleural cavity and was orally treated with the same solution (under the same conditions). These animals were considered as the basal (vehicle, control group).

After 4 h, the animals were sacrificed, their thorax were opened and the pleural cavity were immediately washed with sterile