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Anti-inflammatory effects and acute toxicity of hydroethanolic extract of *Jacaranda decurrens* roots in adult male rats

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ABSTRACT

Ethnopharmacological relevance: Jacaranda decurrens subsp. *symmetrifoliolata* Farias and Proença (Bignoniaceae) is a species traditionally used for the treatment of inflammatory diseases. However, until this moment, there is no scientific evidence of these effects.
Aim of study: To evaluate the anti-inflammatory effects of hydroethanolic root extract of *Jacaranda decurrens* in rats and to determine the safe of this plant after acute exposure.
Materials and methods: The acute toxicity of *Jacaranda decurrens* root extract (EJD) was evaluated by oral administration to male rats as single doses of 0; 500; 1000 or 2000 mg/kg body weight. General behavior and toxic symptoms were observed for 14 days. The anti-inflammatory activity was evaluated in carrageenan-induced inflammatory paw edema and myeloperoxidase activity in male rats.
Results: No signs of acute toxicity were observed, indicating that the LD_{50} is greater than 2000 mg/kg. EJD (100 and 300 mg/kg) significantly reduced edema formation and at higher dose, the reduction was similar to dexamethasone. A significant decrease in myeloperoxidase activity was also observed.
Conclusions: The present study shows that *Jacaranda decurrens* extract has anti-inflammatory properties in rats without causing acute toxicity. These properties observed may be due to the presence of bioactive constituents such as ursolic acid.

1. Introduction

Steroidal or nonsteroidal anti-inflammatory drugs (NSAIDs) have a number of adverse effects (Batlouni, 2010) and, there is considerable interest in identifying new anti-inflammatory agents obtained from plants used in popular medicine.

Jacaranda decurrens subsp. *symmetrifoliolata* Farias and Proença (Bignoniaceae), traditional known as "carobinha-do-campo", "carobinha" or "caroba", is an endemic species found in the Brazilian states of Goias, Mato Grosso, Mato Grosso do Sul, Minas Gerais and São Paulo (Bertoni et al., 2010). According to folk medicine, the leaves and/or the roots were prepared in form of infusion, decoction and "garrafadas" against inflammatory diseases and infections (Tresvenzol et al., 2006).

Pharmacological evaluations have revealed that species from *Jacaranda* genus possess antioxidants (Carvalho et al., 2009), antimicrobials (Zatta et al., 2009), chemopreventives properties (Subbaramaiah et al., 2000). It is suggested that the responsible for these activities are compounds such as ursolic acid. Phytochemical analyses of the leaves of *Jacaranda decurrens* indicated the presence of triterpenes, ursolic acid, oleanolic, flavonoid, saponins and coumarins (Carvalho et al., 2009; Zatta et al., 2009).

Due to the use of *Jacaranda decurrens* roots in folk medicine to combat inflammatory diseases, without scientific evidence of this potential therapeutic application, the aim of the study was to evaluate the anti-inflammatory effects of hydroethanolic extract of *Jacaranda decurrens* (EJD) roots in male rats and to determine the toxicity of this plant after acute exposure.

2. Materials and methods

2.1. Plant material, preparation and isolation of extract

Jacaranda decurrens subsp. *symmetrifoliolata* roots were collected (April 2010) in the Medicinal Plant Garden of the Federal University of Grande Dourados. A voucher specimen was identified by Dr. Rosana Farias Singer and deposited (register: W.G.

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Garcia 14.008) in the Herbarium of the Department of Botany at the Biology Institute of the State University of Campinas.

The dry roots (560 g) of *Jacaranda decurrens* were extracted with 0.7 L of ethanol:water (70:30) at room temperature. Extracts were united, filtered and concentrated under vacuum and lyophilizer. During the treatment, the extract was dissolved in a hydroethanolic solution.

The EJD (1.14 g) was dissolved in water (0.2 L) and fractionated by XAD-2 (Supelco, Bellefonte, PA, USA) resin column chromatography (30 × 3 cm) eluted with water (0.3 L), followed by methanol (0.2 L) and with ethyl acetate (0.2 L). The methanolic fraction (0.59 g) was dissolved in methanol (10 mL) and fractionated by Sephadex LH-20 (*Amersham Pharmacia Biotech*, *Uppsala*, *Sweden*), column chromatography (100 × 3 cm) eluted with methanol (0.3 mL/min). In total, 29 fractions of 5 mL were collected. The fractions were combined according to their behavior by thin-layer chromatography (silica gel plates, ethyl acetate/*n*-propanol/water, 140:8:80 by volume, upper phase). Fractions 17 (12.3 mg) resulted in the isolation of the compound oleanolic acid (3.9 mg). Fractions 18 was purified using polyvinypolypyrrolidone (Sigma, St. Louis, MO, USA) column chromatography (10 × 1 cm) eluted with methanol, leading to the identification of the compounds oleanolic and ursolic acids (9.8 mg).

2.2. Animals

Adult male Wistar rats (90 days old, weighing approximately 340 g) from the Federal University of Mato Grosso do Sul, were maintained under controlled temperature (23° C), with a constant 12 h light–dark cycle and free access to food and water. The experimental procedures were in accordance with the Ethical Principles in Animal Research and approved by the Committee for Ethics in Animal Experimentation at the University Center of Grande Dourados (Protocol no. 334/10).

2.3. Acute oral toxicity

The acute toxicity studies were conducted using the OECD (Organization for Economic Cooperation and Development)—Guideline 425 and ANVISA guidelines (Brazilian Health Surveillance Agency) (Brazilian Health Surveillance Agency (ANVISA), 2010; Organisation for Economic Co-operation and Development (OECD), 2008). After 12 h of fasting, the animals were divided into four groups. The treatments were performed by single oral administration at doses 0; 500; 1000 or 2000 mg/kg of body weight of EJD. Animals were observed for signs of toxicity during the first 0.5, 1, 2, 4, 8, and 12 h and at every 24 h for 14 days each. Behaviors parameters, death, the weight, the amount of water and feed were analyzed.

After 14 days of treatment, the animals were weighed and anesthetized (ketamine and xylazine, 25 and 10 mg/kg, respectively). Next, blood samples were collected from the renal vein, with and without anticoagulant (Heparin sodium, Cristália). The blood samples were used to determine the hematology parameters (total and differential leukocyte count, hematocrit, hemoglobin and erythrocyte count), and the non-anticoagulated serum samples were used for biochemical analysis (aspartate aminotransferase—AST, alanine aminotransferase—ALT, gamma-glutamyl transferase—δ-GT, creatinine and urea) (Balani et al., 2011; Organisation for Economic Co-operation and Development (OECD), 2008). The biochemical parameters were determined using the semi-automatic Bioplus Bio200 equipment (Gold Analysis kits).

After that, the animals were euthanised and the vital organs (liver, lung and right kidney) were removed, weighed (absolute and relative to body weight) were determined. For the histopathological evaluation of these organs, the samples were fixed in 10% buffered formalin and processed for histological study by light microscopy. The parameters investigated were: reversible (degeneration) and irreversible cell damage (necrosis and apoptosis), leukocyte infiltration, congestion, extravasation of blood and fibrosis.

2.4. Carrageenan-induced rat paw edema

Different groups of rats were orally treated with EJD (100 and 300 mg/kg), or vehicle. Another group was treated subcutaneously with dexamethasone (1 mg/kg). After 1 h, the animals received a solution of 50 μL carrageenan injection (300 μg/paw) in one of the hind paws. The other paw received the same volume of sterile 0.9% saline. The thickness of the paw edema was measured using a digital micrometer, before the treatment and at 0.5; 1; 2 and 4 h after the carrageenan. Results were expressed as micrometer and the difference between basal and post-injection values quantified as edema (Kassuya et al., 2009).

2.5. Determination of myeloperoxidase activity

In this experiment, a carrageenan non-injected group was inserted and was called as naïve (N) group. Six hours after carrageenan, the skins of paws were removed after euthanasia and the methodology of De Young et al. (1989) was performed with modifications. The tissue was homogenized in phosphate buffer (80 mM, pH 5.4) containing hexadecyltrimethylammonium bromide (0.5%). The homogenate was centrifuged at 12,000*g* (4 °C, 20 min). Thirty microliter of each supernatant were mixed with 100 μl of buffer (80 mM), 85 μl of phosphate buffer (0.22 M) and 15 μl of H_2O_2 (0.017%) in a 96-well plate. The reaction was initiated with 20 μl of 3,3,3-tetramethylbenzidine (dissolved in N,N-dimethylformamide). The plate was kept at 37 °C for 3 min and the reaction stopped by adding sodium acetate (30 μl, 1.46 M, pH 3.0). The enzymatic activity was determined by measuring the optical density (630 nm) and expressed as OD/mg of protein. Protein levels were measured in the supernatants (Bradford, 1976) using a mixture of each sample with Bradford's reactant and after the absorbance was measured.

2.6. Statistical analyses

Data are presented as mean ± SEM. Difference between groups was evaluated by analyses of variance (one-way ANOVA) followed by Newman–Keuls test. Statistical differences were considered to be significant at $P < 0.05$.

3. Results and discussion

Jacaranda decurrens roots are empirically used for the treatment of inflammatory disorders (Nunes et al., 2003); however, its toxic and pharmacological effects on inflammatory experimental models have not been scientifically investigated. To our knowledge, the present study represents the first research into the anti-inflammatory effects and toxicity study of extract of *Jacaranda decurrens* roots. Several pharmaceutical products currently used to treat inflammation are not completely efficient in chronic disease and produce many adverse side effects. Therefore, it is necessary to develop more effective agents that are also less toxic.

No changes in food consumption, water uptake or behavior (irritability, contortion, tremors, convulsions, lacrimation and piloerection) were observed in the animals after acute treatment with EJD. Additionally, the absolute and relative weight of the vital organs, the hematological parameters, biochemical