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Evaluation of the antiulcerogenic and analgesic activities of *Cordia verbenacea* DC. (Boraginaceae)

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ABSTRACT

Ethnopharmacological relevance: Cordia verbenacea is a medicinal plant popularly used in Brazil as anti-inflammatory, antiulcer and anti-rheumatic agent without detailed pharmacological and toxicological studies.
Aim of the study: The study was aimed to investigate the effects of *Cordia verbenacea* in antiulcer, analgesic and antioxidant assays, as well as to evaluate its toxic effects and phytochemical profile.
Material and methods: Antiulcer activity of plant extract was evaluated using ethanol/HCl, ethanol and piroxican-induced gastric lesions methods. The pH, volume and total acid of gastric juice were determined by pylorus-ligated assay. Analgesic activity was evaluated by writhing, tail-flick and hot-plate tests. Antioxidant activity was determined by *in vitro* lipoperoxidation assay. Acute toxicity and number of deaths were evaluated by Hippocratic screening.
Results: The ethanol leaf extract shows a potent antiulcer activity in the ethanol/HCl and absolute ethanol-induced gastric lesions. The IC_{50} value of plant extract on the lipid peroxidation was 76.11 μg/ml. Preliminary phytochemical tests were positive for flavonoids, steroids, saponins, fixed acids, alkaloids and phenols. In the analgesic models the extract did not present any activity.
Conclusions: Cordial verbenaceae showed a potent antiulcer activity at the dose of 125 mg/kg and this effect may be associated with an improvement in stomach antioxidant mechanisms.

1. Introduction

Cordia verbenacea DC. (Boraginaceae) is a shrub medicinal plant popularly known in Brazil as "erva baleeira" and its leaf infusion or decoction are used as anti-inflammatory, analgesic (Di Stasi et al., 2002), antiulcer and anti-rheumatic agents (Correa, 1984). Recently, it was released in the Brazilian market as a phytomedicine with this plant for anti-inflammatory topical use, but despite the wide use and market of this medicinal, there are few data about its pharmacological effects on gastrointestinal system and painful processes as well as of its possible toxic properties and chemical composition. This prompted us to investigate the effects of pharmacological activities of *Cordia verbenacea* in experimental models of gastric ulcer and pain, as well as to evaluate its acute toxicity and qualitative phytochemical profile.

2. Material and methods

2.1. Plant material

The plant material was collected in June 2004, Ubatuba, State of São Paulo, Brazil. The plant species *Cordia verbenaceae* DC. (Boraginaceae) was authenticated by Dr. Neusa Taroda Ranga, from Herbarium SJRP, IBILCE/UNESP, São José do Rio Preto, State of São Paulo, Brazil, where a voucher specimen was deposited under number 28117.

2.2. Preparation of the extracts

The fresh leaves of *Cordia verbenacea* (7300 g) were dried at 50 °C/66 h in a hot house with forced air circulation and renewal. After drying, the plant material was powdered yielding 1876 g (25.7%). Dry and pulverized leaves were submitted to cold maceration in absolute ethanol (4 times × 48 h each). After filtration, the plant residue was discarded and the filtrate taken to 10% of the original volume under reduced pressure at 40 °C. The extract was

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than submitted to chlorophyll extraction, performed according to the method adapted by Ferri, 1996. Briefly, chlorophyll precipitation was produced by adding distilled water by drips (4.0 ml/min until 40% of total volume of concentrated extract) under constant shaking. After this procedure, the extract was maintained in shaking for 1 h, followed by cooling to 4 °C/16 h and filtrated on celite and Whatman paper. The chlorophyll-free extract was further concentrated at 40 °C until total ethanol elimination and separation of a pasty precipitate. The w/w extraction yield of this defatted extract based on dry plant material was 2.8%. The aqueous phase of extraction was discarded because preliminary studies on ethanol and ethanol/HCl-induced damage were negative.

2.3. Animals

Male or female Swiss albino mice (25–50 g) from the Central Animal House of the São Paulo State University (UNESP/Botucatu) were used. The animals were housed in standard environmental conditions (21 °C, 60–70% humidity) under a 12 h light/dark cycle. Female and male mice were used for acute toxicity evaluation and Hippocratic screening. Experimental protocols meet the "Guidelines of Animal Experimentation" and they were approved by the Commission of Ethics in Animal Experimentation of the Institute of Biosciences, São Paulo State University (Unesp), Botucatu, São Paulo, Brazil.

2.4. Antiulcer activity

Antiulcer activity was evaluated using three different models for induction of acute gastric mucosal lesions: ethanol/HCl 0.3 M (Mizui and Doteuchi, 1983), absolute ethanol (Morimoto et al., 1991) and piroxican (Puscas et al., 1997). Mice, fasted for 24 h and kept in raised mesh-bottomed cages to prevent coprophagy, with water *ad libitum*, were used in these tests. The plant extract was administered by oral route at the doses of 125, 250, 500 or 1000 mg/kg. In positive control groups, mice were treated with carbenoxolone (250 mg/kg, p.o.) or cimetidine (100 mg/kg, p.o.) and, in the negative control groups mice received vehicle (Tween 80® 15% at 10 ml/kg). After 1 h of the administration of the extracts, the animals received ethanol/HCl 0.3 M (7 ml/kg, p.o.) or absolute ethanol (4 ml/kg, p.o.). One hour after the administration of the ulcer inductor agent, animals were killed by cervical dislocation. In the model of piroxican-induced gastric lesions the animals received 30 mg/kg (s.c.) 1 h after the administration of Tween 80® 15%, extracts or cimetidine, and were killed after 6 h. The stomachs were removed, opened along the greater curvature, washed in saline 0.9% and fixed between two glass plates. Each stomach was scanned in an HP Scanner Jet and the images were stored for lesion areas measurement with the Assess (Image analysis software for Plant disease quantification) software. The results were expressed as total lesion area (mm^2) and relative lesion area to total stomach area (%).

2.4.1. Ethanol-induced gastric mucosal lesion in L-NAME pre-treated mice

The experiment was performed according to the method described by Arrieta et al. (2003), with some modifications. The mice were treated with 70 mg/kg (i.p.) of L-NAME (an inhibitor of nitric oxide synthase) or saline (i.p.) 30 min before administration of Tween 80® 15% (10 ml/kg), carbenoxolone (250 mg/kg) or plant extract (250 mg/kg). After 1 h, all groups received absolute ethanol (4 ml/kg p.o.) for gastric ulcer induction. One hour after ethanol administration, animals were killed by cervical dislocation and the stomachs excised and gastric damage determined as previously described.

2.4.2. Shay ulcer

Mice (35–50 g), fasted for 24 h with free access to water, were used. Thirty minutes after oral administration or immediately after pylorus-ligature the animals received Tween 80® 15% (10 ml/kg), cimetidine (100 mg/kg) or plant extract (250 mg/kg) to evaluate the local or systemic activity of the plant extract, respectively as described by Shay et al. (1945), with some modifications. Four hours later, the animals were killed by cervical dislocation, the abdomen was opened and another ligature was placed around the esophagus close to the diaphragm. The stomach was removed and the gastric juice volume and the pH were measured. Then, the contents were drained into graduated centrifuge tubes and the volumes were completed to 10 ml with distilled water. The resultant solution was centrifuged at 2000 × *g* for 10 min and the total acid content of gastric secretion was determined by titration at pH 7.0 with 0.01N NaOH using a digital burette (E.M., Hirschmann Technicolor, Germany).

2.5. Antioxidant activity

In vitro experiments were performed in order to test the antioxidant activity at different concentrations (5.68, 11.36, 22.73, 45.45 and 90.91 μg/ml) of *Cordia verbenacea* leaf extract. This extract was evaluated by inhibition of the iron and ascorbic acid-induced lipid peroxidation in rat liver membrane-enriched fraction P2, as described by Gálvez et al. (1995). The flavonoid quercetin (0.19, 0.38, 0.77 and 1.54 μg/ml) was used as reference and tested in the same assay system.

2.6. Analgesic activity

Analgesic effects of *Cordia verbenacea* leaf extract were evaluated using three different models: the writhing test, tail-flick test and hot-plate test. For all tests only the dose of 250 mg/kg was used because it was the most effective dose in the initial studies of gastric ulcer. Animals were fasted for 24 h prior to free access of water.

The writhing test was performed according to the method described by Koster et al. (1959). The animals were treated, through oral route, with Tween 80® 15% (10 ml/kg), acetylsalicylic acid (300 mg/kg) or leaf extract 30, 60, 90 and 120 min before the 0.6% acetic acid (10 ml/kg, i.p.) administration. The mice, kept in individual boxes, were thereafter observed continuously for 20 min and the number of abdominal contortions was recorded.

The tail-flick test was performed according to the method described by Jansen et al. (1963). The basal reaction time of each mouse was determined using tail-withdrawal response when one-third of the tail was immersed in water bath at 51 ± 1 °C. Mice with basal reaction time between 5 and 10 s were selected 24 h before the test. The animals were treated with Tween 80® 15% (10 ml/kg, p.o.), morphine (10 mg/kg, s.c.) or leaf extract (p.o.) and 30, 60, 90 and 120 min later the reaction time was evaluated. The cutoff time for immersion was 180 s.

The hot-plate test was used to measure the latency of the response as described by Eddy and Leimback, 1953 with minor modifications. The temperature of the hot-plate was maintained at 56 ± 1 °C. The mice were placed in a 24 cm diameter glass cylinder on the heated surface and the time between placement and licking of the paws or jumping was recorded as response latency. The group control was treated with oral distilled water and morphine was used as positive control (10 mg/kg, s.c.). Mice were selected 24 h before the experiment on the basis of their reactivity to the test. The animals were observed 30, 60, 90 and 120 min after oral plant extracts administration. The cutoff time was 30 s.