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Antinociceptive effect of *Croton celtidifolius* Baill (Euphorbiaceae)

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Abstract

Croton celtidifolius Baill (Euphorbiaceae) is a tree found in the Atlantic forest of southern Brazil. This plant is used in folk medicine for the treatment of several inflammatory diseases, leukaemia, ulcers and other pathologies. Previous studies demonstrated anti-inflammatory and antioxidant activities and the objective of this work was to investigate a possible antinociceptive action of ethanolic extract of *Croton celtidifolius* bark (EE) and ethyl acetate fraction (EAF), *n*-butanol fraction (FBuOH), and aqueous fraction (FAq) obtained from EE. Two standard rodent models of pain were employed for this investigation, the writhing test and the formalin test. In the writhing test, the pre-treatment with EE significantly reduced the writhing induced by 0.6% acetic acid injection and its effect persisted for 4 h. In the formalin test, the pre-treatment with EAF caused marked and dose-related inhibition of formalin-induced licking in mice in the first phase, while pre-treatment with EAF, FBuOH and FAq had a similar effect in the second phase, when given by intraperitoneal (i.p.) and orally (p.o.) route. However, given by i.p. route, the effect of fractions was about three to five-fold more potent in inhibiting licking than when administered by p.o. route. EE presented an antinociceptive effect only in the second phase, when given by i.p. or p.o. route. The oedema caused by formalin was significantly reduced in animals treated i.p. with EAF, FBuOH and FAq. Under the same experimental conditions, in animals treated with sub-fractions derived from EAF only the 63 sub-fraction significantly reduced nociception in both phases and oedema caused by formalin. The results obtained suggest that *Croton celtidifolius* possesses antinociceptive properties since the EE, fractions and a sub-fraction significantly reduced the writhing induced by acetic acid and the nociception in both phases of the formalin test.

Keywords: *Croton celtidifolius*; Antinociception; Natural products; Medicinal plants; Writhing test; Formalin test

1. Introduction

Several species of the genus *Croton* are described as medicinal plants (Gupta et al., 2004), meaning that some of them have had their biological activities evaluated. Amongst such plants already studied are *Croton lechleri* (Rossi et al., 2003), *Croton cajucara* (Silva et al., 2001; Hiruma-Lima et al., 2002a, 2002b; Freire et al., 2003), and *Croton urucurana* (Gurgel et al., 2001). Some species of *Croton* present multiple activities, such as *Croton cajucara*, for which cytotoxic (Freire et al., 2003), gastroprotective (Hiruma-Lima et al., 2002a, 2002b), antiulcerogenic (Hiruma-Lima et al., 2002b), glucose- and triglyceride-lowering (Silva et al., 2001) and antigenotoxic (Agner et al., 2001) activities have been described. Besides the cited studies, many others are in development concerning the biological activities of extracts, fractions and active components from the plants of this genus.

Croton celtidifolius Baill (Euphorbiaceae) is native to the regions of the Atlantic Forest, being frequently found from the state of Rio de Janeiro to Goiás, São Paulo and the southern region of Brazil. The plant has numerous popular names, depending on the region where it is found, including "Pau-Sangue", "Sangue-de-Dragão", "Sangue-de-Adáve", and others (Smith et al., 1988). Its use is recommended for the treatment of inflammatory and ulcerative diseases,

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either by chewing the bark or making an infusion of the same.

Studies for the chemical characterisation of the constituents of *Croton celtidifolius* are still limited. Mukherjee and Axt (1984) determined the presence of cyclitols, including 1_L-1-*O*-methyl-myo-inositol, neo inositol and sitosterol. Also, the presence was noted of catechins, gallocatechins and proanthocyanidins in fractions obtained from the ethanolic extract of the bark of *Croton celtidifolius* (Nardi et al., 2003). In addition, other authors have also demonstrated the presence of alkaloids and saponins in the bark of this plant (Farnsworth et al., 1969; Barnes et al., 1980; Amaral and Barnes, 1997). However, studies on the biological activities of *Croton celtidifolius* remain scarce. Research carried out with the ethanolic extract and fractions obtained from the bark has revealed anti-inflammatory, antiedematogenic and antioxidant activities (Nardi et al., 2003).

In this study, we extended our previous findings by examining the antinociceptive activity of *Croton celtidifolius* extracts, fractions and sub-fractions in two chemical models of nociception, the writhing and formalin tests, since numerous studies have demonstrated that anti-inflammatory drugs can attenuate the parameters of nociception analysed in these models (Hunskaar and Hole, 1987; Malmberg and Yaksh, 1992).

2. Materials and methods

2.1. Animals

Male Swiss mice (25–35 g) housed at 22 ± 2 °C under a 12 h light/12 h dark cycle and with free access to food and water, were used in the experiments. All animals were acclimatised to the laboratory for 24 h before the experiments. The experiments were performed after approval of the protocol by the Institutional Ethics Committee (no 157/CEUA) and were carried out in accordance with the current guidelines for the care of laboratory animals and the ethical guidelines for investigations of experimental pain in conscious animals (Zimmermann, 1983).

2.2. Plant material

Bark of *Croton celtidifolius* Baill (Euphorbiaceae) was collected in March 2000 at Orleans, Santa Catarina, Brazil. The plant was classified by Dr Daniel de Barcelos Falkenberg (Departamento de Botânica, UFSC, Florianópolis, Brazil) and a voucher specimen was deposited by the authors in the Herbarium, number FLOR 31272.

2.3. Extraction and isolation

The extraction was performed as described previously by Nardi et al. (2003). Air-dried bark (154 g) was chopped into small pieces and extracted three times with 250 mL of 80% aqueous EtOH at room temperature. The combined extracts were filtered and the solvent was evaporated in a vacuum to give 42.9 g of the ethanolic extract. The aqueous suspension of this extract was successively partitioned with ether, ethyl acetate (EtOAc) and *n*-butanol (*n*-BuOH) furnishing an ether (2.0 g), ethyl acetate (EAF) (17.6 g), *n*-butanol (FBuOH) (22.2 g) and aqueous (FAq) (6.9 g) fractions.

The EAF (9.0 g) was separated by chromatography over a column of silica gel water (20%) inactivated and eluted with hexane/EtOAc (4:1) and the polarity was increased by gradual addition of EtOAc and methanol (MeOH). After thin layer chromatography (TLC) analysis, four sub-fractions were obtained, named 11SF, 19SF, 35SF and 63SF. Sub-fraction 11SF was further purified by flash chromatography using 40% hexane/59% EtOAc/1% AcOH as solvent to give catechin and gallocatechin.

2.4. Writhing test

The methodology was the same described previously by Koster et al. (1959). Writhing was induced by an intraperitoneal (i.p.) injection of acetic acid (0.6%) and evaluated by the number of writhing movements in 20 min of observation immediately after the acetic acid injection. Animals were pre-treated orally (p.o.) with EE at doses of 3–300 mg/kg 1 h before the i.p. injection of acetic acid. A time-course was obtained by administering EE (100 mg/kg, p.o.) at 0, 0.5, 1, 2, 3 and 4 h previously. Control animals received vehicle (10 mL/kg) 1 h before, and the positive control animals received acetylsalicylic acid (AAS) (100 mg/kg, i.p.), 30 min before the irritant agent. The results are expressed as mean ± standard error of mean (S.E.M.) and statistical significance was determined by comparing treated groups with the control group.

2.5. Formalin test

The procedure used was essentially the same as that described previously by Hunskaar and Hole (1987). Animals received 20 μL of a 2.5% formalin solution (0.92% formaldehyde) made up in PBS, injected in the ventral surface of the right hind paw. Following the formalin injection, animals were immediately placed in an acrylic observation chamber (15 cm × 15 cm × 15 cm), and the time spent licking/flinching and biting the injected paw was measured with a stopwatch and considered as an indication of nociception (expressed in seconds). The first phase of the nociceptive response normally peaks at 0–5 min, and the second phase 15–30 min after the formalin injection. This later phase is usually accompanied by the development of paw oedema, due to the release of inflammatory mediators. The paw oedema was measured by comparing the difference in weight (in grams) between the left and the right hind paws. For this purpose, animals were killed at the end of all experiments by cervical dislocation, and the paw was cut off at the knee joint and weighed.

Mice were pre-treated with EE and FAq at doses of 10–300 mg/kg, and FBuOH and EAF at doses of 3–300 mg/kg, administered by p.o. route, 1 h before formalin injection. By the i.p. route, animals were treated with FAq, FBuOH and EAF at doses of 3–300 mg/kg, 30 min before formalin injection.

Other groups of animals were treated with sub-fractions derived from the EAF: 19SF, 26SF, 35SF, 51SF, 63SF and with the isolated compounds from the 11SF, catechin and gallocat-