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Antinociceptive properties of the ethanolic extract and of the triterpene 3β,6β,16β-trihidroxilup-20(29)-ene obtained from the flowers of *Combretum leprosum* in mice

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Abstract

The present study examined the antinociceptive effects of the ethanolic extract (EE) and of the triterpene 3β,6β,16β-trihidroxilup-20(29)-ene obtained from the flowers of *Combretum leprosum* in chemical and thermal behavioural models of pain in mice. The EE (10–1000 mg/kg) given orally (p.o.), 1 h prior to testing, produced dose-dependent inhibition of acetic acid-induced visceral pain, with mean ID_{50} value of 131.9 mg/kg. In the formalin test, the EE (10–300 mg/kg, p.o.) also caused significant inhibition of both the early (neurogenic pain) and the late (inflammatory pain) phases of formalin-induced licking, however, it was more potent and efficacious in relation to the late phase of the formalin test, with mean ID_{50} values for the neurogenic and the inflammatory phases of ~300 and 88.8 mg/kg, respectively. The EE (10–1000 mg/kg, p.o.) also caused significant and dose-dependent inhibition of capsaicin- and glutamate-induced pain, with mean ID_{50} values of 160.5 and 38.3 mg/kg, respectively. Furthermore, the triterpene 3β,6β,16β-trihidroxilup-20(29)-ene (1–30 mg/kg), given p.o., 1 h prior to testing, also produced dose-related inhibition of glutamate-induced pain, with a mean ID_{50} value of 5.6 mg/kg. When assessed in a thermal model of pain, the EE (10–300 mg/kg, p.o.) and fentanyl (100 μg/kg, s.c.) caused a significant and marked increase in the latency response on the hot-plate test (50 °C). The antinociception caused by EE (100 mg/kg, p.o.) in the glutamate test was significantly attenuated by intraperitoneal (i.p.) treatment of mice with naloxone (opioid receptor antagonist, 1 mg/kg), pindolol (a 5-$HT_{1A/1B}$ receptor/β adrenoceptor antagonist, 1 mg/kg), WAY100635 (a 5-HT_{1A} receptor antagonist, 0.7 mg/kg) or ketanserin (a 5-HT_{2A} receptor antagonist, 0.3 mg/kg). In contrast, EE (100 mg/kg, p.o.) antinociception was affected neither by L-arginine (precursor of nitric oxide, 600 mg/kg) nor by ondansetron (a 5-HT_3 receptor antagonist, 0.5 mg/kg) i.p. treatment. It was not associated with non-specific effects such as muscle relaxation or sedation. Together, these results indicate that EE produces dose-related antinociception in several models of chemical and thermal pain through mechanisms that involve an interaction with opioid and serotonergic (i.e., through 5-$HT_{1A/1B}$ and 5-HT_{2A} receptors) systems.

Keywords: *Combretum leprosum*; Triterpene; Antinociception; Opioid; Nitric oxide system; 5-HT receptors; Mice

1. Introduction

The plants of the family Combretaceae comprise 20 genera with approximately 600 species, the largest of which are *Combretum*, with about 370 species, and *Terminalia*, with about 200 species (McGaw et al., 2001; Katerere et al., 2003). Species from the genus *Combretum* and to a lesser extent *Terminalia* are most widely used for medicinal purposes (McGaw et al., 2001). These genera are widespread in parts of Africa where they are often the most abundant species. It has been demonstrated that some of the extracts or active principles obtained from *Combretum* species have a broad spectrum of biological activities, including antibacterial, antiprotozoal, anticancer, cytotoxic, analgesic, anti-inflammatory, hepatoprotective and antiviral activities (Nabha et al.,

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2000; McGaw et al., 2001; Griggs et al., 2001; Asres et al., 2001; Adnyana et al., 2001; Fyrquist et al., 2002; Ancolio et al., 2002; Ali et al., 2002; Lira et al., 2002; Olajide et al., 2003; Cirla and Mann, 2003; Nam, 2003; Young and Chaplin, 2004; Martini et al., 2004; Benssong et al., 2005).

Combretum leprosum Mart., a member of the family Combretaceae, is a plant from the north of Brazil, known by the popular name of "mufumbo" or "mofumbo" or "cipoaba" (Lira et al., 2002). *C. leprosum* is the major species of tree reported in the natural habitat of Ceará State (Facundo et al., 1993). Infusions prepared with the aerial (stems, leaves and flowers) part and roots of *C. leprosum* are used in folk medicine for the treatment of haemorrhages and as a sedative (Lira et al., 2002). In spite of the aerial (leaves and stems) parts of *C. leprosum* being used popularly, until the present moment no data exists about the possible biological activity of the flowers of this plant. However, preliminary studies conducted by Lira et al. (2002) have demonstrated that the ethanolic extract (EE) of the roots from *C. leprosum* has antinociceptive activity in two models of pain (tail immersion and formalin tests) in rats and mice.

Phytochemical studies carried out with some species belonging to the genus *Combretum* have demonstrated the occurrence of many classes of constituents, including triterpenes, flavonoids, lignans, non-protein amino acids, among others (Facundo et al., 1993; Masika and Afolayan, 2002; Katerere et al., 2003; Chowdhury and Islam, 2004).

Taking into account the biological activities of *C. leprosum*, it is surprising that no pharmacological study has been carried out on the possible antinociceptive effects of the EE from the flowers of *C. leprosum* up to now. Here, we have therefore examined the possible antinociceptive action of the EE from the flowers of *C. leprosum* in chemical and thermal models of nociception in mice. Attempts have been made to further investigate some of the possible mechanisms that underlie the antinociceptive action of *C. leprosum* extract. In addition, we also analysed the possible antinociceptive effect of the triterpene 3β,6β,16β-trihidroxilup-20(29)-ene isolated from this plant.

2. Materials and methods

2.1. Animals

Experiments were conducted using male Swiss mice (25–35 g), housed at 22±2 °C under a 12-h light/12-h dark cycle (lights on at 06:00 h) and with access to food and water ad libitum. Animals were acclimatised to the laboratory for at least 1 h before testing and were used only once throughout the experiments. The experiments were performed after approval of the protocol by the Institutional Ethics Committee 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). The numbers of animals and intensities of noxious stimuli used were the minimum necessary to demonstrate the consistent effects of the drug treatments.

2.2. Preparation of ethanolic extract, isolation and chemical identification of the active compound

Botanical material was collected in May 2001 at Viçosa, Ceará State, Brazil, and was classified by Dr. Afrânio Fernandes (Universidade Federal do Ceará, Fortaleza) as *C. leprosum* Mart. A voucher specimen of this plant was deposited in the Herbarium Prisco Bezerra of the Biology Department, Universidade Federal do Ceará, Brazil, under number 12446.

The dried flowers (2.7 kg) were powdered and extracted with ethanol (5 l), being stirred and macerated at room temperature (24±3 °C) for approximately 24 h, with this procedure being repeated three times. The solvent was fully evaporated under reduced pressure, and the extract (yield 58.3 g) was concentrated and stored in a freezer at −20 °C until use.

Part of the extract (32.0 g) was chromatographed on a silica gel column, eluted successively with hexane, $CHCl_3$, EtOAc and MeOH, respectively. The fraction eluted with $CHCl_3$ was subjected to column chromatography over silica gel and then eluted with hexane–EtOAc, with increasing polarity. A total of 3 fractions (30 mL each) eluted with hexane–EtOAc (30:70) were combined on the basis of TLC analysis and after removal of the solvent, the precipitate was observed, which, through recrystallization from Et_2O, was identified as being the triterpene 3β,6β,16β-trihidroxilup-20(29)-ene (Fig. 1), previously identified in leaves of this plant (Facundo et al., 1993).

2.3. Drugs

The following substances were used: acetic acid, formalin and morphine hydrochloride (Merck, Darmstadt, Germany); N^{ω}-nitro-L-arginine, L-arginine hydrochloride, capsaicin, L-glutamic acid hydrochloride, naloxone hydrochloride, pindolol, WAY100635 (Sigma Chemical Co., St. Louis, USA); ketanserin tartarate (Tocris Cookson Inc., Ellisville, USA); ondansetron hydrochloride (Cristália, São Paulo, Brazil). The drugs were dissolved in saline, with the exception of EE, triterpene and capsaicin that were dissolved in Tween 80/DMSO plus saline, Tween 80 plus saline and absolute ethanol. The final concentration of Tween 80, DMSO and ethanol did not exceed 5% and did not cause any "per se" effect.

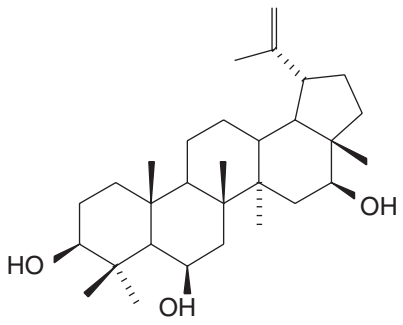

Fig. 1. Molecular structure of triterpene 3β,6β,16β-trihidroxilup-20(29)-ene.