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Original Article

Anti-nociceptive and anti-oedematogenic properties of the hydroethanolic extract of *Sidastrum micranthum* leaves in mice

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ABSTRACT

Sidastrum micranthum (A. St.-Hil.) Fryxell, Malvaceae, grows in the northeastern region of Brazil, where the leaves of this species are traditionally used to treat coughs, bronchitis or asthma. Male Swiss mice (20-22 g) were tested in models of acute pain (acetic acid-induced abdominal writhing, tail flick and formalin test), oedema assessment test (paw oedema test) and model for evaluation of spontaneous motor performance (open field test). The hydroethanolic extract of *S. micranthum* was administered orally at doses of 50-500 mg/kg. In addition were administered water, vehicle, morphine 5.01 mg/kg (evaluation of pain and motor performance) and dexamethasone 2.25 mg/kg (evaluation of oedema formation). The extract showed a significant effect at all doses in the acetic acid-induced abdominal writhing test and at the second phase of the formalin test, while in the first phase of this test and in the paw oedema test only at the highest dose (500 mg/kg). In the formalin and paw oedema tests, the extract had a potentiation of the anti-nociceptive and anti-inflammatory effects by pretreatment with L-NAME and reduction of the effect by pretreatment with L-arginine. The extract was not toxic after oral administration (LD_{50} > 2000 mg/kg).

Introduction

Non-steroidal anti-inflammatory drugs (NSAID) are used worldwide for the treatment of inflammation, pain and fever. However, the side effects of currently available anti-inflammatory drugs include gastric ulcer, renal damage, bronchospasm and cardiac abnormalities, which have limited their use (Burke et al., 2006). Opioids are one of the most powerful drugs for the treatment of pain, nevertheless adverse side effects have limited their use also (Butler et al., 2004). Efforts to eliminate or minimize the undesired adverse effects have led to the search for medicinal plants derived natural products (Cowan et al., 2002; Gilson et al., 2004). In recent years, many researchers have focused on medicinal plants-derived natural products such as flavonoids, steroids, polyphenols, coumarins, terpenes and alkaloids, due to their wide range of pharmacological applications including anti-

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inflammatory, analgesic and antipyretic activities with lesser side effects (Shukla et al., 2010).

Traditional medicine is used throughout the world and has rapidly gained growing economic importance, where modern health services are limited, particularly in developing countries. In these countries traditional medicine represents the only accessible treatment (Agra et al., 2007).

According to estimates by the World Health Organization (WHO, 1999), in many developed countries, a high proportion of the population uses traditional health practices, especially medicinal plants. Although access to modern medicine is available in these countries, the use of medicinal herbs has retained popularity for historical and cultural reasons.

The Malvaceae family consists of 243 genera and 4225 species distributed around the world, predominantly in South America (Gomes et al., 2011b). *Sidastrum micranthum* is an erect shrub, 2-3 m high with a stem up to 4 cm in diameter at base (Shimpale et al., 2009). In Brazil, *Sidastrum micranthum* (A. St.-Hil.) Fryxell, Malvaceae, appears mostly in the northeast region, where the leaves of this species are traditionally used to treat coughs, bronchitis, asthma and rheumatism, among other conditions (Fryxell, 1997). Rural communities in this area do not have access to immediate medical treatment and for this reason the hydroethanolic extract obtained from the leaves of *S. micranthum* is extensively used. In Brazil, *S. micranthum* is commonly known by several names: malva-preta, relógio, vassoura-de-relógio, vassourinha, malva, vassoura-do-campo, guanxuma and mata-pasto (Wagner et al., 1999).

Gomes et al. (2011a) reported the isolation of the triterpene isoarborinol β-sitosterol and the glycosylated steroid 3-*O*-β-D-glucopyranoside, from *S. micranthum* leaves. β-sitosterol and stigmasterol mixture, isolated from *S. paniculatum*, showed a significant anti-inflammatory activity (Cavalcante et al., 2010). Triterpenes and steroids may be responsible for anti-inflammatory and anti-nociceptive effects, properties extensively documented in the literature (Diaz et al., 2000; Gaertner et al., 1999; Geetha and Varalakshmi, 2001; Santos et al., 1995).

The species *Sidastrum micranthum*, despite its popular use, has not been presented with scientific evidence of its effects, especially those related to the control of pain and inflammation. Based on the results of an ethnobotanical survey conducted among traditional medicine practitioners, the aim of this study is to evaluate the anti-nociceptive and anti-oedematogenic activity of the hydroethanolic extract of *Sidastrum micranthum* leaves in mice.

Materials and methods

Plant material

The leaves of *Sidastrum micranthum* (A. St.-Hil.) Fryxell, Malvaceae, were collected in the city of Muniz Freire, ES, Brazil (20º 26' 19'' S e 41º 23' 44'' W) in March 2009. The plant was authenticated by Érika Von Sohsten de Souza Medeiros (Departamento de Botânica Sistemática, Jardim Botânico do Rio de Janeiro, Brazil) and a voucher specimen was deposited in the Herbarium of the Botanical Garden under the number RB492872.

Preparation of the plant extract

The leaves (200 g) were grounded and extracted with 70% hydroethanolic solution for 72 h, filtered and subjected for further extraction for the same amount of time. The filtrates were concentrated in a rotary vacuum evaporator, to obtain the crude hydroethanolic extract. The yield of the extract was about 23% (w/w). The material was stored at -20°C until use.

Phytochemical analysis

The material was partitioned with *n*-hexane, chloroform, ethyl acetate and *n*-buthanol. The *n*-hexane phase was subjected to column chromatography with 7734 silica gel 60, and eluted with *n*-hexane, ethyl acetate and then methanol, gradually increasing the polarity. The identification of the chemical constituents was performed through spectra analysis by infrared and nuclear magnetic resonance ^{1}H and ^{13}C spectroscopy using uni and bi-dimensional techniques, and compared results with the literature.

Chemicals

The following substances were used: acetic acid (Vetec, Rio de Janeiro, Brazil), formalin (Merck, Darmstadt, Germany), dexamethasone, L-NAME, L-arginine, mecamylamine, atropine, λ-carrageenan, 70% ethanol and dimethyl sulfoxide (Sigma-Aldrich, St. Louis, MO, USA), morphine and naloxone (Cristália, São Paulo, Brazil).

Animals

Male Swiss mice (20-22 g) were obtained from the housing facilities of the Department of Physiological Sciences of the Federal Rural University of Rio de Janeiro. The animals were maintained in a room with controlled temperature (22 ± 2°C) and a 12 h light/dark cycle with free access to food and water. Twelve hours before each experiment, the animals received only water, to avoid possible interference of food with the absorption of the drug. The experimental protocol was approved by the Ethics Committee for Animal Research of the Federal Rural University of Rio de Janeiro (COMEP-UFRRJ) (23083.004724/2011-16).

Treatments

Increasing doses of the extract were administered orally (50, 100, 300 and 500 mg/kg *p.o.*). Morphine and dexamethasone were used as positive controls. The doses of the opioid analgesic, morphine (5.01 (2.47-8.68) mg/kg, *p.o.*) and the steroidal anti-inflammatory, dexamethasone (2.25 (1.82-2.79) mg/kg, *s.c.*) were obtained by ED_{50} (confidence limits) calculation in acetic acid-induced abdominal writhing and paw edema tests, that were performed beforehand. The ED_{50} value (the dose producing 50% of the maximal effect) for the anti-nociceptive and anti-oedematogenic actions was obtained by nonlinear regression (sigmoidal dose response) (data not shown).