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Antinociceptive effect of the aqueous extract obtained from roots of *Physalis angulata* L. on mice

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Abstract

In this study, we attempted to identify the possible antinociceptive action of aqueous extract (AE) obtained from roots of *Physalis angulata*, known in Brazil as "Camapu", used to treat various pain-related physiological conditions. The AE of *Physalis angulata* (10–30 mg/kg) given by i.p. or p.o. route, 0.5 and 1 h prior, produced significant inhibition of abdominal constrictions caused by acetic acid, with ID_{50} values of 18.5 (17.4–19.8) and 21.5 (18.9–24.4) mg/kg and inhibitions of 83 ± 8 and $66 \pm 5\%$, respectively. The AE (10–60 mg/kg, i.p.) also caused significant inhibition of the late-phase of formalin-induced pain, with an ID_{50} value of 20.8 (18.4–23.4) mg/kg and inhibition of 100%. Treatment of mice with AE (60 mg/kg, i.p.) or with morphine (10 mg/kg, i.p.) produced a significant increase of the reaction time in the hot-plate test. These results demonstrate, for the first time, that the AE of *Physalis angulata* produce marked antinociception against the acetic acid-induced visceral pain and inflammatory pain responses induced by formalin in mice. The mechanism by which the AE produces antinociception still remains unclear. However, pharmacological and chemical studies are continuing in order to characterize the mechanism(s) responsible for the antinociceptive action and also to identify the active principles present in *Physalis angulata*. Moreover, the antinociceptive action demonstrated in the present study supports, at least partly, the ethnomedical uses of this plant.

Keywords: *Physalis angulata*; Antinociception; Formalin test; Writhing test; Hot-plate test

1. Introduction

Physalis angulata L. belongs to the Solanaceae family and includes about 120 species with herbal characteristics and perennial habits (Corrêa, 1962; Kissmann and Groth, 1995). It is distributed throughout the tropical and subtropical regions of the world (Kissmann and Groth, 1995; Santos et al., 2003). Extracts or infusions from this plant have been used in various countries in popular medicine as a treatment for a variety of illnesses, such as malaria, asthma, hepatitis, dermatitis and rheumatism (Chiang et al., 1992a; Lin et al., 1992; Santos et al., 2003; Soares et al., 2003). In Brazil, *Physalis angulata* is popularly known as "Camapu", "Bucho de Rã", "Juá de Capote" or "Mata-Fome" (Branch and Silva, 1983), and its juice is considered to be sedative and depurative against rheumatism and earache. The leaves are sometimes used against inflammations of the bladder, spleen and liver. The whole plant cooked is recommended in baths for inflammatory processes, such as rheumatism (Lorenzi, 1982). It has been demonstrated that some of the extracts or active principles obtained from *Physalis angulata* have a broad spectrum of biological activities, including antibacterial, molluscicidal, antiprotozoal, anticancer, cytotoxic and immunomodulatory activities (Kastelein and Camargo, 1990; Lee et al., 1991; Chiang et al., 1992a,b; Lin et al., 1992; Cáceres et al., 1995; Freiburghaus et al., 1996; Pietro et al., 2000; Ismail and Alam, 2001; Januario et al., 2002; Santos et al., 2003; Soares et al., 2003).

Phytochemical studies of *Physalis angulata* have demonstrated the presence of steroids, known as physalins (D, I, G, K, B, F, E), physagulins (E, F and G), with anolides and flavonoids (Row et al., 1978, 1980; Lee et al., 1991; Chiang et al., 1992a,b; Shingu et al., 1992; Ismail and Alam, 2001). In the present study, we have attempted to investigate the antinociceptive action of

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the aqueous extract obtained from roots of *Physalis angulata* on chemical and thermal models of nociception in mice for the purpose of validating its ethnomedical use.

2. Material and methods

2.1. Preparation of the aqueous extract (AE) of Physalis angulata

The plant was collected in Pará State, Brazil, during the year 2000 and classified by Dr. Ricardo Seichas (Department of Botany, Museu Emílio Goeldi). A voucher specimen (ref. 653) was deposited in the João Murca Pires herbarium of the Pará's Emílio Goeldi Museum (Belém, PA, Brazil). After collecting the material, roots of *Physalis angulata* were separated for extraction. The roots weighing 150 g were cleaned in a water stream, extracted with 700 ml of Milli-Q water, and concentrated at a final volume of 14%. The decoct was cooled and stored in a freezer at −20 °C for subsequent lyophilization, producing 2.712 g of the extract.

2.2. Animals

Swiss male mice (30–35 g) were obtained from the Evandro Chagas' Animal Resources Centre, Belém, Pará, Brazil. They were randomly assigned to groups of 10 animals and maintained in plastic boxes, with food and water ad libitum, under a 12 h light/12 h dark cycle. The room temperature was maintained at 22 ± 1 °C. The animals were acclimatized to the laboratory for at least 2 h before the experiments that were carried out between 8:00 and 13:00 h in order to avoid circadian influence. All experiments reported in this study were carried out in accordance with current guidelines for the care of laboratory animals and ethical guidelines for investigation of experimental pain in conscious animals. All efforts were made to minimize the number of animals used and their suffering.

2.3. Abdominal constriction by intraperitoneal injection of acetic acid

Abdominal contraction, induced by i.p. injection of acetic acid 1%, consisted of a contraction of the abdominal muscle together with a stretching of the hind limbs (Tonos et al., 1999). The animals were pre-treated intraperitoneally (i.p.) with morphine (10 mg/kg) and aspirin (100 mg/kg), used as positive control, or with the AE of *Physalis angulata* (10, 20 or 30 mg/kg) 0.5 h before, or orally with the AE of *Physalis angulata* (10, 20 or 30 mg/kg) 1 h before, acetic acid injection. The control groups received the same volume, 0.9% of NaCl (10 ml/kg). After challenge, pairs of mice were placed in separate boxes and the number of abdominal constrictions was counted every 5 min over a 1 h period. Antinociceptive activity was expressed as the reduction in the number of abdominal constrictions, i.e. the difference between control animals (NaCl) and animals pre-treated with AE or morphine.

2.4. Formalin-induced licking

The procedure used was similar to that described previously (Santos et al., 1998). Twenty microliters of 2.5% formalin solution (0.92% formaldehyde) was injected intraplantarly (i.pl.) under the ventral surface of the right hindpaw. The animals were placed individually in clear plexiglass cages (33 cm × 23 cm × 21.5 cm) and observed from 0 to 30 min following formalin injection. The amount of time spent licking the injected paw was timed with a chronometer and was considered as indicative of nociception. The initial nociceptive response normally peaked 5 min after formalin injection (early-phase) and 15–30 min after formalin injection (late-phase), representing the tonic and inflammatory pain responses, respectively (Hunskaar and Hole, 1987). The animals were pre-treated intraperitoneally with the AE of *Physalis angulata* (10, 20, 30 or 60 mg/kg), or with morphine (10 mg/kg) or indomethacin (10 mg/kg) which were used as positive controls, 0.5 h beforehand. The control animals received the same volume of vehicle (10 ml/kg, i.p.) used to dilute these drugs. Following intraplantar injection of formalin, each animal was immediately placed into a clear plexiglass cage, and the time it spent licking the injected paw was determined.

2.5. Hot-plate test

The hot-plate test was used to measure the response latencies according to the method described previously (Santos et al., 1998). In the experiments the hot-plate was maintained at 55 ± 1 °C. Before beginning the experiments, the basal reaction time response of all animals was taken. The animals were pre-treated with saline (10 ml/kg, i.p.), morphine (10 mg/kg, i.p.) or AE of *Physalis angulata* (30 and 60 mg/kg, i.p.) and 0, 0.25, 0.5, 1, 1.5, 2 and 2.5 h later, they were put on the heated surface of the plate at 55 ± 1 °C. The time necessary for the initial response to the painful stimulus (elevation of the paws, licking or jumping) was taken as defining the response. In order to minimize damage to the animals' paws, the cut-off time was 30 s.

2.6. Drugs

The drugs used were: formalin and acetic acid (Merck, São Paulo, Brazil), indomethacin and aspirin (Sigma Chemical Co., St. Louis, MO, USA), morphine hydrochloride (Cristalia-Brazil, São Paulo, Brazil). All substances used were dissolved in saline solution, with the exception of indomethacin and aspirin that were dissolved in 5% $NaHCO_3$ and Tween 80 plus 0.9% NaCl solution, respectively. The final concentration of Tween 80 did not exceed 5% and did not cause any effect per se.

2.7. Statistical analysis

The results are presented as mean ± S.E.M., except the ID_{50} values (i.e. the doses of aqueous extract of *Physalis angulata* necessary to reduce response by 50% relative to control value) which are reported as geometric means accompanied by their respective 95% confidence limits. The ID_{50} values were calculated from at least three dosages of AE, determined by linear