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Orofacial antinociceptive effect and antioxidant properties of the hydroethanol extract of *Hyptis fruticosa* salmz ex Benth

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ABSTRACT

Ethnopharmacological relevance: Hyptis fruticosa is a plant native to Brazil with antinociceptive and antiinflamatory properties. This study evaluated the antinociceptive activity of the hydroethanol extract of the plant leaves (CHEE) against orofacial pain as well as its in vitro effect against lipid peroxidation.

Materials and methods: The antinociceptive activity was investigated in mice orally treated with different doses of the CHEE (50, 100, and 200 mg/kg) and morphine (5 mg/kg) using formalin, glutamate, and capsaicin orofacial pain models using. Lipoperoxidation was induced in egg yolk by AAPH and $FeSO_4$ in the absence and presence of the CHEE (5, 50, 100, and 150 μg/mL).

Results: CHEE (200 mg/kg) significantly reduced ($\rho < 0.001$) the pain response in the first (69.6%) and second (81.8%) phases of the formalin test, while the nociception caused by capsaicin was significantly ($\rho < 0.001$) reduced by up to 62% at 200 mg/kg of extract. When glutamate was used as algogen, a significant ($\rho < 0.001$) nociception reduction of up to 85% at 200 mg/kg extract was observed. CHEE showed a higher protection against lipoperoxidation caused by $FeSO_4$ (82.3% TBARS inhibition) than AAPH (35.7% TBARS inhibition) at 150 μg/mL.

Conclusion: Hyptis fruticosa leaf CHEE is of pharmacological interest because it was able to inhibit the peripheral and central transmission of orofacial pain, while reducing the spreading of the inflammatory processes by neutralizing reactive oxygen species, which are by-products in the biosynthesis of pain mediators.

1. Introduction

Pain is a complex multidimensional phenomenon linked to nociception recognition, which can be defined as the perception of a lesion in tissues. It is controlled by a receptor system that sends information to the brain through specialized nervous fibers (Heredia and Rodrigues, 2008; Szumita et al., 2010). In this context, the orofacial region is a common part of the body for pain to occur, which is acute and frequently associated with respiratory, head, and neck syndromes (Macfarlane et al., 2001; Luccarini et al., 2006; Bonjardim et al., 2011). Despite its importance, the mechanisms by which the orofacial pain occurs are still poorly understood, probably due to few animal models to study nociception in this area (Bonjardim et al., 2011). In addition, there are difficulties in treating both acute and chronic orofacial pain when they are caused by neuropeptides or leukotrienes because nonsteroidal anti-inflammatory drugs (NSAIDs) are not effective in inhibiting these inflammatory mediators (Lindenmeyer et al., 2010). Therefore, the development of alternative therapies is necessary.

Hyptis fructicosa (Lamiaceae) is known as "alecrim de tabuleiro" and can be found in the coastal tablelands and lowlands of northeast Brazil, where it is an important medicinal plant whose leaves are directly chewed or the leaf tea is used by the population to treat pain (Menezes et al., 2007; Agra et al., 2008; Franco et al., 2011). *Hyptis fruticosa* has been the subject of some studies, which reported the toxicities of the essential oil and leaf aqueous extract, antibacterial and antineoplasic activities of compounds isolated from the plant roots, and cardiovascular effects of the essential oil (Silva et al., 2006; Menezes et al., 2007; Santos et al., 2007). Regarding pain and inflammation, the analgesic and antinociceptive activities of the *Hyptis fruticosa* essential oil and leaf aqueous extract were also demonstrated (Menezes et al., 2007; Franco et al., 2011). Nevertheless, despite of

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the plant being used to treat oral conditions, no study was done to demonstrate its effect on the orofacial pain. Therefore, the present study aims to improve the knowledge of the antinociceptive activity of this plant by studying its potential to treat orofacial pain using formalin, glutamate, and capsaicin pain models, as well as its possible antioxidant and anti-lipoperoxidative activity.

2. Materials and methods

2.1. Plant material

Hyptis fruticosa Salzm. Ex Benth. leaves were collected in July 2009, in the village Beans, São Cristóvão, Sergipe (10° 56'S, 37° 05'W). The plant was identified by Profa. Dra. Ana Paula Silver, botanist of the Department of Biology, Federal University of Sergipe, Brazil (DB–UFS). A voucher specimen was deposited in the Herbarium of the DB–UFS under the registration number SEA 13649.

2.2. Extract preparation

Leaves were dried at environment temperature and triturated in a knife mill to give a fine powder (900.5 g), which was subjected to extraction by maceration with 90% ethanol for 7 days. The extract was concentrated by solvent evaporation in a rotary evaporator under reduced pressure to give the crude hydroethanol extract (CHEE, 381.3 g, yield 42.3%).

2.3. Phytochemical analysis

The qualitative identification of the main classes of the secondary metabolites presents in the CHEE was done by using the colorimetric methods (Table 1) proposed by Sousa et al. (2007) and Matos (2009).

2.4. Total phenol quantification

The total phenol content was spectrophotometrically determined using the Folin–Ciocalteu method (Sousa et al., 2007). The reaction mixture was composed of extract (0.1 mL), distilled water (7.9 mL), Folin–Ciocalteu reagent (0.5 mL), and 20% sodium carbonate (1.5 mL). After 2 h, the absorbance of the resulting solutions was measured at 750 nm and applied in the calibration curve built with known concentrations of gallic acid (GA) standards (0.01 to 0.35 mg/mL). Results were expressed as milligram equivalents of gallic acid per g of extract (EAG mg/g). All analyses were carried out in triplicate.

Table 1
Phytochemical analysis of the crude methanol extract of *Hyptis fructicosa* leaves and its fractions.

Metabolites	Assays	CHEE
Alkaloids	Mayer's reagent/Dragendorff's reagent	–
Phenols in general	Folin Ciocaultey reagent	+
Flavonoids in general	Hydrochloric acid/magnesium	+
Flavones	pH change	+
Flavononols	pH change	+
Tannins	Cerric sulphate	+
Saponins	Clorofórmio	+
Steroids	Lieberman–Bouchard's reagent	+
Triterpenes	Anysaldehyde/Sulfuric acid	+
Xanthones	pH change	+

2.5. In vitro lipid peroxidation inhibitory activity

The degree of lipid peroxidation prevented by the hydroethanol extract was monitored by measuring the production of thiobarbituric acid-reactive substances (TBARS) (Budni et al., 2007). Briefly, egg yolk homogenate (1%w/v, 1 mL) in phosphate buffer (pH 7.4) was sonicated (10 s) and mixed with freshly prepared solutions of the hydroethanol extract and controls at 5, 50, 100 and 150 μg/mL. Lipid peroxidation was induced by adding either 2′-azobis(2-amidinopropane) dihydrochloride (AAPH, 0.17 mol/L, 0.1 mL) or ferrous sulphate ($FeSO_4$, 0.17 mol/L, 0.1 mL). Trolox was used a positive control, while the negative control was the vehicle (water or ethanol). The mixture was incubated at 37 °C for 30 min. Upon cooling, samples (0.5 mL) were centrifuged with 15% trichloroacetic acid (TCA, 0.5 mL) at 1200 rpm for 10 min. Supernatant was taken (0.5 mL), mixed with 0.67% thiobarbituric acid (TBA, 0.5 mL), incubated at 95 °C for 60 min and, after cooling, the formation of TBARS was measured by reading the supernatant absorbance at 532 nm. Results were expressed as inhibition percentage of TBARS formation.

2.6. Antinociceptive activity

2.6.1. Animals

Male Swiss mice weighing 20–35 g each, obtained from the Central Animal House of the Federal University of Sergipe, were randomly maintained in cages under controlled temperature (22 ± 3 °C) with light/dark cycles of 12 h (lights on from 06h00 to 18h00). The animals had free access to food (Purina™) and tap water. Animal Care and Use Committee of Federal University of Sergipe approved the experimental protocols and procedures under the registration CEPA/UFS number 65/09.

2.6.2. Formalin test

Orofacial nociception was induced in mice by injection of 2% formalin (20 μL, s.c) in the right upper lip (perinasal area) using a 27-gauge needle (Luccarini et al., 2006). The volume and formalin concentration were selected from pilot studies that showed a nociceptive-related biphasic behavioral response of great intensity at periods from 0 to 5 min (first phase) and 15 to 40 min (second phase). Nociception was quantified by measuring the time (sec) animals spent rubbing their faces in the injected area with their fore- or hind paws. To assess the effects of the test drugs, groups of mice ($n=8$) were systematically pretreated with vehicle (one drop of 0.2% Tween 80 in distilled water) and CHEE (50, 100, and 200 mg/kg, v.o.) 1 h before the formalin injection. Morphine (MOR, 5 mg/kg, i.p.), administered 1 h before the algogen, was included as positive control.

2.6.3. Capsaicin test

Orofacial nociception was induced by capsaicin (Pelissier et al., 2002) as described for formalin. Capsaicin (20 μL, 2.5 μg, s.c.) was injected in the perinasal area and the drug effects were evaluated using the same groups as described previously, which were administered 1 h before the algogen. Capsaicin was dissolved in ethanol, dimethyl sulfoxide and distilled water (1:1:8), and the face-rubbing behavior was observed during 42 min. MOR (5 mg/kg, i.p.) was used as positive control.

2.6.4. Glutamate test

In an attempt to provide evidence of the CHEE interaction with the glutamatergic system, its antagonistic effect on glutamate-induced orofacial nociception in mice was studied using the method previously described by Beirith et al. (2002) with modifications. Glutamate (20 μL, 25 μmol/L in phosphate buffered