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Original Article

Evaluation of the orofacial antinociceptive profile of the ethyl acetate fraction and its major constituent, rosmarinic acid, from the leaves of *Hyptis pectinata* on rodents

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ABSTRACT

Hyptis pectinata (L.) Poit., Lamiaceae, popularly known as "sambacaitá," is an aromatic shrub largely grown in the Brazilian northeastern. We investigated the antinociceptive effects of the ethyl acetate fraction obtained from the leaves of *H. pectinata* and of its main constituent rosmarinic acid, on formalin (2%)-, glutamate (25 μM)- and capsaicin (2.5 μg)-induced orofacial nociception in rodents. Male mice were pretreated with ethyl acetate fraction (100, 200 or 400 mg/kg, *p.o.*), rosmarinic acid (10 or 20 mg/kg, *p.o.*), morphine (5 mg/kg, *i.p.*), or vehicle (distilled water + 0.2% Tween 80). Ethyl acetate fraction reduced the nociceptive face-rubbing behavior during the two phase of the formalin test, whereas pretreatment with rosmarinic acid decreased the pain behavior in the second phase. Ethyl acetate fraction produced significant antinociceptive effects in the capsaicin and glutamate tests. This study showed that oral administration of ethyl acetate fraction produced potent antinociceptive effects compared to treatment with rosmarinic acid.

Introduction

Pain in the oral and craniofacial system represents a major medical and social problem (Hargreaves, 2011). Indeed, a report from the U.S. Surgeon General on orofacial health concludes that, ". . .oral health means much more than healthy teeth. It means being free of chronic oral-facial pain conditions. . ." (National Institutes of Health, 2000). Moreover, orofacial pain is derived from many unique target tissues, such as the meninges, cornea, tooth pulp, oral/nasal mucosa, and temporomandibular joint, and thus has several unique physiologic characteristics compared with the spinal nociceptive system (Bereiter et al., 2008; Hargreaves, 2011). Thus, the management or treatment of orofacial pain conditions represents a significant health care problem and a challenge for the pharmaceutical industry.

In the last couple of decades, important progress has been made regarding the development of natural therapies. However, there is an urgent need to discover effective and safe analgesic agents (Calixto et al., 2000) and natural products have been shown to be strong candidates for development of new drugs for pain control (Quintans et al., 2014; Siqueira-Lima et al., 2014). Besides, a current approach is to develop new biological compounds from natural products that manage orofacial pain with enhanced efficacy and minimal side effects; these compounds are derived from medicinal plants or their secondary metabolites (Bonjardim et al., 2012; Guimarães et al., 2013; Quintans-Júnior et al., 2010; Venâncio et al., 2011).

Hyptis pectinata (L.) Poit., Laminaceae, is a medicinal plant known as "sambacaitá" or "canudinho" in northeastern Brazil that is widely used to treat gastrointestinal disorders, skin infections, nasal congestion, fever, cramps, inflammation and pain (Bispo et al., 2001; Raymundo et al., 2011). Some studies have demonstrated that *H. pectinata* possesses antinociceptive and anti-inflammatory activities (Bispo et al., 2001; Lisboa et al., 2006; Raymundo et al.,

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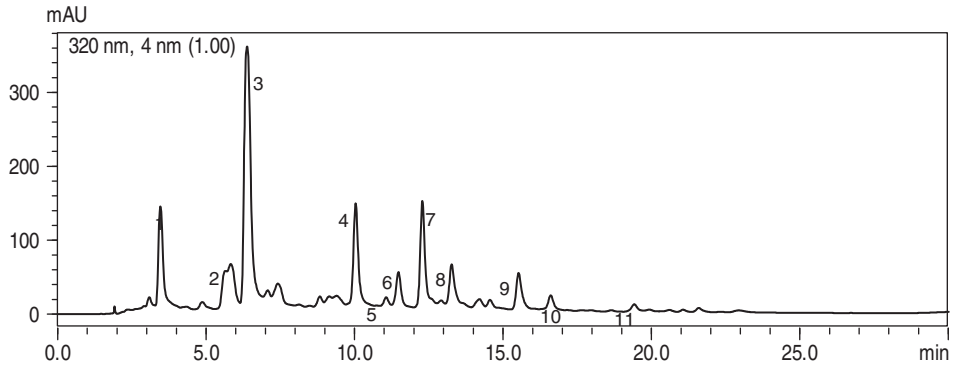

Fig. 1. Chromatogram from HPLC-DAD analysis and the compounds isolated from the EtOAc fraction of the leaves of *Hyptis pectinata*.

2011). Nascimento et al. (2008) described how *H. pectinata* leaves are used as treatment for several oral diseases, such as dental caries and orofacial pain. This pharmacological profile was corroborated by Paixão et al. (2013, 2015), who demonstrated an important neurogenic and inflammatory orofacial antinociceptive profile of the crude aqueous extract obtained from *H. pectinata* leaves and its possible application against other orofacial diseases such as periodontitis.

Besides, Arrigoni-Blank et al. (2005) demonstrated antiedematogenic effect of the aqueous extract of *H. pectinata*. Hasanein and Mohammad Zaheri (2014) showed that rosmarinic acid (**1**), an ester of caffeic acid found in *Hyptis* species, reduces nociception in painful diabetic neuropathy model. Nevertheless, little is known how *H. pectinata* extract modulates orofacial pain transmission.

The present study proposed to verify the antinociceptive properties of the ethyl acetate fraction (EtOAc) of *H. pectinata* leaves and, in particular, rosmarinic acid (**1**) (RA), which was the major compound isolated from this fraction. The antinociceptive properties of EtOAc fraction were tested on mice following orofacial nociception induced by formalin, capsaicin and glutamate, three algogens agents that promote pain through different mechanisms and also by activation of different neuronal populations.

Materials and methods

Plant material

Plant material was obtained and extracted according to protocols described in Falcão et al. (2013). Voucher specimen (88157) is deposited at the Instituto Agronômico de Pernambuco, Recife, PE. Briefly, the leaves were dried, crushed and successively extracted with EtOH to obtain 7 g dry extract. This extract was dissolved in MeOH:H_2O (1:1) and successively fractionated with hexane and EtOAc. A portion of the EtOAc fraction (3.5 g) was subjected to chromatography on a Sephadex LH-20 column, and the compounds were purified using a semi-preparative HPLC column. This fractionation resulted in the isolation of sambacaitaric acid (**2**), 3-*O*-methyl-sambacaitaric acid (**3**), rosmarinic acid (**1**), 3-*O*-methyl-rosmarinic acid (**4**), ethyl caffeoate (**5**), nepetoidin A (**6**), nepetoidin B (**7**), cirsiliol (**8**), cirsimaritin (**9**), 7-*O*-methylluteolin (**10**) and genkwanin (**11**) (Falcão et al., 2013). Rosmarinic acid was the major compound isolated from the EtOAc fraction, as shown in the chromatogram (Fig. 1) obtained by HPLC-DAD analysis.

Animals

Male Swiss mice (28–34 g), 2–3 months of age, were used throughout this study. The animals were randomly housed in appropriate cages at 22 ± 2 °C on a 12 h light/dark cycle (lights on between 6 am and 6 pm) with free access to food and water. All experiments were carried out between 9 am and 2 pm in a quiet room. All nociception tests were carried out by the same visual observer, and behavioral tests were performed under blind conditions. Experimental protocols were approved by the Animal Care and Use Committee at the Federal University of Sergipe (CEPA/UFS # 10/11).

Drug and treatments

Morphine hydrochloride (União Química, Brazil), 37% formaldehyde (Vetec, Brazil), diazepam (Roche, Brazil), Tween 80 (polyoxyethylene-sorbitan monooleate), glutamate, capsaicin and rosmarinic acid (RA) were purchased from Sigma (USA). During the nociception tests, the extract or agent was administrated by oral gavage (*p.o.*, *per os*) or intraperitoneally (*i.p.*) at a dose volume of 0.1 ml/10 g, with the exception of the nociception inducing agents, such as formalin, glutamate and capsaicin, which were injected subcutaneously (*s.c.*) into the right upper lip.

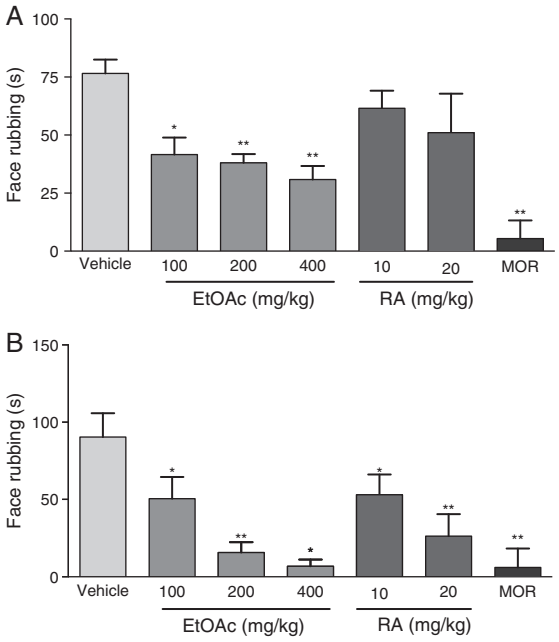

Fig. 2. Effects of EtOAc fraction (*Hyptis pectinata*), rosmarinic acid (RA) or morphine (MOR) on formalin-induced orofacial nociception in mice. Vehicle (control), EtOAc (100, 200 or 400 mg/kg, *p.o.*), RA (10 or 20 mg/kg, *p.o.*) or MOR (5 mg/kg, *i.p.*) were administered 1 h before formalin injection. (A) First phase (0–5 min) and (B) second phase (15–40 min). Each column represents the mean ± S.E.M. ($n = 8$ per group). $^{*}p < 0.05$ or $^{**}p < 0.001$ *vs.* the control (ANOVA followed by Tukey's test).