Revista Brasileira de Farmacognosia 26 (2016) 203–208

Brazilian Journal of Pharmacognosy
REVISTA BRASILEIRA DE FARMACOGNOSIA
www.elsevier.com/locate/bjp

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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ARTICLE INFO

Article history:
Received 5 June 2015
Accepted 19 July 2015
Available online 31 December 2015

Keywords:
Hyptis pectinate
Rosmarinic acid
Orofacial pain
Nociception

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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http://dx.doi.org/10.1016/j.bjp.2015.07.029
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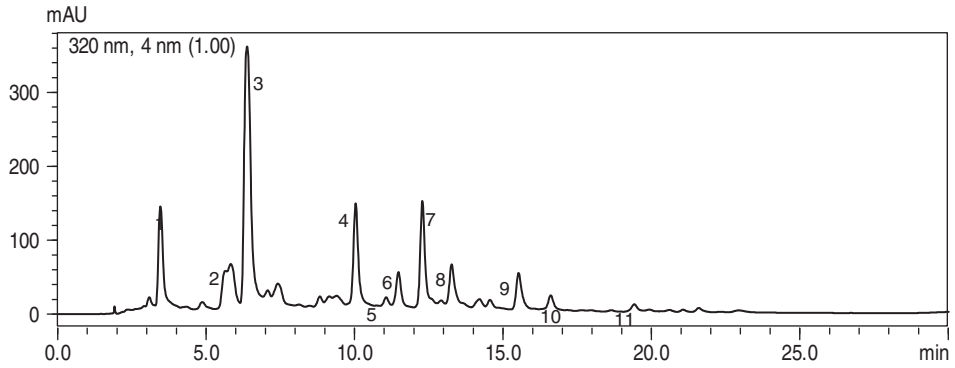


Fig. 1. Chromatogram from HPLC-DAD analysis and the compounds isolated from the EtOAc fraction of the leaves of *Hyptis pectinata*.

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.

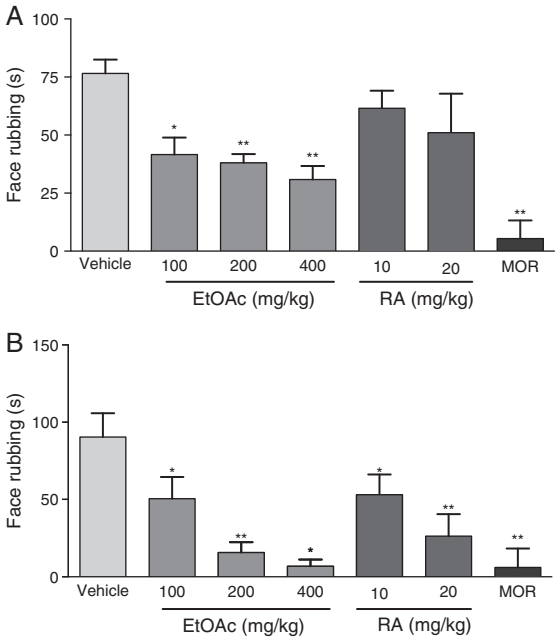
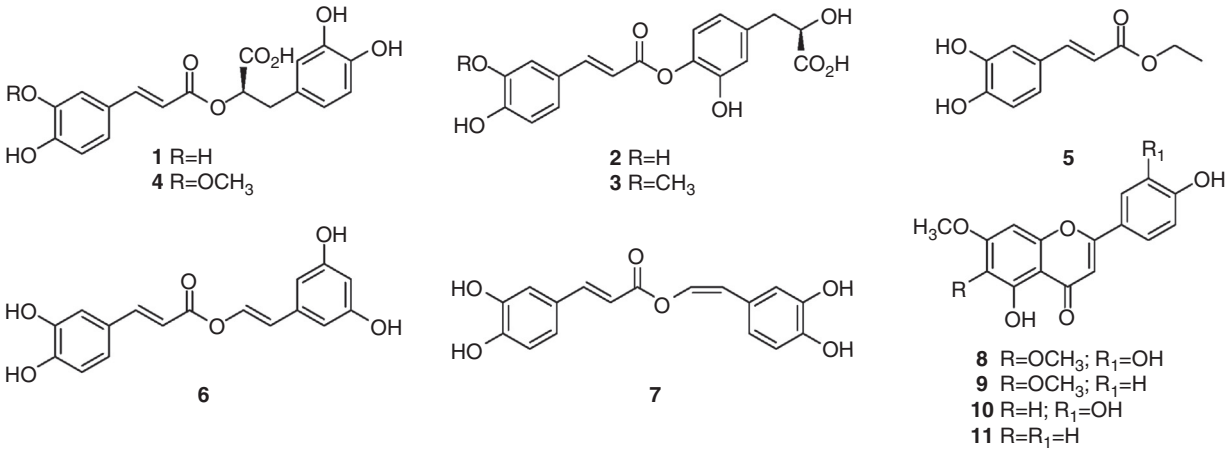


Fig. 2. Effects of EtOAc fraction (*Hyptis pectinata*), rosmarinic acid (RA) or morphine (MOR) on formalin-induced orofacial nociception in mice. Vehicle (control), EtOAc (100, 200 or 400 mg/kg, *p.o.*), RA (10 or 20 mg/kg, *p.o.*) or MOR (5 mg/kg, *i.p.*) were administered 1 h before formalin injection. (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).



Nociception studies

Formalin test

Orofacial nociception was induced in mice by *s.c.* injection of 20 μl 2% formalin into the right upper lip (perinasal area), using a 27-gauge needle (Luccarini et al., 2006; Pelissier et al., 2002) according to previously reported methods with changes (Quintans-Júnior et al., 2010). This volume and concentration of formalin was selected during our pilot studies because it showed an intense nociception-related biphasic behavioral response (face-rubbing) at periods of 0–5 min (first phase) and 15–40 min (second phase) (Quintans-Júnior et al., 2010). Nociceptive behavior was quantified during these periods by measuring the time (s) that the mice spent rubbing their face at the injection area with its fore or hind paws (Luccarini et al., 2006). To assess the effects of the test drugs, groups of mice ($n = 8$ per group) were pretreated systemically with a vehicle control (distilled water + 0.2% Tween 80), EtOAc (100, 200 or 400 mg/kg, *p.o.*), or RA (10 or 20 mg/kg, *p.o.*) 1 h before the injection of formalin. Morphine (MOR, 5 mg/kg, *i.p.*) was administered 1 h before of nociceptive agent, as a positive control.

Capsaicin and glutamate tests

The orofacial nociception induced by capsaicin or glutamate in rodents was performed as described previously by Quintans-Júnior et al. (2010). Mice ($n = 8$ per group) were injected with capsaicin (20 μl, 2.5 mg) or glutamate (40 μl, 25 μM prepared in phosphate buffered saline) subcutaneously into the right upper lip (perinasal area), using a 27-gauge needle. Capsaicin was dissolved in ethanol, dimethyl sulfoxide and distilled water (1:1:8). An additional group received a similar volume of the capsaicin vehicle. Nociception quantification was performed by measuring the time(s) that the animals spent rubbing the orofacial region with greater intensity for a period of 42 and 30 min after the injection of capsaicin and glutamate, respectively. The EtOAc fraction (100, 200 or 400 mg/kg) or vehicle (distilled water + 0.2% Tween 80) was given by oral gavage to animals 1 h before the injection of capsaicin or glutamate, similar to the formalin test. MOR was used as a positive control and was administered (5 mg/kg, *i.p.*) 1 h before the nociception-inducing agent.

Evaluation of motor activity

In order to evaluate a possible non-specific muscle-relaxant or sedative effect of the extract, mice were submitted to the Rota-rod apparatus, as described by Quintans-Júnior et al. (2010). Initially, 24 h before the test, the mice that were able to remain on the Rota-rod apparatus (AVS®, Brazil) longer than 180 s, at a speed of 9 rpm, were selected. Mice were treated with the EtOAc fraction (100, 200 or 400 mg/kg, *p.o.*), vehicle or diazepam (3 mg/kg, *i.p.*) 1, 2 and 4 h before being placed on the Rota-rod; each animal was tested on the Rota-rod apparatus, and the time (s) that the animal remained on the bar for up to 180 s was recorded.

Statistical analysis

Data obtained from the animal experiments were expressed as the mean ± the standard error of the mean (mean ± S.E.M.). Significant differences between the treated and the control groups were evaluated using the ANOVA and Tukey tests. Differences were considered to be statistically significant when $p < 0.05$. All statistical analyses were performed using GraphPad Prism 5 (Graph Pad Prism Software Inc., San Diego, CA, USA).

Results

The chemical analysis of the EtOAc fraction from *H. pectinata* leaves showed the presence of eleven compounds and the chromatogram (HPLC-DAD) is shown in Fig. 1. As seen in the chromatogram, rosmarinic acid (**1**) was the main component of this fraction. Four of the eleven isolated compounds are derived from rosmarinic acid, and compounds **2** (sambacaitaric acid) and **3** (3-*O*-methyl sambacaitaric acid) were identified as new natural compounds.

Acute treatment of mice with the EtOAc fraction (100, 200 or 400 mg/kg, *p.o.*) produced a significant reduction ($p < 0.05$ or $p < 0.001$) in the face-rubbing behavior (Fig. 2A and B) in both phases of formalin-induced nociception. Additionally, treatment with rosmarinic acid (10 or 20 mg/kg, *p.o.*) alone was effective in significantly reducing of the nociceptive behaviors during the second phase, $p < 0.05$ or $p < 0.001$ respectively.

As shown in Fig. 3A, pretreatment with the EtOAc fraction (100, 200 or 400 mg/kg, *p.o.*) significantly reduced ($p < 0.001$) the face-rubbing behavior induced by capsaicin treatment. As expected, the group that received the vehicle treatment (ethanol, dimethyl sulfoxide and distilled water 1:1:8) did not present any nociceptive behavior (data not shown). Besides, acute treatment with the EtOAc fraction produced a significant reduction ($p < 0.05$ or $p < 0.001$) of glutamate-induced nociception when compared with the control group (Fig. 3B).

In the rota-rod test, EtOAc-treated mice did not show any significant motor performance alterations with the doses of 100, 200 or 400 mg/kg (Fig. 4). As expected, the CNS depressant diazepam (3 mg/kg, standard drug), reduced the time of animals on the rota-rod after 60, 120 and 240 min.

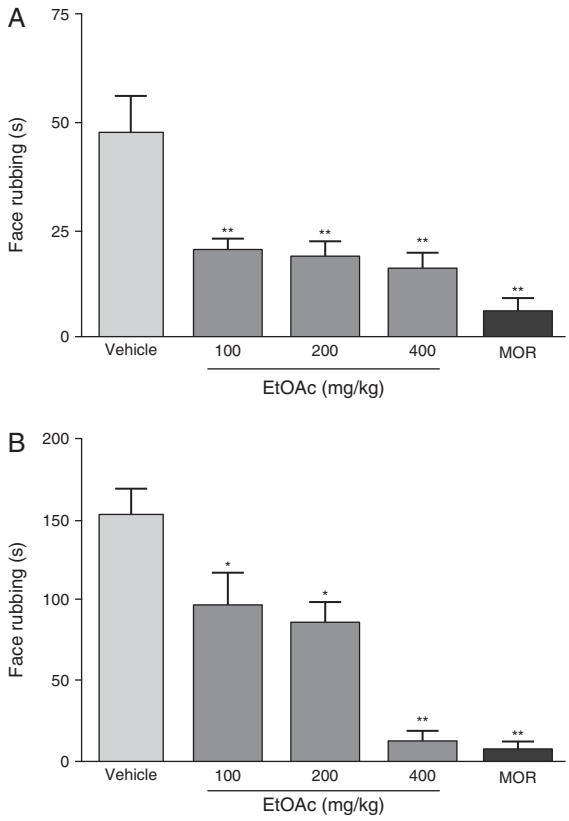


Fig. 3. Effects of EtOAc (*Hyptis pectinata*) on (A) capsaicin- or (B) glutamate-induced orofacial nociception in mice. Vehicle (control), EtOAc (100, 200 or 400 mg/kg, *p.o.*), or MOR (5 mg/kg, *i.p.*) was administered 1 h before capsaicin or glutamate injection. Each column represents the mean ± S.E.M. ($n = 8$ per group). $^{*}p < 0.05$ or $^{**}p < 0.001$ *vs.* the control (ANOVA followed by Tukey's test).

Discussion

In folk medicine used in northeastern Brazil, the *H. pectinata* plant ("sambacaita") is used in the treatment of several orofacial pathological disorders, including orofacial pain. However, there are no pharmacological studies that report this effect. For the first time, we demonstrate that oral administration of the EtOAc fraction from *H. pectinata* leaves exerts protective effects against formalin-, capsaicin-, and glutamate-induced orofacial pain in mice; this effect may be related to the presence of rosmarinic acid in this fraction.

In this study, the eleven compounds of the EtOAc fraction from *H. pectinata* leaves were isolated and identified according to the protocols described by Falcão et al. (2013). Rosmarinic acid (RA), a polyphenol commonly found in other species of this genus as *Hyptis atrorubens*, was identified as the main component of this fraction.

During the formalin test, the presence of two distinct phases is at least partially due to the mechanisms of nociception. The first phase is associated with the direct stimulation of the C-nociceptors by mechanisms dependent on TRPA1, whereas the second phase reflects the integration between the peripheral (nociceptors) and central (spinal/brainstem) signaling pathways (Capuano et al., 2009; Dallel et al., 1995; McNamara et al., 2007). All tested doses of EtOAc produced antinociception in both the first and second phase of the formalin test compared to the vehicle control. Synergistic effect of compounds in the extract, as polyphenols and flavonoids, certainly contributed to the reduction of formalin-induced nociception during the first phase, since flavonoids and phenols are among the main natural products studied pain, as demonstrated by Quintans et al. (2014).

Interestingly, Bispo et al. (2001) demonstrated that pretreating mice with the aqueous extract of *H. pectinata* at the doses of 200 and 400 mg/kg had no significant effect during the first phase of the formalin test, when the painful agent was applied in the paw. However, Paixão et al. (2013) showed a marked antinociceptive effect of the aqueous extract of *H. pectinata* in both phases of the orofacial formalin test and attributed this profile to a possible antioxidant effect of the extract. The discrepancies in the results of Bispo et al. (2001) and Paixão et al. (2013) may be due to the greater sensitivity of the orofacial region, the involvement of the trigeminal pathway (Sessle, 2011), or due the chemical variation of plant secondary metabolites generated by environmental and genetic (Moore et al., 2014).

RA reduced only late phase of formalin test. A previous study showed that the administration of RA at higher doses reduces both phases of formalin-induced nociception, with the involvement of opioid mechanisms (Boonyarikpunchai et al., 2014; Hasanein and Mohammad Zaheri, 2014). Furthermore, this compound and some analogs could be useful in the prevention and treatment of inflammatory diseases, and inhibit inflammatory responses, such as neutrophilic migration and edema in the lungs of mice (Gamaro et al., 2011).

Capsaicin, an active ingredient in hot chili peppers, directly stimulates transient receptor potential cation channel subfamily

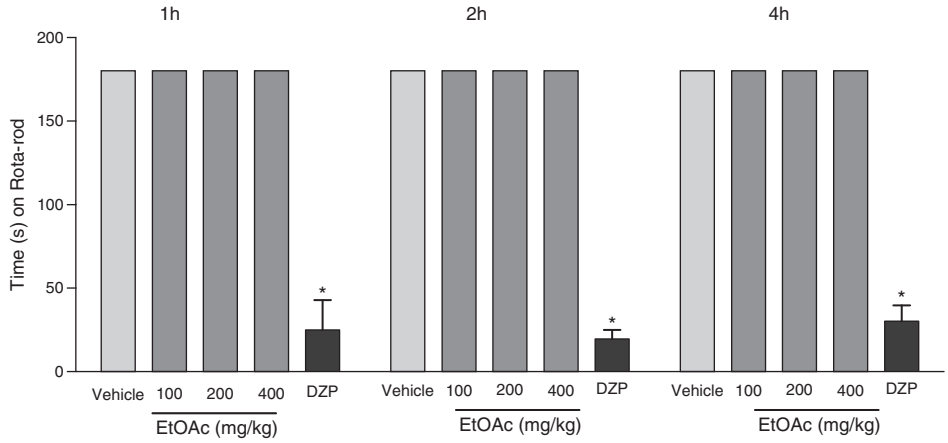


Fig. 4. Time (s) on the Rota-rod observed in mice after *p.o.* treatment with vehicle (Control), EtOAc (100, 200 or 400 mg/kg, *p.o.*), or diazepam (DZP, 3 mg/kg, *i.p.*). The motor response was recorded for 1, 2, and 4 h after drug treatment, and the time (s) that the animal remained on the bar for up to 180 s was recorded. Significant differences *vs.* the control group were calculated using an ANOVA test, followed by Tukey's test ($n = 8$ per group). $^{*}p < 0.001$.

V member 1 (TRPV1), which are involved in the transmission and modulation of nociceptive activity, through selective actions on unmyelinated C-fibers and thinly myelinated A primary sensory neurones (Dray, 1992). Antagonists of TRPV1 receptors have been reported to exhibit a pain-relieving activity (Trevisani, 2013) and were effective in reducing nociception from inflammatory as well as neuropathic pain models in rats (Khairatkar-Joshi and Szallasi, 2009). Based on our finding, the oral administration of EtOAc produced a neurogenic inhibition against capsaicin-induced nociception indicating its ability to inhibit nociceptive transmission initiated by TRPV1 activation.

When glutamate is injected into the right upper lip (perinasal area) elicits a noxious stimulus characterized by a behavioral response of rubbing the orofacial region (Quintans-Júnior et al., 2010; Siqueira et al., 2010). Noxious stimulation of primary afferent fibers results in the release of glutamate from the peripheral and central terminals of the trigeminal and spinal afferent fibers (Keast and Stephensen, 2000; Lam et al., 2005). Our results demonstrated that EtOAc fraction reduced orofacial pain induced by glutamate. Thus, suggest that the suppression of glutamate-induced nociception by EtOAc treatment can be associated to its interaction with the glutamatergic system. These results are similar to those found by Guginski et al. (2009), who demonstrated that rosmarinic acid blocks the pain induced by glutamate on the mouse paw.

In summary, we demonstrated the EtOAc effect on orofacial pain induced by three algogens agents, which promote nociception by different mechanisms as TRPA1, TRPV1 and glutamatergic receptors (Caterina et al., 1997; McNamara et al., 2007; Willcockson and Valtschanoff, 2008) with the possible participation of peptidergic and non-peptidergic primary afferent neurons (Kobayashi et al., 2005; Willcockson and Valtschanoff, 2008). Additionally, previous study has shown the CNS action of the aqueous extract from *H. pectinata* leaves on rodent central nervous system (Bueno et al., 2006), with possible involvement of opioid system (Bispo et al., 2001). Thus, it is suggested that EtOAc has central antinociceptive actions and may be acting on perception of nociceptive stimuli, signal transduction or its conduction to the central nervous system (CNS).

In this sense drugs that act on the CNS, as diazepam or morphine, can impair motor coordination and generate confusion in analysis of results of nociceptive behavioral tests, such as those used here (Quintans-Júnior et al., 2010). Therefore, we evaluated whether pretreatment with EtOAc can impair the motor coordination of mice using a Rota-rod apparatus. However, all tested doses of EtOAc were ineffective in producing changes in motor coordination.

Taken altogether, our data led to the hypothesis that EtOAc had a protective role in orofacial nociception in mice. These effects may be related to the presence of rosmarinic acid and other compounds. These findings also support the hypothesis that *H. pectinata* has potential therapeutic use for painful facial and, perhaps, dental disorders. Nevertheless, further studies are needed to determine the precise mechanism of action.

Taken altogether, our data led to the hypothesis that EtOAc had a protective role in orofacial nociception in mice. These effects may be related to the presence of rosmarinic acid and other compounds. These findings also support the hypothesis that *H. pectinata* has potential therapeutic use for painful facial and, perhaps, dental disorders. Nevertheless, further studies are needed to determine the precise mechanism of action.

Authors' contributions

REAF, SAS and CAC contributed in collecting plant sample and identification, confection of herbarium, running the laboratory work, analysis of the data and drafted the paper. JSSQ, PLS, and AANL contributed to biological studies. REAF, SAS and CAC contributed in plant identification, herbarium confection and contributed to chromatographic analysis. JSSQ, LJQJ, MTSC, TMSS contributed to critical reading of the manuscript. AGG and LJQJ designed the study, supervised the laboratory work and contributed to critical reading of the manuscript. All the authors have read the final manuscript and approved the submission.

Conflicts of interest

The authors declare no conflicts of interest.

Acknowledgements

We would like to thank the CNPq/Brazil – grant number 555076/2010-5 and FACEPE (grant number PRONEM APQ-1232.1.06/10) for financial support.

References

Arrigoni-Blank, M.F., Silva-Mann, R., Campos, D.A., Silva, P.A., Antoniolli, A.R., Caetano, L.C., Sant'Ana, A.E.G., Blank, A.F., 2005. Morphological, agronomical and pharmacological characterization of *Hyptis pectinata* (L.) Poit germplasm. Rev. Bras. Farmacogn. 15, 298–303.

Bereiter, D.A., Hargreaves, K.M., Hu, J.W., 2008. Trigeminal mechanisms of nociception: peripheral and brainstem organization. In: Masland, R.H., Albright, T.D., Albright, T.D., Masland, R.H., Dallos, P., Oertel, D., Firestein, S., Beauchamp, G.K., Bushnell, M.C., Basbaum, A.I., Kaas, J.H., Gardner, E.P. (Eds.), The Senses: A Comprehensive Reference. Academic Press, New York, pp. 435–460.

Bispo, M.D., Mourão, R.H., Franzotti, E.M., Bomfim, K.B., Arrigoni-Blank, M.F., Moreno, M.P., Marchioro, M., Antoniolli, A.R., 2001. Antinociceptive and antiedematogenic effects of the aqueous extract of *Hyptis pectinata* leaves in experimental animals. J. Ethnopharmacol. 76, 81–86.

Bonjardim, L.R., Cunha, E.S., Guimarães, A.G., Santana, M.F., Oliveira, M.G.B., Serafini, M.R., Araújo, A.A.S., Antoniolli, A.R., Cavalcanti, S.C.H., Santos, M.R.V., Quintans-Júnior, L.J., 2012. Evaluation of the anti-inflammatory and antinociceptive properties of *p*-cymene in mice. Z. Naturforschung. C: J. Biosci. 67, 15–21.

Boonyarikpunchai, W., Sukrong, S., Towiwat, P., 2014. Antinociceptive and anti-inflammatory effects of rosmarinic acid isolated from *Thunbergia laurifolia* Lindl. Pharmacol. Biochem. Behav. 124, 67–73.

Bueno, A.X., Moreira, A.T.S., Silva, F.T., Estevam, C.S., Marchioro, M., 2006. Effects of the aqueous extract from *Hyptis pectinata* leaves on rodent central nervous system. Rev. Bras. Farmacogn. 16, 317–323.

Calixto, J.B., Beirith, A., Ferreira, J., Santos, A.R., Filho, V.C., Yunes, R.A., 2000. Naturally occurring antinociceptive substances from plants. Phytother. Res. PTR 14, 401–418.

Capuano, A., De Corato, A., Treglia, M., Tringali, G., Dello Russo, C., Navarra, P., 2009. Antinociceptive activity of buprenorphine and lumiracoxib in the rat orofacial formalin test: a combination analysis study. Eur. J. Pharmacol. 605, 57–62.

Caterina, M.J., Schumacher, M.A., Tominaga, M., Rosen, T.A., Levine, J.D., Julius, D., 1997. The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature 389, 816–824.

Dallel, R., Raboisson, P., Clavelou, P., Saade, M., Woda, A., 1995. Evidence for a peripheral origin of the tonic nociceptive response to subcutaneous formalin. Pain 61, 11–16.

Dray, A., 1992. Mechanism of action of capsaicin-like molecules on sensory neurons. Life Sci. 51, 1759–1765.

Falcão, R.A., do Nascimento, P.L.A., de Souza, S.A., da Silva, T.M.G., de Queiroz, A.C., da Matta, C.B.B., Moreira, M.S.A., Camara, C.A., Silva, T.M.S., 2013. Antileishmanial phenylpropanoids from the leaves of *Hyptis pectinata* (L.) Poit. Evid.-Based Complement. Altern. Med. 2013, 1–7.

Gamaro, G.D., Suyenaga, E., Borsoi, M., Lermen, J., Pereira, P., Ardenghi, P., 2011. Effect of rosmarinic and caffeic acids on inflammatory and nociception process in rats. ISRN Pharmacol. 2011, 1–6.

Guginski, G., Luiz, A.P., Silva, M.D., Massaro, M., Martins, D.F., Chaves, J., Mattos, R.W., Silveira, D., Ferreira, V.M.M., Calixto, J.B., Santos, A.R.S., 2009. Mechanisms involved in the antinociception caused by ethanolic extract obtained from the leaves of *Melissa officinalis* (lemon balm) in mice. Pharmacol. Biochem. Behav. 93, 10–16.

Guimarães, A.G., Quintans, J.S.S., Quintans-Júnior, L.J., 2013. Monoterpenes with analgesic activity – a systematic review. Phytother. Res. PTR 27, 1–15.

Hargreaves, K.M., 2011. Orofacial pain. Pain 152, S25–S32.

Hasanein, P., Mohammad Zaheri, L., 2014. Effects of rosmarinic acid on an experimental model of painful diabetic neuropathy in rats. Pharm. Biol. 52, 1398–1402.

Keast, J.R., Stephensen, T.M., 2000. Glutamate and aspartate immunoreactivity in dorsal root ganglion cells supplying visceral and somatic targets and evidence for peripheral axonal transport. J. Comp. Neurol. 424, 577–587.

Khairatkar-Joshi, N., Szallasi, A., 2009. TRPV1 antagonists: the challenges for therapeutic targeting. Trends Mol. Med. 15, 14–22.

Kobayashi, K., Fukuoka, T., Obata, K., Yamanaka, H., Dai, Y., Tokunaga, A., Noguchi, K., 2005. Distinct expression of TRPM8, TRPA1, and TRPV1 mRNAs in rat primary afferent neurons with aδ/c-fibers and colocalization with trk receptors. J. Comp. Neurol. 493, 596–606.

Lam, D.K., Sessle, B.J., Cairns, B.E., Hu, J.W., 2005. Neural mechanisms of temporomandibular joint and masticatory muscle pain: a possible role for peripheral glutamate receptor mechanisms. Pain Res. Manag. J. Can. Pain Soc. J. Société Can. Pour Trait. Douleur 10, 145–152.

Lisboa, A.C.C.D., Mello, I.C.M., Nunes, R.S., Dos Santos, M.A., Antoniolli, A.R., Marçal, R.M., de Cavalcanti, S.C.H., 2006. Antinociceptive effect of *Hyptis pectinata* leaves extracts. Fitoterapia 77, 439–442.

Luccarini, P., Childeric, A., Gaydier, A.-M., Voisin, D., Dallel, R., 2006. The orofacial formalin test in the mouse: a behavioral model for studying physiology and modulation of trigeminal nociception. J. Pain Off. J. Am. Pain Soc. 7, 908–914.

McNamara, C.R., Mandel-Brehm, J., Bautista, D.M., Siemens, J., Deranian, K.L., Zhao, M., Hayward, N.J., Chong, J.A., Julius, D., Moran, M.M., Fanger, C.M., 2007. TRPA1 mediates formalin-induced pain. Proc. Natl. Acad. Sci. U. S. A 104, 13525–13530.

Moore, B.D., Andrew, R.L., Külheim, C., Foley, W.J., 2014. Explaining intraspecific diversity in plant secondary metabolites in an ecological context. New Phytol. 201, 733–750.

Nascimento, P.F.C., Alviano, W.S., Nascimento, A.L.C., Santos, P.O., Arrigoni-Blank, M.F., de Jesus, R.A., Azevedo, V.G., Alviano, D.S., Bolognese, A.M., Trindade, R.C., 2008. *Hyptis pectinata* essential oil: chemical composition and anti-*Streptococcus mutans* activity. Oral Dis. 14, 485–489.

National Institutes of Health, 2000. Oral health in America: A Report of the Surgeon General (Executive Summary). Department of Health and Human Services, National Institute of Dental and Craniofacial Research, N.I. of H., Rockville, USA.

Paixão, M.S., Melo, M.S., Oliveira, M.G.B., Santana, M.T., Lima, A.C.B., Damascena, N.P., Dias, A.S., Araujo, B.S., Estevam, C.S., Botelho, M.A., Quintans-Júnior, L.J., 2013. *Hyptis pectinata*: redox protection and orofacial antinociception. Phytother. Res. PTR 27, 1328–1333.

Paixão, M.S., Melo, M.S., Damascena, N.P., Araújo, A.A.S., Soares, A.F., Oliveira, D.V.A., Oliveira, J.S., Almeida, F.T.C., Amaral, F.S., Araújo, B.S., Estevam, C.S., Botelho, M.A., Quintans-Júnior, L.J., 2015. *Hyptis pectinata* gel prevents alveolar bone resorption in experimental periodontitis in rats. Rev. Bras. Farmacogn. 25, 35–41.

Pelissier, T., Pajot, J., Dallel, R., 2002. The orofacial capsaicin test in rats: effects of different capsaicin concentrations and morphine. Pain 96, 81–87.

Quintans, J.S.S., Antoniolli, A.R., Almeida, J.R.G.S., Santana-Filho, V.J., Quintans-Júnior, L.J., 2014. Natural products evaluated in neuropathic pain models – a systematic review. Basic Clin. Pharmacol. Toxicol. 114, 442–450.

Quintans-Júnior, L.J., Melo, M.S., De Sousa, D.P., Araujo, A.A.S., Onofre, A.C.S., Gelain, D.P., Gonçalves, J.C.R., Araújo, D.A.M., Almeida, J.R.G.S., Bonjardim, L.R., 2010. Antinociceptive effects of citronellal in formalin-, capsaicin-, and glutamate-induced orofacial nociception in rodents and its action on nerve excitability. J. Orofac. Pain 24, 305–312.

Raymundo, L.J.R.P., Guilhon, C.C., Alviano, D.S., Matheus, M.E., Antoniolli, A.R., Cavalcanti, S.C.H., Alves, P.B., Alviano, C.S., Fernandes, P.D., 2011. Characterisation of the anti-inflammatory and antinociceptive activities of the *Hyptis pectinata* (L.) Poit essential oil. J. Ethnopharmacol. 134, 725–732.

Sessle, B.J., 2011. Peripheral and central mechanisms of orofacial inflammatory pain. Int. Rev. Neurobiol. 97, 179–206.

Siqueira, R.S., Bonjardim, L.R., Araújo, A.A.S., Araújo, B.E.S., Melo, M.G.D., Oliveira, M.G.B., Gelain, D.P., Silva, F.A., De Santana, J.M., Albuquerque-Júnior, R.L.C., Rocha, R.F., Moreira, J.C.F., Antoniolli, A.R., Quintans-Júnior, L.J., 2010. Antinociceptive activity of atranorin in mice orofacial nociception tests. Z. Naturforschung. C: J. Biosci. 65, 551–561.

Siqueira-Lima, P.S., Araújo, A.A.S., Lucchese, A.M., Quintans, J.S.S., Menezes, P.P., Alves, P.B., de Lucca Júnior, W., Santos, M.R.V., Bonjardim, L.R., Quintans-Júnior, L.J., 2014. β-Cyclodextrin complex containing *Lippia grata* leaf essential oil reduces orofacial nociception in mice – evidence of possible involvement of descending inhibitory pain modulation pathway. Basic Clin. Pharmacol. Toxicol. 114 (2), 188–196.

Trevisani, M.G.R., 2013. TRPV1 antagonists as analgesic agents. Open Pain J. 6, 108–118.

Venâncio, A.M., Marchioro, M., Estavam, C.S., Melo, M.S., Santana, M.T., Onofre, A.S.C., Guimarães, A.G., Oliveira, M.G.B., Alves, P.B., de Pimentel, H.C., Quintans-Júnior, L.J., 2011. *Ocimum basilicum* leaf essential oil and (−)-linalool reduce orofacial nociception in rodents: a behavioral and electrophysiological approach. Rev. Bras. Farmacogn. 21, 1043–1051.

Willcockson, H., Valtschanoff, J., 2008. AMPA and NMDA glutamate receptors are found in both peptidergic and non-peptidergic primary afferent neurons in the rat. Cell Tissue Res. 334, 17–23.