Evaluation of the Anti-Inflammatory and Antinociceptive Properties of *p*-Cymene in Mice

Leonardo R. Bonjardim*, Edisleide S. Cunha, Adriana G. Guimarães, Michele F. Santana, Makson G. B. Oliveira, Mairim R. Serafini, Adriano A. S. Araújo, Ângelo R. Antoniolli, Sócrates C. H. Cavalcanti, Márcio R. V. Santos, and Lucindo J. Quintans-Júnior

Department of Physiology, Universidade Federal de Sergipe (DFS/UFS), Aracaju, SE, Brazil. Fax: (0055) 79-2105-6494. E-mail: lbonjardim@yahoo.com.br

* Author for correspondence and reprint requests

Z. Naturforsch. **67c**, 15–21 (2012); received March 11/November 21, 2011

We attempted to identify the antinociceptive and anti-inflammatory actions of the monoterpene *p*-cymene. Firstly, behavioural screening was carried out to verify the influence of *p*-cymene [25, 50, and 100 mg/kg intraperitoneal (i.p.)] on the central nervous system (CNS) activity. The antinociceptive activity of *p*-cymene was evaluated by the acetic acid-induced writhing response, formalin, and hot-plate test, respectively. The leukocyte migration induced by injection of carrageenan was used to assess the anti-inflammatory activity. *p*-Cymene showed depressant activity on CNS after 4 h of treatment and also a possible action on the autonomous nervous system (ANS), mainly at the dose of 100 mg/kg (i.p.). It was found that *p*-cymene (50 and 100 mg/kg, i.p.) significantly ($p < 0.05$) reduced the writhing responses induced by acetic acid. *p*-Cymene also decreased the licking time in the first and second phase, respectively, of the formalin test. The results of the hot-plate test showed that all doses of *p*-cymene increased significantly the latency time of the response to the thermal stimulus in both licking and jumping parameters. In addition, there was a significantly ($p < 0.05$) decreased leukocyte migration at all doses of *p*-cymene. The experimental data demonstrate that *p*-cymene possesses antinociceptive and anti-inflammatory activities.

Key words: Monoterpene, *p*-Cymene, Pain, Inflammation

Introduction

Pain is a sensorial modality which in many cases represents the only symptom for the diagnosis of several diseases and often has a protective function. Throughout history man has used many different forms of therapy for the relief of pain, among them medicinal herbs are prominent due to their wide-spread popular use (Almeida *et al*., 2001).

Monoterpenes represent a large group of naturally occurring organic compounds whose basic structure consists of two linked isoprene units. They constitute 90% of the essential oils and have a great variety of structures (Bakkali *et al*., 2008). Among their numerous pharmacological activities, these compounds have strong antinociceptive and anti-inflammatory effects (Guimarães *et al*., 2010; Melo *et al*., 2010; Quintans-Júnior *et al*., 2010, 2011).

p-Cymene [1-methyl-4-(1-methylethyl)benzene; Fig. 1] is an aromatic monocyclic monoterpene (Dewick, 2009). This compound is present in volatile oils from over 100 plants and occurs naturally in more than 200 foodstuffs, such as orange juice, oregano, and almost any other spice (Philis, 2005).

The commercial importance of *p*-cymene is due to its utilization as an intermediate in the industrial syntheses of fragrances, flavours, herbicides and, principally, pharmaceuticals (Luengo *et al*., 2008). Recently, we suggested that *p*-cymene might represent an important biomolecule for the management of orofacial painful conditions (Santana *et al*., 2011). However, no data exists on the possible antinociceptive and anti-inflammatory activities of this compound. Therefore the purpose of the present study was to evaluate the specific effects of *p*-cymene on the central nerv-

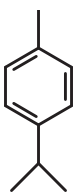

Fig. 1. Chemical structure of *p*-cymene.

ous system (CNS) as well as its antinociceptive and anti-inflammatory actions.

Materials and Methods

Chemicals

For all *in vivo* experiments, the following agents were used: *p*-cymene (99.7% purity), polyoxyethylene-sorbitan monooleate (Tween 80), naloxone, acetic acid, carrageenan lambda (all from Sigma, St. Louis, MO, USA), acetylsalicylic acid (ASA) (Roche, Jaguaré, São Paulo, Brazil), morphine (Cristalia, Itapira, São Paulo, Brazil), dexamethasone (Aché, Guarulhos, São Paulo, Brazil), and formaldehyde (Vetec, Duque de Caxias, Rio de Janeiro, Brazil). The vehicle was saline solution and 0.2% Tween 80 which was used to dissolve the test drugs.

Animals

Adult (2–3 months of age) male albino Swiss mice (20–30 g) were randomly housed in appropriate cages at (25 ± 2) °C with a 12 h/12 h light/dark cycle (light from 06:00 a.m. to 06:00 p.m.), with free access to food (Purina, São Paulo, Brazil) and water. All experimental observations were conducted between 12:00 a.m. and 05:00 p.m. All procedures were carried out in accordance with the Animal Care and Use Committee at the Federal University of Sergipe, Aracaju, SE, Brazil (CEPA/UFS 03/09).

Behavioural screening

Initially, the mice were divided into four groups ($n = 8$ per group). Subsequently, the three experimental groups were treated with *p*-cymene [25, 50, and 100 mg/kg, intraperitoneal (i.p.)] and the control group received only the vehicle (saline + 0.2% Tween 80). Specific behavioural responses (piloerection, palpebral ptosis, abdominal contortions, locomotion, hypothermia, muscular tonus, trembling, forepaw paralysis, sedation, ambulation reduction, response to touch, analgesia, and defecation) were observed and graded as presence (+) or absence (-) according to Almeida *et al.* (1999) during 4 h after i.p. administration of *p*-cymene or vehicle.

Acetic acid-induced writhing

This study was performed according to Koster *et al.* (1959) and Broadbear *et al.* (1994). Mice ($n = 8$ per group) were injected i.p. with 0.6% acetic acid at a dose of 10 ml/kg 30 min after the i.p. administration of *p*-cymene (25, 50, and 100 mg/kg, i.p.), ASA (300 mg/kg, i.p.) or vehicle (saline + 0.2% Tween 80, i.p.). Subsequently, the writhing responses were counted for 15 min, after a latency period of 5 min.

Formalin-induced pain

This test was carried out as described by Hunskaar and Hole (1987). Each mouse was placed in the chamber for 30 min before the treatment in order to allow acclimatization to the new environment. Then, nociception was induced by 20 μl of 1.0% formalin solution (0.92% formaldehyde) in phosphate buffer (137 mM NaCl, 10 mM phosphate, 2.7 mM KCl, and pH 7.4) injected into the dorsal surface of the left hind paw using a microsyringe with a 26-gauge needle. Mice ($n = 8$ per group) were treated with *p*-cymene (25, 50, and 100 mg/kg, i.p.), ASA (300 mg/kg, i.p.), and vehicle (saline + 0.2% Tween 80, i.p.) 30 min prior to formalin injection. Each animal was then returned to the chamber, and the duration of paw licking was measured at 0–5 min (early phase) and 15–30 min (late phase) after formalin administration.

Hot-plate test

The hot-plate test was carried out as described by Eddy and Leimbach (1953). The animals ($n = 8$ per group) were placed individually on a metal plate heated to (50 ± 0.5) °C. The reaction time was noted by observing the licking of the hind paws at 0 (baseline), 0.5, 1.0, 1.5, and 2.0 h after administration of *p*-cymene (25, 50, and 100 mg/kg, i.p.) or vehicle (saline + 0.2% Tween 80). Morphine (5 mg/kg, i.p.) was used as reference drug. The effect of pre-treatment with naloxone (0.5 mg/kg, i.p.) on the antinociception produced by *p*-cymene (100 mg/kg, i.p.) and morphine (5 mg/kg, i.p.) was also determined.

Leukocyte migration induced by carrageenan

The leukocyte migration was induced by injection of carrageenan (1%, 0.25 ml/cavity) into the peritoneal cavity of mice 30 min after administration of *p*-cymene (25, 50, and 100 mg/kg, i.p.), dexamethasone (2 mg/kg, i.p.) or vehicle (saline + 0.2% Tween 80) by a modification of the technique previously described by Bastos *et al.* (2007).

The mice were euthanized by cervical dislocation 4 h after carrageenan injection. Shortly after, phosphate-buffered saline (PBS) containing EDTA (1 mM, i.p., 3 ml) was injected. Immediately a brief massage was done for further fluid collection, which was centrifuged (5000 rpm, 6 min) at room temperature. The supernatant was disposed and 1 ml of PBS was added to the precipitate. An aliquot of 10 μl from this suspension was dissolved in 200 μl of Turk solution, and the total cells were counted in a Neubauer chamber under a microscope. The results were expressed as the number of leukocytes/ml. The percentage of the leukocyte inhibition was determined as follows: % leukocyte inhibition = $(1-T/C) \cdot 100$, where T represents the leukocyte counts of the treated group and C represents the leukocyte counts of the control group.

Statistical analyses

Data analyses were performed using the GraphPad Prism 5.0 software. The obtained data were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's test (post hoc test). Differences were considered significant if $p < 0.05$.

Results

Behavioural screening

p-Cymene at doses of 50 and 100 mg/kg (i.p.) induced depressant activity in the CNS of mice based on the following behavioural alterations observed during the 4 h after the treatment: decrease of the spontaneous activity, analgesia, and sedation. The behavioural screening also demonstrated a possible action on the autonomous nervous system (ANS), since the animals showed decreased urination and defecation. These effects were more pronounced at the dose of 100 mg/kg, i.p.

Acetic acid-induced writhing

p-Cymene at doses of 50 and 100 mg/kg, i.p. significantly ($p < 0.05$ and $p < 0.01$, respectively) reduced writhing induced by acetic acid (Fig. 2).

Formalin-induced pain

The results shown in Fig. 3 indicate that in the early and late phases, all doses of *p*-cymene significantly reduced the licking time compared with the control group.

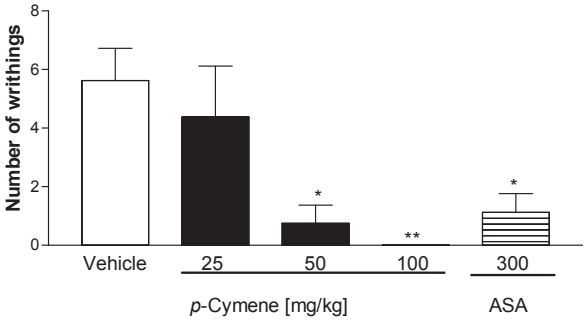

Fig. 2. Antinociceptive effect of vehicle (control group), ASA (acetylsalicylic acid; 300 mg/kg, i.p.), and *p*-cymene (25, 50, and 100 mg/kg, i.p.) on acetic acid-induced writhings in mice. Each column represents the mean ± S.E.M. of 8 animals. Statistical differences between the treated and the control groups were evaluated by ANOVA and Tukey's tests, and the asterisks denote the levels of significance in comparison with control groups; $^{**}p < 0.01$; $^{*}p < 0.05$.

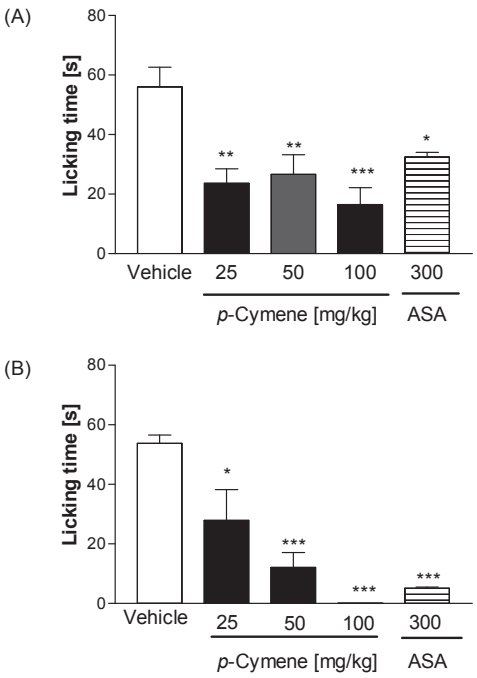

Fig. 3. Antinociceptive effect of vehicle (control group), ASA (acetylsalicylic acid; 300 mg/kg, i.p.), and *p*-cymene (25, 50, and 100 mg/kg, i.p.) against (A) first phase (0–5 min) and (B) second phase (15–30 min) of formalin-induced nociception in mice. Each column represents the mean ± S.E.M. of 8 animals. Statistical differences between the treated and the control groups were evaluated by ANOVA and Tukey's tests, and the asterisks denote the levels of significance in comparison with control groups; $^{*}p < 0.05$; $^{***}p < 0.001$.

Hot-plate test

Table I shows the results of the hot-plate test. The parameter "reaction time" corresponding to the latency time of response to the thermal stimulus was significantly increased by the treatment with *p*-cymene (25, 50, and 100 mg/kg, i.p.) at 0.5 and 1 h. At 1.5 h, this effect was observed only at doses of 50 and 100 mg/kg, i.p., and at 2 h only the highest dose provoked an antinociceptive effect. Naloxone did not reverse the effect of *p*-cymene (100 mg/kg, i.p.) but antagonized the antinociceptive effect of morphine.

Leukocyte migration induced by carrageenan

Fig. 4 shows that *p*-cymene (25 and 50 mg/kg, i.p., $p < 0.05$; 100 mg/kg, i.p., $p < 0.001$) and dexamethasone (DEXA) (2 mg/kg, i.p.) significantly reduced the leukocyte migration (predominantly neutrophil migration) induced by injection of carrageenan into the peritoneal cavity.

Discussion

The present study demonstrated that intraperitoneal administration of *p*-cymene appears to exert a depressant effect on the central nervous system and produces a suppressive effect on nociceptive responses evoked by chemical and thermal stimuli in mice. Further, this work also revealed that *p*-cymene reduces the leukocyte migration induced by carrageenan, maybe by modulation of the production of inflammatory mediators.

In behavioural screening, mice treated with *p*-cymene at higher doses exhibited changes in the behaviour, indicating possible effects on the CNS, similar to those of drugs that reduce central activity as described for other monoterpenes (Melo *et al.*, 2010).

Additionally, *p*-cymene exhibited both peripheral and central antinociceptive properties in different tests of nociception. In the acetic acid-induced abdominal writhing test, nociception was elicited by the injection of an irritant such as acetic acid into the peritoneal cavity which produced episodes of characteristic stretching movements and abdominal constriction (writhing), and inhibition of the number of episodes by analgesics was easily quantifiable (Quintans-Júnior *et al.*, 2011).

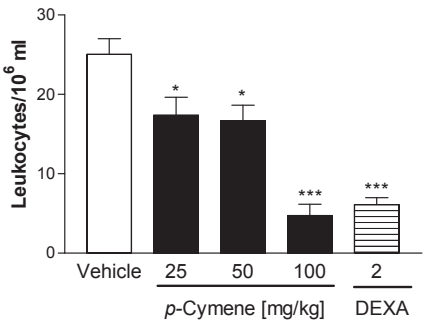

Fig. 4. Effect of *p*-cymene (25, 50, and 100 mg/kg, i.p.) or DEXA (dexamethasone; 2 mg/kg, i.p.) on leukocyte migration into the peritoneal cavity induced by carrageenan (1%, i.p.). Each column represents the mean ± S.E.M. of 8 animals. Statistical differences between the treated and the control groups were evaluated by ANOVA and Tukey's tests, and the asterisks denote the levels of significance in comparison with control groups; $*p < 0.05$; $***p < 0.001$.

Table I. Effects of *p*-cymene (25, 50, and 100 mg/kg, i.p.) or morphine on thermal nociception. Data represents the mean ± S.E.M. of latency time to the thermal stimulus.

Treatment	Dose (mg/kg)	Reaction time [s][a]				
		Baseline	0.5 h	1 h	1.5 h	2 h
Vehicle	-	6.7 ± 2.4	8.1 ± 0.76	8.1 ± 0.6	8.7 ± 2.0	6.1 ± 0.6
p-Cymene	25	7.1 ± 3.2	14.2 ± 3.6[c]	15.6 ± 1.8[b]	8.7 ± 2.3	7.7 ± 3.2
p-Cymene	50	8.0 ± 2.2	13.8 ± 1.3[b]	15.2 ± 1.7[b]	16.5 ± 2.0[b]	8.2 ± 2.9
p-Cymene	100	7.7 ± 2.9	14.2 ± 1.8[c]	18.2 ± 1.5[d]	17.6 ± 1.9[d]	14.1 ± 2.2[d]
p-Cymene + naloxone	100 + 0.5	8.1 ± 3.1	11.9 ± 3.7[NS]	14.1 ± 4.9[NS]	12.4 ± 5.8[NS]	10.0 ± 3.9[NS]
Morphine	5	7.1 ± 2.8	26.5 ± 4.5[c]	29.3 ± 3.5[c]	28.7 ± 4.1[c]	29.1 ± 5.9[c]
Morphine + naloxone	5 + 0.5	5.9 ± 4.1	11.4 ± 4.4[e]	12.7 ± 5.8[e]	19.5 ± 4.9[e]	13.7 ± 6.2[e]

[a] Corresponds to the latency time in response to the thermal stimulus.
[b] $p < 0.05$ statistical differences between the treated and the control groups (ANOVA and Tukey's test).
[c] $p < 0.01$ statistical differences between the treated and the control groups.
[d] $p < 0.001$ statistical differences between the treated and the control groups.
[e] $p < 0.001$ statistical differences between morphine and morphine + naloxone.
[NS] No statistical differences between *p*-cymene and *p*-cymene + naloxone (ANOVA and Tukey's test).

In this test, *p*-cymene significantly reduced the nociceptive behavioural response.

This is a standard, simple, and sensitive test for measuring analgesia induced by both opioids and peripherally acting analgesics (Guimarães *et al.*, 2010). This method has been associated with prostanoids in general, *e.g.* increased levels of PGE_2 and $PGF_2\alpha$ in peritoneal fluids as well as of lipoxygenase products (Verma *et al.*, 2005). Although this test is nonspecific (*e.g.* anticholinergic, tricyclic antidepressant, antihistaminic and other agents show activity in this test), it is widely used for analgesic screening and involves local peritoneal receptors (cholinergic and histaminic receptors) and the mediators acetylcholine and histamine (Queiroz *et al.*, 2010).

This deficiency can be overcome by using other experimental protocols, such as the formalin test. This method is used as a primary behavioural screen for the assessment of the antinociceptive activity of compounds used in moderate, long-lasting clinical pain. In mice, the spontaneous nociceptive responses are typically characterized by lifting, biting/licking, and flinching of the hind paw after the injection of formalin into either the dorsal or plantar surface of the paw (Khan *et al.*, 2010).

Further, this test allows evaluating two distinct phases of painful sensitization: the first phase, that occurs during 5 min after the formalin injection (neurogenic nociception), characterized by the direct stimulation of nociceptors presents on afferent C- and, in part, Aδ-fibers (glutamate and substance P release) (Dos Santos *et al.*, 2009); the second phase of nociception, that occurs between 15 and 30 min after formalin injection (inflammatory nociception), is related with the release of pro-inflammatory mediators (Tjolsen *et al.*, 1992) such as adenosine, bradykinin, histamine, prostaglandin, and serotonin (Dos Santos *et al.*, 2009).

For these reasons, the formalin-induced nociception model was also employed to evaluate the antinociceptive effect of *p*-cymene. The treatment with *p*-cymene significantly reduced the nociceptive response in both phases of formalin-induced nociception. This data allows us to suggest that *p*-cymene could be responsible for both peripheral and central antinociceptive action.

Moreover, the hot-plate test was used to study the involvement of supraspinal and spinal components (Le Bars *et al.*, 2001). This test is selective for analgesics acting centrally, such as morphine. Experimental evidence obtained in this study indicated that *p*-cymene at all doses increases significantly the latency time of mice on the hot plate. These findings corroborate the results obtained in the first phase of the formalin test and suggest that *p*-cymene may modulate conduction pathways of nociceptive stimulation. However, this effect was not antagonized by naloxone, discarding the hypothesis of the possible involvement of the opioid system in the mechanism of action of *p*-cymene.

To confirm the probable anti-inflammatory activity of *p*-cymene, peritonitis was induced by carrageenan in mice. Peritonitis is a frequent inflammatory condition with severe morbidity and mortality and can be caused by bacteria, fungi, and parasites (Queiroz *et al.*, 2010). Carrageenan-induced peritonitis represents a well characterized model for investigation of compounds with an anti-inflammatory effect on acute peritoneal inflammation (Loram *et al.*, 2007).

Cell migration during inflammation depends on the release of local mediators which are responsible for local vascular and tissue changes as well as for the recruitment of defence cells. The inflammation induced by carrageenan involves cell migration, plasma exudation, and production of mediators, such as nitric oxide, prostaglandins E_2, IL-1β, IL-6, and TNF-α (Salvemini *et al.*, 1996; Loram *et al.*, 2007). These mediators recruit leukocytes, such as neutrophils, in several experimental models (Thomazzi *et al.*, 2010). Treatment with anti-inflammatory drugs, such as dexamethasone, indomethacin, and nimesulide, inhibits cell migration induced by carrageenan (Dapino *et al.*, 1994; Arruda *et al.*, 2003).

In this test, *p*-cymene inhibited leukocyte migration induced by carrageenan. A putative mechanism explaining this activity may be inhibition of the synthesis of inflammatory mediators whose involvement in cell migration is well established (Quintans-Júnior *et al.*, 2011). This supposed anti-inflammatory activity of *p*-cymene may contribute to the control of nociceptive responses induced by acetic acid and during the second phase of the response to formalin.

In addition, it was previously found that *p*-cymene is metabolized to carvacrol and hydroxycarvacrol in some rodent species (Walde *et al.*, 1983). Carvacrol possesses antinociceptive activity associated with the inhibition of prostaglandin synthesis (Guimarães *et al.*, 2010), since it is

a potent suppressor of COX-2 expression and an activator of peroxisome proliferator-activated receptors (PPAR) α and γ (Hotta *et al*., 2010). Thus, *p*-cymene could be acting as a pro-drug, which when biotransformed into carvacrol may have its antinociceptive and anti-inflammatory effects enhanced.

In this regard, it can be concluded that *p*-cymene possesses peripheral and central antinociceptive properties as well as anti-inflammatory activity. The precise mechanisms involved in antinociceptive and anti-inflammatory effects are currently under investigation, but possibly *p*-cymene and its metabolites could act on the arachidonic acid cascade and/or modulation of the production of pro-inflammatory molecules.

Acknowledgements

We would like to thank the Fundação de Amparo à Pesquisa do Estado de Sergipe (FAPITEC-SE) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) of Brazil for financial support.

Almeida R. N., Falcão A. C. G. M., Diniz R. S. T., Quintans-Júnior L. J., Polari R. M., Barbosa-Filho J. M., Agra M. F., Duarte J. C., Ferreira C. D., Antoniolli A. R., and Araújo C. C. (1999), Metodologia para avaliação de plantas com atividade no sistema nervoso central e alguns dados experimentais. Rev. Bras. Farm. **80**, 72–76.

Almeida R. N., Navarro D. S., and Barbosa-Filho J. M. (2001), Plants with central analgesic activity. Phytomedicine **8**, 310–322.

Arruda V. A., Ferreira de Araújo M., Bom C., and Lobo de Araújo A. (2003), *Bothrops lanceolatus* (Fer de Lance) venom stimulates leukocyte migration into the peritoneal cavity of mice. Toxicon **41**, 99–107.

Bakkali F., Averbeck S., Averbeck D., and Idaomar M. (2008), Biological effects of essential oils – a review. Food Chem. Toxicol. **46**, 446–475.

Bastos L. F., Merlo L. A., Rocha L. T., and Coelho M. M. (2007), Characterization of the antinociceptive and anti-inflammatory activities of doxycycline and minocycline in different experimental models. Eur. J. Pharmacol. **576**, 171–179.

Broadbear J. H., Negus S. S., Butelman E. R., Costa B. R., and Woods J. H. (1994), Differential effects of systemically administered nor-binaltorphimine (nor-BNI) on k-opioid agonists in mouse writhing assay. Psychopharmacology **15**, 311–319.

Dapino P., Ottonello L., and Dallegri F. (1994), The anti-inflammatory drug, nimesulide inhibits neutrophil adherence to an migration across monolayers of cytokine activated endothelial cells. Respirations **61**, 336–341.

Dewick P. M. (2009), Medicinal Natural Products: a Biosynthetic Approach, 3rd ed. Wiley, Chichester, UK.

Dos Santos D. A., Fukui M. D. J., Dhammika Nanayakkara N. P., Khan S. I., Sousa J. P. B., Bastos J. K., Andrade S. F., Silva Filho A. A., and Quintão N. L. M. (2009), Anti-inflammatory and antinociceptive effects of *Baccharis dracunculifolia* DC (Asteraceae) in different experimental models. J. Ethnopharmacol. **127**, 543–550.

Eddy N. B. and Leimbach D. (1953), Synthetic analgesics: IIR dithienyl-butenyl and dithienylbutylamines. J. Pharmacol. Exp. Ther. **107**, 385–393.

Guimarães A. G., Oliveira G. F., Melo M. S., Cavalcanti S. C., Antoniolli A. R., Bonjardim L. R., Silva F. A., Santos J. P., Rocha R. F., Moreira J. C., Araújo A. A. S., Gelain D. P., and Quintans-Júnior L. J. (2010), Bioassay-guided valuation of antioxidant and antinociceptive activities of arvacrol. Basic Clin. Pharmacol. Toxicol. **107**, 949–957.

Hotta M., Nakata R., Katsukawa M., Hori K., Takahashi S., and Inoue H. (2010), Carvacrol, a component of thyme oil, activates PPAR α and γ and suppresses COX-2 expression. J. Lipid Res. **51**, 132–139.

Hunskaar S. and Hole K. (1987), The formalin test in mice: dissociation between inflammatory and non-inflammatory pain. Pain **30**, 103–104.

Khan H., Saeed M., Gilani A. H., Khan M. A., Dar A., and Khan I. (2010), The antinociceptive activity of *Polygonatum verticillatum* rhizomes in pain models. J. Ethnopharmacol. **127**, 521–527.

Koster R., Anderson M., and Beer E. J. (1959), Acetic acid for analgesic screening. Federal Proc. **18**, 412–416.

Le Bars D., Gozariu M., and Cadden S. W. (2001), Animal models of nociception. Pharmacol. Rev. **53**, 597–652.

Loram L. C., Fuller A., Fick L. G., Cartmell T., Poole S., and Mitchell D. (2007), Cytokine profiles during carrageenan-induced inflammatory hyperalgesia in rat muscle and hind paw. J. Pain **8**, 127–136.

Luengo M. A. M., Yates M., Domingo M. J. M., Casal B., Iglesias M., Esteban M., and Ruiz-Hitzky E. (2008), Synthesis of *p*-cymene from limonene, a renewable feedstock. Appl. Catal. B: Environ. **81**, 218–224.

Melo M. S., Sena L. C. S., Barreto F. J. N., Bonjardim L. R., Almeida J. R. G. S., Lima J. T., De Sousa D. P., and Quintans-Júnior L. J. (2010), Antinociceptive effect of citronellal in mice. Pharm. Biol. **48**, 411–416.

Philis J. G. (2005), The S1←S0 spectrum of jet-cooled *p*-cymene. Spectrochim. Acta Part A **61**, 1239–1241.

Queiroz A. C., De Lira D. P., Dias T. L., De Souza E. T., Da Matta C. B., De Aquino A. B., Silva L. H., Da

Silva D. J., Mella E. A., Agra M. F., Filho J. M., De Araújo-Júnior J. X., Santos B. V., and Alexandre-Moreira M. S. (2010), The antinociceptive and anti-inflammatory activities of *Piptadenia stipulacea* Benth. (Fabaceae). J. Ethnopharmacol. **128**, 377–383.

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

Quintans-Júnior L. J., Guimarães A. G., Santana M. T., Araújo B. E. S., Moreira F. V., Bonjardim L. R., Araujo A. A. S., Antoniolli A. R., Botelho M. A., Almeida J. R. G. S., and Santos M. R. V. (2011), Citral reduces nociceptive and inflammatory response in rodents. Braz. J. Pharmacogn. **21**, 497–502.

Salvemini D., Wang Z. Q., Wyatt P. S., Bourdon D. M., Marino M. H., Manning P. T., and Currie M. G. (1996), Nitric oxide: a key mediator in the early and late phase of carrageenan-induced rat paw inflammation. Br. J. Pharmacol. **118**, 829–838.

Santana M. F., Quintans-Júnior L. J., Cavalcanti S. C. H., Oliveira M. G. B., Guimarães A. G., Cunha E. S., Melo M. S., Santos M. R. V., Araújo A. A. S., and Bonjardim L. R. (2011), *p*-Cymene reduces orofacial nociceptive response in mice. Rev. Bras. Farmacogn. http://dx.doi.org/10.1590/S0102-695X2011005000156

Thomazzi S. M., Silva C. B., Silveira D. C., Vasconcellos C. L., Lira A. F., Cambui E. V., Estevam C. S., and Antoniolli A. R. (2010), Antinociceptive and anti-inflammatory activities of *Bowdichia virgilioides* (sucupira). J. Ethnopharmacol. **127**, 451–456.

Tjolsen A., Berge D. G., Hunskaar S., Rosland J. H., and Hole K. (1992), The formalin test: an evaluation of the method. Pain **51**, 5–17.

Verma P. R., Joharapurkar A. A., Chatpalliwar V. A., and Asnani A. J. (2005), Antinociceptive activity of alcoholic extract of *Hemidesmus indicus* R. Br. in mice. J. Ethnopharmacol. **102**, 298–301.

Walde A., Ve B., Scheline R. R., and Monge P. (1983), *p*-Cymene metabolism in rats and guinea-pigs. Xenobiotica **13**, 503–512.