Anti-nociceptive and anti-inflammatory actions of *Nepeta cataria* L. var. citriodora (Becker) Balb. essential oil in mice

Efeitos antinociceptivos e anti-inflamatórios do óleo essencial de Nepeta cataria L. *var. citriodora (Becker) Balb. em camundongos*

Esther Lopes Ricci[1], Daniela Oliveira Toyama[1], João Henrique Guilardi Lago[2], Paulete Romoff[3], Thiago Berti Kirsten[4], Thiago Marinho Reis-Silva[4], Maria Martha Bernardi[3,5]

[1]Biological and Health Sciences Center, Mackenzie Presbyterian University, São Paulo-SP, Brazil; [2]Department of Exact Sciences and Earth, University of São Paulo, Diadema-SP, Brazil; [3]Sciences and Humanity Center, Mackenzie Presbyterian University, São Paulo-SP, Brazil; [4]School of Veterinary Medicine, University of São Paulo, São Paulo-SP, Brazil; [5]School of Veterinary Medicine, University Paulista, São Paulo-SP, Brazil.

Abstract

Objective – *Nepeta cataria* (catnip) is a plant used to treat human diseases and is also found in pet toys. This study was performed to analyze the anti-nociceptive and anti-inflammatory effects of *N. cataria* essential oil (NCEO) in female mice. **Methods** – Phytochemical analyses of NCEO were performed. In addition, female mice treated with the oil were observed in an open field for its general activity and to investigate the dose and time responses. The anti-nociceptive effects were evaluated by tail immersion and acetic acid writhing reflex tests. The anti-inflammatory oil properties were investigated by the carrageenan-induced edema test. **Results** – The results showed that 0.0005 and 0.001 mL/kg i.p. doses of NCEO increased the general activity of female mice, and the 0.0005 mL/kg dose reduced their immobility. Moreover, NCEO (0.0005 mL/kg) has anti-nociceptive properties, as the treated animals exhibited an increased latency of tail withdrawal and reduced acetic acid-induced abdominal constrictions. Furthermore, NCEO (0.0005 mL/kg) presented peripheric anti-inflammatory properties by reducing the induced edema after carrageenan injection. **Conclusions** – These effects may be due to the nepetalactone *trans-trans* and *trans-cis* nepetalactone isomers, which were detected as the predominant active components in the phytochemical analysis. It was suggested that the main effect of NCEO occurs on the central nervous system mechanism of pain.

Descriptors: Nepeta/chemistry; Plant oils; Edema/chemically induced; Immersion; Colic/chemically induced

Resumo

Objetivo – A *Nepeta cataria* (catnip) é uma planta utilizada para tratar doenças humanas e também é encontrada em brinquedos de animais de estimação. O objetivo deste estudo foi analisar os efeitos antinociceptivos e anti-inflamatório do óleo essencial de *N. cataria* (NCEO) em camundongos fêmeas. **Métodos** – A análise fitoquímica do NCEO foi realizada. Além disso, os animais que foram tratados com o óleo e foram observados em um campo aberto para mensurar a sua atividade em geral e investigar a dose e tempo de respostas. Os efeitos antinociceptivos foram avaliados pelos testes de imersão da cauda e reflexo de contorções abdominais causadas pelo ácido acético. As propriedades antiinflamatórias do óleo foram investigadas pelo teste de edema induzido por carragenina. **Resultados** – Os resultados mostraram que 0,0005 e 0,001mL/ kg doses ip. de NCEO aumentou a atividade geral de camundongos fêmeas, e dose de 0,0005 mL/kg reduziu sua imobilidade. Além disso, NCEO (0,0005 mL/kg), tem propriedades antinociceptiva, como os animais tratados apresentaram uma maior latência de retirada de cauda e reduziu as contorções abdominais induzidas pelo ácido acético. Além disso, NCEO (0,0005 mL/kg) apresentou efeito periférico e propriedades antiinflamatórias, reduzindo o edema induzido após a injeção de carragenina. **Conclusões** – Estes efeitos podem ser devido aos isômeros nepetalactone *trans-trans* e nepetalactone *trans-cis*, que foram detectados como os componentes ativos predominante na análise fitoquímica. Foi sugerido que o principal efeito da NCEO ocorre no mecanismo do sistema nervoso central da dor.

Descritores: Nepeta/química; Óleos vegetais; Edema/induzido quimicamente; Imersão; Cólica/induzido quimicamente

Introduction

The *Nepeta cataria* plant (catnip or catmint) belongs to the mint family (Lamiaceae)[1]. *Nepeta* is a genus composed of perennial or annual herbs, with a cosmopolitan distribution in Asia, Europe and North Africa. For a long time, *N. cataria* has been used in North American popular medicine and in teas, dyes or infusions. *Nepeta* species are widely used because of their antispasmodic, expectorant, diuretic, antiseptic, febrifuge, antitussive and antiasthmatic effects[2]. Moreover, the fresh or dried plant, juice or extract can induce extreme pleasure manifestations in cats. For these reasons, *N. cataria* is also used in toys for pets[3].

Klimek and Modnick[4] (2005) determined the presence of 50% nepetalactone, 33% nepetalic acid, and 14% viscous yellow neutral in *N. cataria* essential oil. Ursolic acid, flavonoids and phenolic acids were also detected in *N. cataria* essential oil.

Aydin *et al.*[5] (1998) investigated the anti-nociceptive effects of essential oils from *Nepeta* species, including *N. phyllochlamys* P. H. Davis, *N. nuda* L. ssp. nuda, and *N. caesarea Boiss*, using tail flick and tail immersion tests. These authors detected central and peripheral anti-nociceptive effects in these plants.

Furthermore, *N. cataria* L. var. citriodora (Becker) Balb. (Lamiaceae) also can be used as an anti-inflammatory agent[4]. More specially, ursolic acid from *N. cataria* is described as having anti-inflammatory proprieties[6].

Despite several studies about *Nepeta* species showing anti-nociceptive and anti-inflammatory properties, no study has been performed in laboratory animals with the *N. cataria* essential oil. Thus, we investigated the anti-nociceptive and anti-inflammatory effects of *N. cataria* essential oil (NCEO) in mice. First, phytochemical analyses of NCEO were performed. The effects of two oil doses on the mice general activity were assessed by the open-field test. The immobility duration was taken as an index of motor impairment and the locomotion frequency was used to determine the time dose effect. The anti-nociceptive effect was evaluated by tail immersion and acetic acid writhing reflex tests. The anti-inflammatory oil properties were investigated by the carrageenan-induced edema test.

Methods

Botanical material collection and NCEO production

N. cataria was collected in Serra Azul Street, 308, Piracicaba, SP, Brazil, number 001/205. Specimens were identified by the botanist Oriana Favero, PhD from Mackenzie Presbyterian University. A voucher specimen was deposited in the Herbário Municipal de São Paulo, collection number PMSP8986.

The leaves of *N. cataria* (357.77 g) were subjected to hydrodistillation using a modified Clevenger apparatus. After four hours, the crude volatile oil was extracted with CH_2Cl_2 and dried with anhydrous $MgSO_4$. After filtration and evaporation of the solvent, 70 mg of crude oil (yield 0.02%) were obtained. The essential oil was diluted ten times in almond oil to be administered in animals.

Animals

Female Balb-C mice weighing 25-30 g from our colony were used. The animals were housed in groups of five in polypropylene cages (38 x 32 x 16 cm) with controlled room temperature (22 ± 2ºC), humidity (65–70%), and artificial lighting (12 h light/12 h dark cycle, lights on at 6:00 a.m.), and free access to Nuvilab® rodent chow (Nuvital Company, São Paulo, Brazil) and filtered water. Animals were divided randomly into control and experimental groups. To minimize the influence of possible circadian changes on mice behavior, the animals were observed at the same time of day (2:00 – 4:00 p.m.). The mice used in this study were maintained in accordance with the guidelines of the Committee on Care and Use of Laboratory Animal Resources of the School of Veterinary Medicine, University of São Paulo, Brazil.

Phytochemical analysis of the NCEO

The NCEO was analyzed by gas chromatography-flame ionization detector (GC–FID) in a HP 5890 series II gas chromatograph. The temperature program for the GC analysis was isothermal at 100°C for 2 min, 100-180°C at 5°C/min, isothermal at 180°C for 2 min, 180–250°C at 10°C/min, and then isothermal at 250°C for 5 min. The temperatures of injection and detection (flame ionization) were 180 and 220°C, respectively. A capillary column (HP-5) coated with 5% PhMe silicone (30 m x 0.32 mm i.d., film thickness 0.25 µm) was used. In addition, the crude NCEO was analyzed by GC/MS at 70 eV in an INCOS 50 Finnigan-Mat-quadrupole spectrometer using a capillary column (DB-5) coated with crosslinked methyl silicone gum (25 m x 0.200 mm i.d., film thickness 0.33 µm). The temperature program was isothermal at 100°C for 1 min, 100–250°C at 10°C/min, and then isothermal at 250°C for 20 min. The temperatures of injection and detection were 230 and 280°C, respectively. Structures of the identified derivatives were confirmed by ^{13}C nuclear magnetic resonance (^{13}C NMR and DEPT 135°), according to the methodology for the analysis of mixed terpenoids[7], using a Bruker DRX-500 operating at 125 MHz for the ^{13}C nucleus. Deuterated chloroform ($CDCl_3$) was used as the solvent and internal standard.

General activity in the open field

Female mice were observed in an open field to evaluate the effects of NCEO on their general activity and to determinate the effects of dose and time for subsequent studies. Twenty female mice were treated intraperitoneally (i.p.) with two doses of NCEO: 0.0005 and 0.001 mL/kg i.p. (n = 10 for both groups), and the control group (n = 10) received the vehicle solution (almond oil). The open-field device consisted of a round wooden arena (40 cm in diameter, 25.5-cm walls) painted gray with an acrylic washable covering and subdivided into 25 parts[8-9]. Immediately and 15, 30, 45, 180 and 300 min after these treatments, each mouse was individually placed in the center of the apparatus and the following parameters were measured over a period of 5 min: locomotion frequency (number of floor units entered with both feet), rearing frequency (number of times the rodents stood on their hind legs) and immobility duration (seconds of absence of activity). Hand-operated counters and stopwatches were employed to count these behaviors. The device was washed with a 5% alcohol/water solution before placing the animals to obviate possible biasing effects due to odor clues left by earlier mice.

The open field test was employed to determinate the general activity. Moreover, the open field test was employed to determinate the doses and time effects of NCEO. Based on the results of the open-field studies, the NCEO group received a dose of 0.0005 mL/kg for the subsequent anti-nociceptive and anti-inflammatory studies, because this level produced behavioral interferences. Based in these data, the remained experiments were performed between 15 and 180 min because this interval produced interferences on general activity.

Tail immersion test

Noxious thermal stimulation was attained by immersing the tip of the tail in hot water. First, three consistent baseline readings were taken with the distal 5 cm of the tail immersed in 52 ± 0.2°C water for a maximum period of 15 s. Nociception was estimated by the latency of flicking the tail. The interval between the three immersions was 30 s. Next, 0.0005 mL/kg of NCEO, 1 mL/Kg of almond oil (the NCEO vehicle), or 20 mg/kg of morphine (Cristália®, Itapira, SP, Brazil) were given by i.p route (n=10 both groups). Three further readings were taken (experimental test) 15, 30 and 45 min after the treatments. Data were taken as the difference between the baseline and the experimental test latencies.

Acetic acid writhing reflex

The mouse writhing test used was based on the method of Koster *et al.*[10] (1959). Contortions were induced by i.p. administration of acetic acid (Merck, 60 mg/Kg). Ten female mice were treated with NCEO (0.0005 mL/kg); the control group (n = 10) received the vehicle solution (almond oil); and the positive control group received an injection of morphine (20 mg/kg)[11], i.p. Fifteen min after the treatment, mice were injected with the acid acetic solution to induce the characteristic writhing. Results are presented as the number of contortions observed during the 20 min interval subsequent to the injection of the acid.

Carrageenan-induced edema

Carrageenan-induced edema was conducted according to Winder *et al.*[12] (1957). Briefly, 0.1 mL of carrageenan was injected subcutaneously (s.c.) into the left hind paw of each female mouse. The volume (mL) of induced edema was measured with a pachimeter immediately before and 30, 60, 120 and 180 min after carrageenan injection to determine the differences in paw volume up to the tibio-tarsal joint. A total of thirty female mice were injected with carrageenan. Sixty min later, they were divided into three equal groups (n = 10): an NCEO (0.0005 mL/kg) group; a control (almond oil) group; and an indomethacin (5 mg/Kg, i.p.) group as a positive control.

Statistical analysis

The results are expressed as mean ± SEM. Homoscedasticity was verified through the F test. Normality was verified through the Kolmogorov-Smirnov test. In the open-field test two way ANOVA followed by the Bonferroni *post hoc* test were used to analyze the results. In tail immersion test and carrageenan-induced edema test, one way ANOVA followed by the Turkey *post hoc* test were used to analyze the results. The Student's t test (unpaired, two-tailed) was used on acetic acid writhing reflex test. In all cases, results were considered significant if $p < 0.05$.

Results

Four major components were identified in *N. cataria* leaves: *trans,trans*-nepetalactone, *cis,trans*-nepetalactone, *trans,cis*-nepetalactone and nepetalactol, using GC-FID and GC-MS (Table 1) as well as ^{13}C NMR (Table 2). The obtained data showed the predominance of a diateriomeric mixture of *trans,trans*- and *trans,cis*-nepetalactone isomers (Figure 1), indicated by the different signs for each stereoisomer of carbons C-1 (δ 171.5/170.0), C-3 (δ 136.3/135.8), C-4a (δ 41.7/37.2), C-7 (δ 32.5/29.9), C-7a (δ 52.3/49.0), and C-9 (δ 20.4/17.6) and comparison with literature data[13].

Figure 2 shows the effects of NCEO (0.0005 and 0.001 mL/kg) i.p. administration on the general activity of female, as observed in the open field. As depicted in Figure 2A, a two-way ANOVA revealed significant differences between groups for various treatments [$F(2/162) = 23.42$, $p < 0.0001$] and times after treatment [$F(5/162) = 2.44$, $p = 0.037$]. For the control group, a multiple comparisons test

Table 1. Phytochemical composition obtained by GC/MS analysis of *N. cataria* essential oil

Composition	Relative %	MS data
trans-trans nepetalactone	50.38	166 (100), 151 (18), 123 (97), 109 (67), 95 (81), 81 (96), 69 (80)
cis-trans nepetalactone	6.66	166 (100), 151 (11), 123 (86), 109 (73), 95 (67), 81 (100), 69 (63)
trans-cis nepetalactone	21.74	166 (90), 151 (15), 123 (96), 109 (66), 95 (80), 81 (100), 69 (71)
Nepetalactol	0.49	168 (23), 150 (7), 135 (32), 121 (14), 111 (28), 97 (47), 81 (55), 58 (58), 41 (100)
Total	79.27	

showed an increased locomotion frequency in mice treated with NCEO after 0, 15 and 30 min in the open field for both the 0.0005 and 0.001 mL/kg doses. By contrast, the 0.0005 mL/kg dose only induced an increased locomotion frequency at 45 and 300 min after treatment. The data of the rearing frequencies are shown in Figure 2B. A two-way ANOVA revealed significant differences between groups for various treatments [$F(2/162) = 26.69$; $p < 0.0001$] and times after treatment [$F(5/162 = 0.40$, $p = 0.84$]). Mice treated with 0.001 mL/kg of NCEO presented a high rearing frequency in comparison to the control group in all sessions. No significant differences were detected in the rearing frequency between the 0.0005 mL/kg–treated and control mice. Figure 2C shows the duration of immobility. The two-way ANOVA revealed significant differences between the groups in terms of the treatment [$F(2/162 = 3.37$, $p = 0.037$]; however, no differences were detected between times after treatment [$F(5/162 = 1.71$, $p = 0.1343$]. Mice treated with 0.0005 mL/kg of NCEO presented low levels of immobility duration, whereas no differences were detected in this parameter with a 0.001 mL/kg dose.

The effects of 0.0005 mL/kg of the NCEO in the tail immersion test are shown in Figure 3. One-way ANOVA revealed differences between treatments ($F(2/81) = 267.59$, $P<0.0001$) and sessions ($F(2/81) = 70.32$, $P < 0.0001$). The Turkey test revealed that NCEO produced a significant anti-nociceptive effect compared to that on the control group, because NCEO animals increased the latency of the tail withdraw 15 and 45 min after treatment ($p < 0.0001$). Moreover, significant differences were detected between NCEO and morphine groups 30 and 45 min after treatments, being the morphine values higher than the NCEO data ($p < 0.0001$), showing that the NCEO is less potent than morphine.

A Student's t-test indicated a significant decrease in the number of writhing in the NCEO-treated group in comparison to controls (control group: 17.50 ± 1.50; NCEO group: 6.30 ± 0.50, $p<0.0001$). Data from morphine group were null, i.e., no mice presented the writhing reflex. Therefore, according to the acetic acid writhing reflex test, NCEO induced anti-nociceptive effects.

Figure 4 shows the effects of NCEO on carrageenan-induced edema. One-way ANOVA analysis revealed significant differences between treatments [$F(2/135) = 40.35$, $p < 0.001$] and time after carrageenan administration [$F(4/135) = 92.27$, $p < 0.0001$]. In comparison to the control group, the edema of NCEO-treated female mice was reduced at 30 ($p<0.001$), 60 ($p < 0.001$) and 90 ($p < 0.05$) min after carrageenan. As expected, comparisons between the indomethacin group and the control showed that the indomethacin-treated mice presented reduced edema at all times after treatment ($p < 0.001$).

Table 2. ^{13}C NMR data (d/ppm, 125 MHz, $CDCl_3$) of *trans-trans* and *trans-cis* nepetalactone isomers

Carbon	*trans-trans*		*trans-cis*	
	obtained	literature[12]	obtained	literature[12]
1	171.5	171.5	170.0	170.1
3	136.3	136.3	135.8	135.8
4	120.6	120.6	120.3	120.4
4a	41.7	41.8	37.2	37.3
5	25.5	25.6	26.0	26.1
6	31.6	31.6	31.8	32.0
7	32.5	32.5	29.9	29.9
7a	52.3	52.5	49.0	49.0
8	14.0	14.0	14.2	14.3
9	20.4	20.4	17.6	17.6

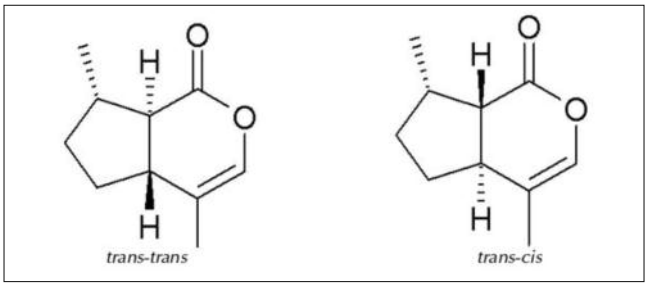

Figure 1. Nepetalactone *trans-trans* and *trans-cis* nepetalactone isomers of *N. cataria* essential oil

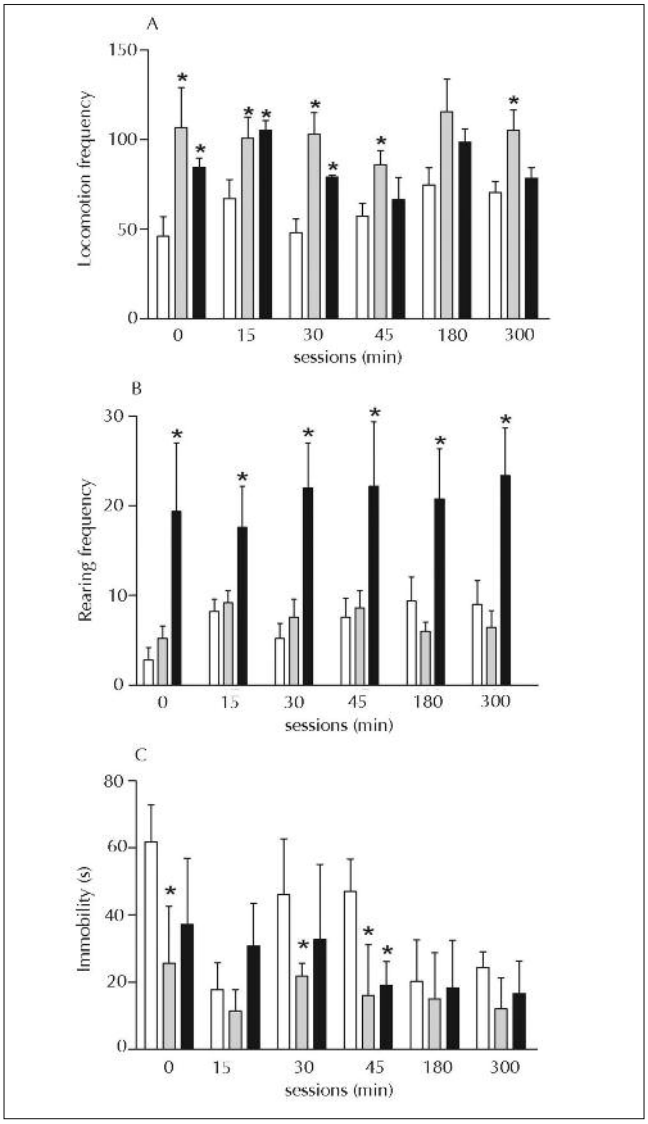

Figure 2. Effects of *N. cataria* essential oil (NCEO) on open-field behavior of female mice. (A) Locomotion frequency; (B) Rearing frequency; (C) Immobility duration. Clear bars = control group; gray bars = 0.0005 mL/kg of NCEO; black bars = 0.001 mL/kg of NCEO. * $p < 0.05$, compared with the control group (two-way ANOVA). Values represent mean ± S.E.M. n = 10 for all groups

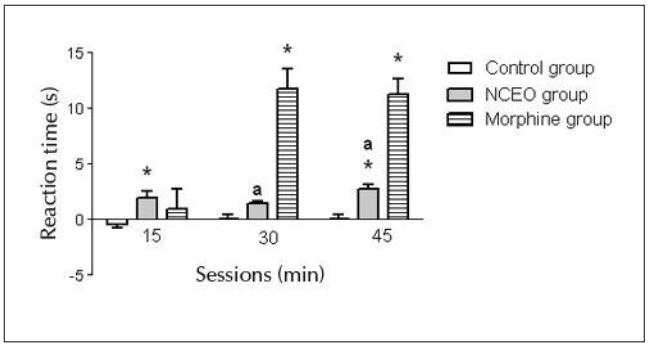

Figure 3. Effects of *N. cataria* essential oil (NCEO, 0.0005 mL/kg) on reaction time in tail immersion test of female mice. Clear bars = control group; gray bars = NCEO group * p < 0.05, compared with the control group (one-way ANOVA). Values represent mean ± S.E.M. n = 10 for both groups

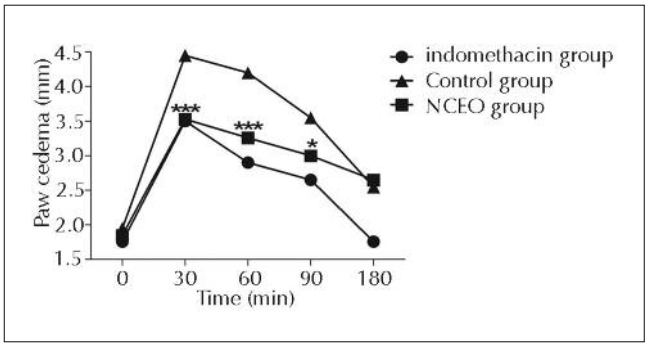

Figure 4. Effects of *N. cataria* essential oil (NCEO, 0.0005 mL/kg) on carrageenan-induced edema of female mice. Triangles = control group; squares = NCEO group; circles = indometacin group. *** p < 0.001, compared with the control group (one-way ANOVA). Values represent mean ± S.E.M. n = 10 for both groups

Discussion

The NCEO demonstrated antinociceptive properties in both, visceral and central nociceptive mouse models. Intraperitoneal injection of acetic acid induces peritoneal inflammation (acute peritonitis), which leads to a response characterized by contraction of the abdominal muscle accompanied by an extension of the forelimbs and elongation of the body. This writhing response is considered to be a visceral inflammatory pain model[10], and has been associated with increased levels of prostaglandins in peritoneal fluids[14]. Relative to controls, NCEO reduced significantly the mice writhing responses. In addition, morphine treatment, the positive control group, impaired this reflex.

In the tail immersion test, which consists of a thermal stimulus, an increase in the reaction time is generally considered as an important parameter for evaluating central anti-nociceptive activity[15]. This test can differentiate between central opioid-like and peripheral analgesics[16]. The anti-nociceptive activity of NCEO was reported to be similar to that of morphine, despite less potent. This reduced response could be a consequence of the NCEO dose employed. In addition, the NCEO effects started before the morphine effects, i.e., 15 min after the treatment. Results from this test also revealed that the anti-nociceptive effect of NCEO on mice remained up to 45 minutes after administration of the oil.

Moreover, NCEO presented a local anti-inflammatory property because the reduction of the induced edema occurred 30, 60 and 90 min after the carrageenan injection[17]. In this experiment, indomethacin, used as positive control group, revealed a peak of effects 30 min after treatment, remaining at least 45 min after the injection.

In all tests the relative potencies of the NCEO and the positive controls were not compared because only one NCEO dose was employed. Future, studies need to be done to investigate this aspect.

In experiments designed to evaluate the analgesic action of new agents, the pharmacological treatment can cause other behavioral alterations, such as motor incoordination and sedation, which might be misinterpreted as analgesia. Therefore, an altered exploratory behavior in the open field may be caused by changes in the anxiety state and/or sedation[18] as well as motor interferences[19]. In this study, the dose applied in the anti-nociceptive and anti-inflammatory studies was chosen based on results from open-field studies. Both doses (0.0005 and 0.001 mL/kg) of NCEO increased the locomotion frequency of female mice in the open field. However, the 0.0005 mL/kg dose was chosen for subsequent studies, because it inhibits immobility in the open field. Furthermore, severe interference of motor behavior could impair the nociceptive behavior analyses[20].

Previous studies with the essential oil from *N. caesarea Boiss*, another *Nepeta* species, showed significant analgesic activity in the tail flick and tail immersion tests. This effect was accompanied by a marked sedation, which was also blocked by naloxone, indicating the involvement of opioid receptors. By contrast, in our experiments, we observed a stimulant effect in the open-field test, similar to the effects from morphine (20 mg/kg) in mice reported by[21].

These behavioral effects might be due to the nepetalactone *trans-trans* and trans-cis nepetalactone isomers, which were detected in the phytochemical analysis as the active components.

Conclusions

Based on our findings from the acetic acid writhing reflex and the carrageenan-induced edema tests, NCEO presents peripheral anti-inflammatory analgesic effects. The tail immersion test suggests that the pharmacological actions were mediated by mu opioid receptors rather than by kappa and delta receptors[22]. Moreover, the peripheral analgesic action of NCEO on acetic acid-induced pain was comparable to that of morphine[23]. Thus, it is possible that the main effect of NCEO occurs on the central nervous system mechanism of pain.

This study is a preliminary study about the analgesic effects of NCEO. To confirm these findings and the central nervous system mechanism underlying these analgesic effects, future detailed studies using several NCEO doses as well as agonists and antagonists of opioid receptors are being planned. Further investigations of the possible anti-inflammatory effect of NCEO will be conducted.

Acknowledgments

This research, which was supported by the State of São Paulo Research Foundation (FAPESP, grant 05/60291-2), is part of the dissertation presented by Esther Lopes Ricci to Mackenzie Presbyterian University. Special thanks are given to Mr. Laudo Landi Bernardes for plant collection.

References

1. Adiguzel A, Ozer H, Sokmen M, Gulluce M, Sokmen A, Kilic H *et al.* Antimicrobial and antioxidant activity of the essential oil and methanol extract of Nepeta cataria. Pol J Microbiol. 2009;58:69-76.

2. Smitherman LC, Janisse J, Mathur A. The use of folk remedies among children in an urban black community: remedies for fever, colic, and teething. Pediatrics. 2005;115:e297-304.

3. Hatch RC. Effect of drugs on catnip (Nepeta cataria)-induced pleasure behavior in cats. Am J Vet Res. 1972;33:143-55.

4. Klimek B, Modnicki D. Terpenoids and sterols from Nepeta cataria L. var. citriodora (Lamiaceae). Acta Pol Pharm. 2005;62:231-5.

5. Aydin S, Beis R, Ozturk Y, Baser KH. Nepetalactone: a new opioid analgesic from Nepeta caesarea Boiss. J Pharm Pharmacol. 1998;50:813-7.

6. Taviano MF, Miceli N, Monforte MT, Tzakou O, Galati EM. Ursolic acid plays a role in Nepeta sibthorpii Bentham CNS depressing effects. Phytother Res. 2007;21:382-5.

7. Lago JHG, Brochini CB, Roque NF. Terpenes from leaves of Guarea macrophylla (Meliaceae). Phytochemistry. 2000;55:727-31.

8. Broadhurst PL. Experiments in phychogenetics. *In*: Eysenk HJ, editor. Experiments in personality. London: Routledge and Kegan Paul; 1960. p.31-71.

9. Faggin BM, Palermo-Neto J. Differential alterations in brain sensitivity to amphetamine and pentylenetetrazol in socially deprived mice. Gen Pharmacol. 1985; 16:299-302.

10. Koster R, Anderson M, Beer EJ. Acetic acid for analgesic screening. Fed Proc.1959;18:412-6.

11. Giorgi R, Bernardi MM, Cury Y. Analgesic effect evoked by low molecular weight substances extracted from Crotalus durissus terrificus venom. Toxicon. 1993;31:1257-65.

12. Winder CV, Wax J, Been MA. Rapid foot volume measurements on unanesthetized rats, and the question of a phenylbutazone effect on anaphylactoid edema. Arch Int Pharmacodyn Ther. 1957;112:174-87.

13. Liblikas I, Santangelo EM, Sandell J, Baeckström P, Svensson M, Jacobsson U *et al.* Simplified isolation procedure and interconversion of the diastereomers of nepetalactone and nepetalactol. J Nat Prod. 2005;68:886-90.

14. Chapman CR, Casey KL, Dubner R, Foley KM, Gracely RH, Reading AE. Pain measurement: an overview. Pain. 1985;22:1-31.

15. Rujjanawate C, Kanjanapothi D, Panthong A. Pharmacological effect and toxicity of alkaloids from Gelsemium elegans Benth. J Ethnopharmacol. 2003;89:91-5.

16. Asongalem EA, Foyet HS, Ekobo S, Dimo T, Kamtchouing P. Antiinflammatory, lack of central analgesia and antipyretic properties of Acanthus montanus (Ness) T. Anderson. J Ethnopharmacol. 2004;95:63-8.

17. Nonato FR, Barros TA, Lucchese AM, Oliveira CE, Santos RR, Soares MB *et al.* Antiinflammatory and antinociceptive activities of Blechnum occidentale L. extract. J Ethnopharmacol. 2009;125:102-7.

18. Chen YF, Huang Y, Tang WZ, Qin LP, Zheng HC. Antinociceptive activity of Paederosidic Acid Methyl Ester (PAME) from the n-butanol fraction of Paederia scandens in mice. Pharmacol Biochem Behav. 2009;93:97-104.

19. Bernardi MM, Neto JP. Effects of abrupt and gradual withdrawal from long-term haloperidol treatment on open field behavior of rats. Psychopharmacology (Berl). 1979;65:247-50.

20. Tabarelli Z, Rubin MA, Berlese DB *et al*. Antinociceptive effect of novel pyrazolines in mice. Braz J Med Biol Res. 2004;37:1531-40.

21. Patti CL, Frussa-Filho R, Silva RH, Carvalho RC, Kameda SR, Takatsu-Coleman AL *et al.* Behavioral characterization of morphine effects on motor activity in mice. Pharmacol Biochem Behav. 2005;81:923-7.

22. Schmauss C, Yaksh TL. *In vivo* studies on spinal opiate receptor systems mediating antinociception. II. Pharmacological profiles suggesting a differential association of mu, delta and kappa receptors with visceral chemical and cutaneous thermal stimuli in the rat. J Pharmacol Exp Ther. 1984;228:1-12.

23. Labuz D, Mousa SA, Schafer M, Stein C, Machelska H. Relative contribution of peripheral versus central opioid receptors to antinociception. Brain Res. 2007;1160:30-8.

Corresponding author:

Maria Martha Bernardi
Department of Pathology
School of Veterinary Medicine, University of São Paulo
Rua Prof. Orlando Marques de Paiva, 87
São Paulo-SP, CEP 05508-000
Brazil

E-mail: marthabernardi@gmail.com

Received April 4, 2010
Accepted May 28, 2010