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ORIGINAL ARTICLE

Evaluation of chemical composition and antiedematogenic activity of the essential oil of *Hyptis martiusii* Benth

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1,8 cineole

Abstract Evaluations of the therapeutic potential of medicinal plants and their components have been the subject of many studies. Furthermore, the biological activities of various plant species have been reported in various pieces of literature. *Hyptis martiusii* Benth (Lamiaceae), popularly known as "mad balm" is commonly found in the North, Southeast, and Northeast of Brazil. Its leaves are used ethnobiologically as antiulcerogenic, antimicrobial, antitumor and as insecticide. This study aimed to analyze the chemical composition of the essential oil of *H. martiusii* Benth (OEHM) by GC/MS as well as its possible topical activity as an antiedematogenic. This is verified by the models of ear edema induced by single (acute edema) and multiple (chronic edema) applications of croton oil topically, and systemically verified through the model of paw edema induced by carrageenan 1%. Doses of 50, 75 and 100 mg/kg OEHM were used in all tests. Chemical analysis of the oil revealed the 1,8-cineole (34.58%) and δ-carene (21:58%) as major components present in the

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essential oil. On the model of ear edema, acute and chronic OEHM in all the tested doses showed no significant antiedematogenic activity ($p < 0.05$). The systemic model of paw edema induced by carrageenin showed that a dose of 100 mg/kg effectively reduced swelling by 55.37% in the second hour evaluation when compared to the saline group. The anti-inflammatory systemic effect can give greater bioavailability of the components present in the essential oil and your interference in cytokines and leukotriene, thromboxane and prostaglandin biosynthesis. It is therefore concluded that OEHM presents systemic antiedematogenic activity but not topical activity at these doses.

1. Introduction

Medicinal plants represent an important therapeutic option for maintaining the health of people, especially for the low-income population (Lima et al., 2013). There are many materials to be extracted from medicinal plants that can be used in folk medicine and targeted for study, such as, essential oils, extracts, and latex. These materials were analyzed in order to contribute to the research and manufacture of new drugs (Elisabetsky and Wannmacher, 1993).

Essential oils are the volatile elements contained in many plant organs and related to various functions necessary for plant survival playing a key role in defense against microorganism (Siani et al., 2000). Essential oils were derived from the secondary metabolism of plants and have a complex chemical composition, especially the presence of terpenes and phenylpropanoids (Silva et al., 2003).These compounds are responsible for presenting answers to biological activity.

The genus *Hyptis* is considered of great economic importance due to the presence of essential oil (Falcão and Menezes, 2003) used in folk medicine as an alternative therapy, with some pharmacological properties already described as antioxidant (Santos et al., 2010), antiulcerogenic (Caldas et al., 2011), antiseptic (Pereda-Miranda et al., 1993), insecticide (Araújo et al., 2003), antibacterial (Souza et al., 2003), antifungal (Oliveira et al., 2004) and antinociceptive (Menezes et al., 2007).

Hyptis martiusii Benth is a small shrub, commonly found in northern, southeastern and northeastern Brazil (de Almeida and de Albuquerque, 2002) popularly known as "cidreira brava" or "cidreira do mato". This is an important source of essential oil, with the predominance of mono-and sesquiterpenes, presenting its chemical compounds as: 1,8-cineole, δ-3-carene and β-caryophyllene and bicyclogermacrene (Araújo et al., 2003).

Even in studies of Araújo et al. (2003), the insecticidal potential of the *H. martiusii* essential oil and its isolated compound 1,8-cineole was proven. Moreover, Costa et al. (2005) confirmed its insecticidal activity against *Aedes aegypti*. In studies by Coutinho et al. (2008), the ethanol extract from the leaves of *H. martiusii* showed *in vitro* antibacterial activity against *Staphylococcus aureus* and *Escherichia coli*.

Caldas et al. (2011) studied the anti-inflammatory activity of the essential oil of *H. martiusii* in models of gastric mucosal injury in rodents. They were able to demonstrate a significant gastroprotective activity of the oil by increasing factors of gastric mucosa defense, encouraging the development of new research on different models of inflammation and their mechanisms of action.

The main effect of the inflammatory cascade is the reaction of the blood vessels that leads to the accumulation of fluid and leukocytes into extravascular tissues featuring edema, a consequence of the inflammatory process. These inflammatory responses may have beneficial or harmful effects to the body and are closely linked to the repair process (Brito et al., 2006).

Considering the aspects already discussed about the Lamiaceae family, as well as previous literature on anti-inflammatory properties of other species of the genus *Hyptis* and the high prevalence of diseases involving inflammatory reactions, this claim investigates the potential antiedematogenic effects of the species *H. martiusii* Benth essential oil through the classical pharmacologic model of inflammations as topical acute and chronic ear edema, and systemic effects by paw edema.

2. Materials and methods

2.1. Ethical aspects

This research was conducted in strict compliance with the current rules and bioethical guidelines for trials involving living beings as according to the Ethics Committee on Animal Research (CEUA) of the Regional University of Cariri – URCA, for the review of experimental protocols, and approved under section number 18/2012.2.

2.2. Botanical material

The collection of plant material (leaves) of *H. martiusii* Benth, was held in the savannah area of the Chapada of Araripe (Barreiro Grande Farm, Crato-Ce, 7° 21′ 50″ S; 39° 28′ 39″ W; altitude: 930 m) in May of 2012. A voucher specimen of the plant specimen was deposited in the Herbarium Carirense Dárdano de Andrade Lima – HCDAL the Regional University of Cariri – URCA, under registration number 8394.

2.3. Animals

For implementation of *in vivo* tests, male mice (*Mus musculus* specimen) restricted to a body mass between 20 and 30 g were chosen randomly. They were kept in polypropylene cages and kept in a temperature of 22 ± 3 °C, accompanied by light/dark cycles of 12 h with free access to drinking water and specific to rodents (Labina, Purina®).

2.4. Essential oil extraction

The fresh leaves collected (3950 g) were subjected to distillation with water vapor drag in Clevenger type apparatus for

2 h. At the end of that period, essential oil from the leaves of *H. martiusii* Benth (OEHM) collected was treated with anhydrous sodium sulfate to eliminate water residual, deposited in an amber vial, and held in the refrigerator a −20 °C for later analysis.

2.5. Chemical analysis of the essential oil from the leaves of H. martiusii Benth (OEHM)

Oil analysis was performed using the Shimadzu GCMS-QP2010 series (GC/MS system), Rtx-5MS capillary column (30 m × 0.25 mm, 0.25 mm film thickness) with helium as the carrier gas at 1.5 ml/min, injector temperature of 250 °C, detector temperature of 290 °C, column temperature increased from 60 °C to 180 °C at 5 °C/min, then from 180°C to 280 °C at 10 °C/min (10 min).The scanning speed was 0.5 scan/s m/z 40 and 350, compared Split (1:200). The injected volume was 1 ml [25 μL (essential oil)/5 μL $CHCl_3$] (1:200), solvent cut time = 2.5 min. The mass spectrometer was operated at 70 eV of ionization energy and identification of individual components was based on their fragmentation mass spectrum based on the NIST Mass Spectral library 08, retention rates, and compared with Kovax index and other published data.

2.6. Croton oil single application-induced mouse ear edema

In this model, the inflammation was induced using 20 μL of 5% croton oil (v/v) in acetone onto the inner and outer surfaces of the right ears, while the left ear received 20 μL of acetone (to verify that the method used to dilute the irritant agent is not interfering with the results). Fifteen minutes before the application of the irritant agent, the animals were pre-treated with 20 μL of OEHM diluted in acetone, at concentrations 50, 75 and 100 mg/kg (2, 4 and 8 mg/ear). The negative control received 20 μL of acetone (to verify that the method used to dilute OEHM is not interfering the activity of the tested product) while the positive control received dexamethasone 0,4 mg/kg (0.08 mg/ear). After 6 h, the animals were euthanized and followed by the subsequent removal of the ears, which were then cut into disks of 6 mm diameter (with metallic leather punch) and weighed on an analytical balance (Tubaro et al., 1986).

2.7. Croton oil multiple application-induced mouse ear edema

The following model demonstrates a chronic persistent skin inflammation with the bioassay conducted over a period of 9 days. Croton oil 5% (v/v) in acetone (20 μL) was applied on the right ear and acetone on the left ear of Swiss mice (n = 7/group), on alternate days. On the days 4–7, groups of six mice were treated on the inner and outer surfaces of the right ear with 20 μL of OEHM (100 mg/kg equivalent of 8 mg/ear), acetone (negative control) or dexamethasone 0,4 mg/kg (0.08 mg/ear) twice a day. The ear edema evaluation occurred every day, by measurement of the ear thickness using a digital caliper (Jomarca®) and on the 8th day, the mice were sacrificed and 6 mm-diameter ear punch biopsies were collected and recorded for the edema weight evaluation (Stanley et al., 1991).

2.8. Carrageenan-induced paw edema

In this assay, six animals per group were tested. To test inhibitory effects on acute inflammation in a systemic animal model, paw edema was induced by subcutaneous injection of 0.05 mL of carrageenan (1%) into the right hind paw. Right after the carrageenan administration, the animals received saline solution orally, as well as indomethacin (10 mg/kg) and OEHM in doses of 50, 75 and 100 mg/kg. Paw volumes were determined using a water plethysmometer measuring instrument (Insight® Paw plethysmometer) at times 0, 1, 2, 3 and 5 h point after edema induction. The increase in percentage of paw volume was calculated based on the volume difference between the abnormal and normal paws (with or without carrageenan injection respectively).

2.9. Statistical analysis

The results are expressed by mean ± standard error of the mean (SEM). The statistical analysis used was ANOVA one-way with post hoc Newman–Keuls test or ANOVA two-way with post hoc Bonferroni test using Prism for Windows software (GraphPad Software). $p < 0.05$ was considered statistically significant.

3. Results and discussion

3.1. Chemical analysis

The yield of essential oil from leaves of *H. martiusii* was 0.34%. The GC–MS analysis identified 18 constituents, representing 87.63% of the total constituent present in essential oil of OEHM, identifying the existence of mono and sesquiterpenes. The major components of the oil were identified as: 1,8-cineole (34.58%/IK^b1038), δ-carene (21.58%/IK^b1021), camphor (5.17%/IK^b1088), limonene (4.94%/IK^b1033), and germacrene B (3.39%/IK^b1534) as seen in Table 1.

Phytochemical characterization of the essential oil from fresh leaves of *H. martiusii* demonstrated the presence of mono-and sesquiterpenes compounds. Other species of the Lamiaceae family when pressed by the same method of obtaining the oil, present similar chemical composition to those identified in the species components of the study in question, such as species of the genus *Teucrium* (Menichini et al., 2009) and the species *Satureja hortensis* (Hajhashemi et al., 2011).

Rebelo et al. (2009) studied the species *Hyptis crenata* which was identified in the essential oil obtained from the leaves of the species as its major components, α-pinene, 1,8-cineole, β-pinene, camphor, limonene and γ-terpinene. 1,8-cineole was the second most commonly found compound in different species (17.6%).

In previous studies, Caldas et al. (2011) reported the chemical composition of the oil obtained from the leaves of the same species, introduced from the majority of compounds 1,8-cineole and δ-carene. These constituents are also significantly present in the essential oil of the study in question. Juergens et al. (2003) and Nascimento et al. (2009) showed the anti-inflammatory and bronchodilator action induced by

Table 1 Chemical composition of the essential oil of *H. martiusii*.

Components	Tr(min)[a]	IK[b]	(%)
α-pinene	3.58	935	2.98
Camphene	3.79	961	0.44
β-pinene	4.17	982	1.73
β-myrcene	4.24	993	1.33
α-phellandrene	4.54	1007	0.74
δ-carene	4.65	1021	21.58
o-cymene	4.86	1028	2.22
Limonene	4.94	1033	4.94
1,8-cineole	5.05	1038	34.58
γ-terpinene	5.47	1058	0.73
Linalool	6.23	1099	0.57
Camphor	7.40	1088	5.17
1-terpinen-4-ol	8.09	1142	0.63
α-terpineol	8.40	1190	0.36
(E)-caryophyllene	14.16	1422	2.97
Germacrene B	16.08	1534	3.39
δ-cadinene	16.68	1542	0.71
Caryophyllene oxide	18.29	1585	2.56
Total			87.63

[a] TR = Retention time.
[b] IK = Kovat's index.

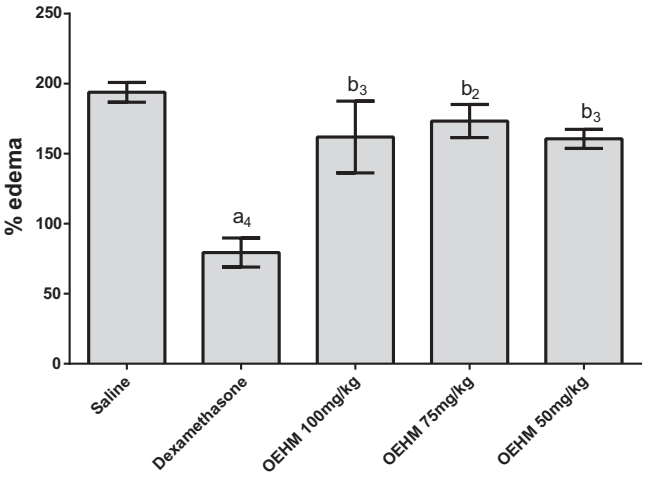

Figure 1 Effect of OEHM at doses of 100, 75 and 50 mg/kg on single application croton oil-induced mouse ear edema. The vertical bars represent the mean and SEM of 6 animals. Statistical analysis: one-way ANOVA followed by Student–Newman–Keuls test. $p < 0.01$ (b2), $p < 0.001$ (b3) compared to dexamethasone and $p < 0.0001$ (a4) compared to the negative control group (saline).

1,8-cineole. These studies corroborated the relationship between these compounds with biological activity and of those present in *H. martiusii* essential oil. These actions may be explained alone or by synergistic effects of these compounds with other constituents present in essential oil.

In the classical model of acute ear edema induction by croton oil, topical application of OEHM in concentrations of 50 mg/kg, 75 mg/kg and 100 mg/kg did not show significant inhibition of ear edema (161.8 ± 25.58, 11.79 ± 173.3, 160.5 ± 6.75, respectively) when compared to the saline group (negative control – 193.8 ± 9.7). Simultaneously, the corticosteroid dexamethasone (positive control) showed a significant reduction of 59.02% of the mass percentage of ear edema relative to saline (Fig. 1).

Topical application of croton oil is a method used to evaluate the activity of steroidal or nonsteroidal anti-inflammatory agents as with topical or systemic activity. The main irritant present in croton oil is 12-O-Tetradecanoylphorbol-13-acetate (TPA) (Stanley et al., 1991), whose action is exerted by cyclooxygenase, histamine, serotonin, and other inflammatory mediators (Saraiva et al., 2011).

The topical application of croton oil induced an acute inflammatory response, mainly characterized by edema, neutrophil infiltration, production of prostaglandins and leukotrienes, as well as increased vascular permeability. The swelling is initially due to histamine and serotonin, followed by the synthesis of prostaglandins and leukotrienes; these are produced by the conversion of arachidonic acid via phospholipase A2, depending on the inflammatory activity of arachidonic acid. Therefore, inhibitors of phospholipase A2, cyclooxygenase and the lipoxygenase pathways, such as dexamethasone, are effective in suppressing ear edema (Zhang et al., 2007). The OEHM showed negligible antiedematogenic activity within the topical concentrations used, indicating that this oil cannot inhibit the activation of phospholipase A2 pathways of COX, LOX or the release of inflammatory mediators.

The application of OEHM 100 mg/kg on croton oil multiple application-induced ear edema on day 5 (in an established inflammatory process) did not cause any significant reduction in ear thickness when compared to the saline-treated group 48 h after the first treatment. However, dexamethasone (positive control) showed a significant reduction in edema in the fifth (34.64%), eighth (27.01%) and ninth days (24.84%) of treatment (Fig. 2A). The results observed on day 9 were confirmed by the evaluation of edema weight. Dexamethasone reduced the edema by 44.16% (Fig. 2B).

In this model, the increased enzymatic activity of phospholipase A2 by persistent activation of protein kinase C (PKC) by TPA leads to increased arachidonic acid metabolites such as leukotrienes and prostaglandins (Garg et al., 2008). The anti-inflammatory and hence antiedematogenic activity of these compounds on the action of TPA occur by their ability to inhibit the arachidonic acid cascade, interfering directly in the activation of PKC, similar to the anti-inflammatory class of corticosteroids; important inhibitors of phospholipase A2, COX and LOX.

Many cases of dermatitis and allergic reactions were caused by treatments that involve the use of concentrated essential oils (Veiga Junior et al., 2005). An example of plant species that fits this context is the essential oil *Tanaecium nocturnum*, which at certain concentrations can cause contact dermatitis (Fazolin et al., 2007). Similarly, the essential oil from *Lippia sidoides* and its isolated constituent, thymol, presents a pro-inflammatory effect in chronic topic models (Veras et al., 2013) and therefore a significant edematogenic activity. In this sense, the absence of anti-inflammatory potential of OEHM when applied topically could be related to this peculiar characteristic of essential oils and the low bioavailability promoted by this administration way.

The essential oil at 100 mg/kg had no significant antiedematogenic effect when compared to the saline group. This fact can be justified by minor bioavailability of the compound

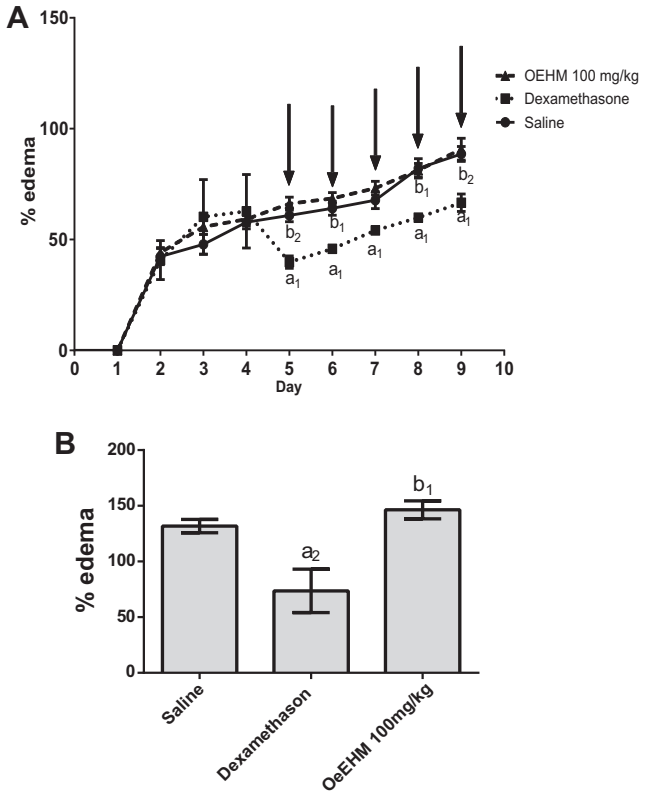

Figure 2 Effect of OEHM on croton oil multiple application-induced ear edema. (A) shows the time–response curve of effect from days 0 to 9. The croton oil in acetone was applied on alternate days. The thickness of the ear was measured daily, using a digital caliper. On days 5–9, the ear of the animals received saline solution, dexamethasone or OEHM 100 mg/kg (arrows indicate the days when the treatment occurred). The effects of the compounds were examined by varying thickness of the ear, calculated as the difference between the initial and final thickness. The points represent the mean of 6 animals and vertical bars ± SEM (two-way ANOVA followed by Bonferroni test) a2 = $p < 0.01$ vs saline; a3 = $p < 0.001$ vs saline; a4 = $p < 0.0001$ vs saline; b4 = $p < 0.0001$ vs Dexamethasone. (B) shows the percentage of edema weight of each group on day 9 (one-way ANOVA followed by Student–Newman–Keuls test). a2 = $p < 0.01$ vs saline; b1 = $p < 0,05$ vs Dexamethasone.

1,8-cineole and other compounds present in essential oil when applied topically. The edematogenic activity of 1,8 cineole was first perceived when applied locally (Santos and Rao, 1997, 1998). This effect can be a result of activation and degranulation of mast cells and thus release of mediators such as histamine and serotonin (Schwartz and Austen, 1984).

The systemic anti-inflammatory activity was demonstrated by paw edema induced by carrageenan. OEHM administered at doses of 50 and 75 mg/kg, showed no significant reduction in the percentage of edema compared to the saline group. In contrast, a concentration of 100 mg/kg was able to reduce edema in the second time (T2) (21.13 ± 2.82), a decrease of 55.37% compared to controls (47.36 ± 10.5). Indomethacin showed significant antiedematogenic effects, at times two (T2) to five (T5), with percentage reduction of edema of 57.10%, 71.58%, 76.45%, 80.66% respectively in relation to the saline group (Fig. 3).

The inflammatory response produced by the carrageenan was characterized by a multiphasic profile, involving the release of various mediators (Fantone and Ward, 1982; Posadas et al., 2004). The first stage was characterized by increased vascular permeability promoted by mediators such as histamine, serotonin and bradykinin (Vinegar et al., 1987). This increase in vascular permeability is mediated at a later time by the actions of kinins and peak edema is characterized by the action of prostaglandins (Di Rosa et al., 1971).

The OEHM at a dose of 100 mg/kg was effective in reducing edema during the peak action of carrageenan. This is a strong indication that the oil may inhibit different aspects that are involved in the inflammatory process, such as the release of mediators as histamine, which will interfere with the production of arachidonic acid metabolites as prostaglandins, responsible for the peak of edema caused by carrageenan.

Studies with the essential oil of *Rosmarinus officinalis* L. species (Lamiaceae), evaluating the anti-inflammatory activity through the paw edema model, found a significant reduction in edema produced by carrageenan. In the same study, the phytochemical analysis of the oil identified among its chemicals 1,8-cineole (Takaki et al., 2008).

Santos and Rao (2000) orally administered the compound 1,8-cineole in isolation order to prove its anti-inflammatory activity while performing the paw edema model, and found a significant reduction of edema caused by carrageenan at all doses tested of 1,8-cineol. This same constituent, found in the form of oil in major studies, may explain in part the potential of oil to reduce the edema induced by carrageenan.

In studies by Juergens et al. (1998a,b) on human blood monocytes, it was found that 1,8-cineol influences the production of and inhibits cytokines, leukotriene B4, thromboxane and prostaglandin. Other components as camphor and limonene can be influence in inflammations process. Silva-Filho et al. (2015) showed that camphor affects inflammatory response, inhibits neutrophil migration *in vitro*, has antiedematogenic activity, and decreases neutrophil infiltration in inflamed tissue and the results yet demonstrated that oral treatment with camphor significantly reduced MPO activity.

Chaudhary et al. (2012) have investigated the effects of D-limonene in (DMBA)-initiated and (TPA)-promoted skin tumor development. The topical treatment of D-limonene inhibits Ras/Raf/ERK1/2 signaling pathway and promoted to pro-apoptotic state attenuating the skin inflammatory responses (edema, hyperplasia and COX-2 expression). D-limonene demonstrates that the potent cellular anti-inflammatory activity of LPS-activated RAW 264.7 cells models indicate that production of NO, PGE2, IL-1β, IL-6, and TNF-α is reduced by actions of D-limonene at the protein level in activated macrophages (Yoon et al., 2010). Another study showed that limonene played a potent anti-inflammatory role in LPS-induced ALI in mice. The results indicated that limonene attenuated LPS-induced inflammatory cell infiltration and reduced yet the activity of MPO. The anti-inflammatory actions can be explained by reduction of inflammatory cytokines (TNF-α, IL-1β, and IL-6) in BALF, and inhibition of ERK, JNK, p38, MAPK, and NF-κB activation (Chi et al., 2013). They soon realized that its systemic action differs mechanistically from its topical action, involved in its biological activity, justifying the results obtained in the present study.

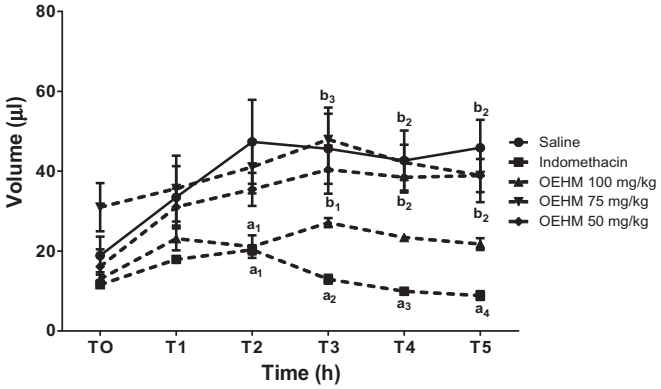

Figure 3 Effect of OEHM at doses of 100, 75 and 50 mg/kg on single application by carrageenan induced paw edema model. The vertical bars represent the mean and SEM. of 6 animals. Statistical analysis: two-way ANOVA followed by Bonferroni test. $p < 0.01$ (b2), $p < 0.001$ (b3) compared to dexamethasone and $p < 0.0001$ (a4) compared to the negative control group (saline).

4. Conclusion

The development of this study allows us to conclude that the OEHM does not have a significant topical antiedematogenic activity; however, the oil at a dose of 100 mg/kg was effective in reducing edema caused by intraplantar injection of carrageenan in the systemic model of paw edema. These results can be related to the presence of the constituents 1,8-cineole, δ-carene, camphor and limonene. The study also indicates the potential application of OEHM as an important herbal medicine to be used against inflammatory diseases.

References

de Almeida, C.F.C.B.R., de Albuquerque, U.P., 2002. Check-list of the family Lamiaceae in Pernambuco, Brazil. Braz. Arch. Biol. Technol. 45, 343–353.

Araújo, E.C.C., Silveira, E.R., Lima, M.A.S., Neto, M.A., Andrade, I. L., Lima, M.A.A., 2003. Insecticidal activity and chemical composition of volatile oils from *Hyptis martiusii* Benth. J. Agric. Food Chem. 51, 3760–3762.

Brito, M.V.H., Figueiredo, R.C., Tavares, M.L.C., Silveira, T.S., Cantanhêde, G., 2006. Efeito dos óleos de andiroba e copaíba na miosite induzida em ratos. Rev. Para. Med. 20, 17–24.

Caldas, G.F.R., Amaral, C.I.M., Silva, J.B.R., Nóbrega, R.F., Rodrigues, F.F.G., Costa, J.G.M., 2011. Antiulcerogenic activity of the essential oil of *Hyptis martiusii* Benth. (Lamiaceae). J. Ethnopharmacol. 137, 886–892.

Chaudhary, S.C., Siddiqui, M.S., Athar, M., Alam, M.S., 2012. D-Limonene modulates inflammation, oxidative stress and Ras-ERK pathway to inhibit murine skin tumorigenesis. Hum. Exp. Toxicol. 31, 798–811.

Chi, G., Wei, M., Xie, X., Soromou, L.W., Liu, F., Zhao, S., 2013. Suppression of MAPK and NF-κB pathways by limonene contributes to attenuation of lipopolysaccharide-induced inflammatory responses in acute lung injury. Inflammation 36, 501–511.

Costa, J.G.M., Rodrigues, F.F.G., Angélico, E.C., Silva, M.R., Mota, M.L., Santos, N.K.A., 2005. Estudo químico-biológico dos óleos essenciais de *Hyptis martiusii*, *Lippia sidoides* e *Syzigium aromaticum* frente às larvas do *Aedes aegypti*. Rev. Bras. Farmacogn. 15, 304–309.

Coutinho, H.D.M., Costa, J.G.M., Siqueira-Júnior, J.P., Lima, E.O., 2008. In vitro anti-staphylococcal activity of *Hyptis martiusii* Benth against methicillin-resistant *Staphylococcus aureus*: MRSA strains. Rev. Bras. Farmacogn. 18, 670–675.

Di Rosa, M., Giroud, J.P., Willoughby, D.A., 1971. Studies of the mediators of the acute inflammatory response induced in rats in different sites by carrageenan and turpentine. J. Pathol. 104, 15–29.

Elisabetsky, E., Wannmacher, L., 1993. The status of ethnopharmacology in Brazil. J. Ethnopharmacol. 38, 129–135.

Falcão, D.Q., Menezes, F.S., 2003. Review ethnopharmacological, pharmacological and chemical of genus *Hyptis*. Braz. J. Pharm. 84, 69–74.

Fantone, J.C., Ward, P.A., 1982. Role of oxygen-derived free radicals and metabolites in leukocyte-dependent inflammatory reactions. Am. J. Pathol. 107, 395.

Fazolin, M., Estrela, J.L.V., Catani, V., Alécio, M.R., Lima, M.S., 2007. Atividade inseticida do óleo essencial de *Tanaecium nocturnum* (Barb. Rodr.) Bur. & K. Shum (Bignoneaceae) sobre *Sitophilus zeamais* Motsch. (Coleoptera: Curculionidae). Acta Amaz. 37, 599–604.

Garg, R., Ramchandani, A.G., Maru, G.B., 2008. Curcumin decreases 12-O-tetradecanoylphorbol-13-acetate-induced protein kinase C translocation to modulate downstream targets in mouse skin. Carcinogenesis 29, 1249–1257.

Hajhashemi, V., Zolfaghari, B., Yousefi, A., 2011. Antinociceptive and anti-inflammatory activities of *Satureja hortensis* seed essential oil, hydroalcoholic and polyphenolic extracts in animal models. Medical principles and practice. Int. J. Kuwait Univ. 21, 178–182.

Juergens, U.R., Stöber, M., Schmidt-Schilling, L., Kleuver, T., Vetter, H., 1998a. Antiinflammatory effects of eucalyptol (1.8-cineole) in bronchial asthma: inhibition of arachidonic acid metabolism in human blood monocytes ex vivo. Eur. J. Med. Res. 3, 407–412.

Juergens, U.R., Stöber, M., Vetter, H., 1998b. Inhibition of cytokine production and arachidonic acid metabolism by eucalyptol (1.8-cineole) in human blood monocytes in vitro. Eur. J. Med .Res. 3, 508–510.

Juergens, U.R., Dethlefsen, U., Steinkamp, G., Gillissen, A., Repges, R., Vetter, H., 2003. Anti-inflammatory activity of 1.8-cineol (eucalyptol) in bronchial asthma: a double-blind placebo-controlled trial. Respir. Med 97, 250–256.

Lima, L.R., Cavalcante, R.R.L., Martins, M.C.C., Parente, D.M., Cavalcante, A., 2013. Assessment of the antiedematogenic, antimicrobial and mutagenic activity of *Amburana cearensis* seeds (AC Smith) (Imburana-de-cheiro). Rev. Bras. Plantas Med. 15, 415–422.

Menezes, I.A.C., Marques, M.S., Santos, T.C., Dias, K.S., Silva, A.B. L., Mello, I.C.M., 2007. Antinociceptive effect and acute toxicity of the essential oil of *Hyptis fruticosa* in mice. Fitoterapia 78, 192–195.

Menichini, F., Conforti, F., Rigano, D., Formisano, C., Piozzi, F., Senatore, F., 2009. Phytochemical composition, anti-inflammatory and antitumour activities of four *Teucrium* essential oils from Greece. Food Chem. 115, 679–686.

Nascimento, N.R.F., Refosco, R.M.D.C., Vasconcelos, E.C.F., Kerntopf, M.R., Santos, C.F., Batista, F.J.A., 2009. 1,8-Cineole induces relaxation in rat and guinea-pig airway smooth muscle. J. Pharm. Pharmacol. 61, 361–366.

Oliveira, C., Silva, M.R.R., Kato, L., Silva, C.C., Ferreira, H.D., Souza, L.K.H., 2004. Chemical composition and antifungal activity of the essential oil of *Hyptis ovalifolia* Benth. (Lamiaceae). J. Braz. Chem. Soc. 15, 756–759.

Pereda-Miranda, R., Hernández, L., Villavicencio, M.J., Novelo, M., Ibarra, P., Chai, H., 1993. Structure and stereochemistry of pectinolides AC, novel antimicrobial and cytotoxic 5, 6-dihydro-α-pyrones from *Hyptis pectinata*. J. Nat. Prod. 56, 583–593.

Posadas, I., Bucci, M., Roviezzo, F., Rossi, A., Parente, L., Sautebin, L., 2004. Carrageenan-induced mouse paw oedema is biphasic, age-weight dependent and displays differential nitric oxide cyclooxygenase-2 expression. Br. J. Pharmacol. 142, 331–338.

Rebelo, M.M., Silva, J.K.R., Andrade, E.H.A., Maia, J.G.S., 2009. Antioxidant capacity and biological activity of essential oil and methanol extract of *Hyptis crenata* Pohl ex Benth. Rev. Bras. Farmacogn. 19 (1B), 230–235.

Santos, F.A., Rao, V.S.N., 1997. Mast cell involvement in the rat paw oedema response to 1,8-cineole, the main constituent of eucalyptus and rosemary oils. Eur. J. Pharmacol. 331, 253–258.

Santos, F.A., Rao, V.S.N., 1998. Inflammatory edema induced by 1,8-cineole in the hindpaw of rats: a model for screening antiallergic and anti-inflammatory compounds. Phytomedicine 5, 115–119.

Santos, F.A., Rao, V.S.N., 2000. Antiinflammatory and antinociceptive effects of 1,8-cineole a terpenoid oxide present in many plant essential oils. Phytother. Res. 14, 240–244.

Santos, A.K., Costa, J.G., Menezes, I.R., Cansanção, I.F., Santos, K. K., Matias, E.F., Coutinho, H.D., 2010. Antioxidant activity of five Brazilian plants used as traditional medicines and food in Brazil. Pharmacogn. Mag. 6, 335–338.

Saraiva, R.A., Araruna, M.K.A., Oliveira, R.C., Menezes, K.D.P., Leite, G.O., Kerntopf, M.R., 2011. Topical anti-inflammatory effect of *Caryocar coriaceum* Wittm. (Caryocaraceae) fruit pulp fixed oil on mice ear edema induced by different irritant agents. J. Ethnopharmacol. 136, 504–510.

Schwartz, L.B.T., Austen, K.F., 1984. Structure and function of the chemical mediators of mast cells (Part 3 of 3). Prog. Allergy 34, 271–321.

Siani, A.C., Sampaio, A.L.F., Souza, M.C., Henriques, M., Ramos, M.F.S., 2000. Óleos essenciais: potencial antiinflamatório. Biotecnolog. Ciênc. Desenvolv. 16, 38–43.

Silva, A.F., Barbosa, L.C.A., Silva, E.A.M., Casali, V.W.D., Nascimento, E.A., 2003. Composição química do óleo essencial de *Hyptis suaveolens* (L.) Poit. (Lamiaceae). Rev. Bras. Plantas Med. 6, 1–7.

Silva-Filho, S.E., de Souza Silva-Comar, F.M., Wiirzler, L.A.M., do Pinho, R.J., Grespan, R., Bersani-Amado, C.A., Cuman, R.K.N., 2015. Effect of camphor on the behavior of leukocytes in vitro and in vivo in acute inflammatory response. Trop. J. Pharm. Res. 13, 2031–2037.

Souza, L.K.H., Oliveira, C., Ferri, P.H., Oliveira Júnior, J.G., Souza Júnior, A.H., Fernandes, O.F.L., 2003. Antimicrobial activity of *Hyptis ovalifolia* towards dermatophytes. Mem. Inst. Oswaldo Cruz 98, 963–965.

Stanley, P.L., Steiner, S., Havens, M., Tramposch, K.M., 1991. Mouse skin inflammation induced by multiple topical applications of 12-O-tetradecanoylphorbol-13-acetate. Skin Pharmacol. Physiol. 4, 262–271.

Takaki, I., Bersani-Amado, L.E., Vendruscolo, A., Sartoretto, S.M., Diniz, S.P., Bersani-Amado, C.A., 2008. Anti-inflammatory and antinociceptive effects of *Rosmarinus officinalis* L. essential oil in experimental animal models. J. Med. Food 11, 741–746.

Tubaro, A., Dri, P., Delbello, G., Zilli, C., Loggia, R.D., 1986. The croton oil ear test revisited. Inflammation Res. 17, 347–349.

Veiga Junior, V.F., Pinto, A.C., Maciel, M.A.M., 2005. Medicinal plants: safe cure? Quím. Nova 28, 519–528.

Veras, H.N.H., Araruna, M.K.A., Costa, J.G.M., Coutinho, H.D.M., Kerntopf, M.R., Botelho, M.A., 2013. Topical antiinflammatory activity of essential oil of *Lippia sidoides* cham: possible mechanism of action. Phytother. Res. 27, 179–185.

Vinegar, R., Truax, J.F., Selph, J.L., Johnston, P.R., Venable, A.L., McKenzie, K.K., 1987. Pathway to carrageenan-induced inflammation in the hind limb of the rat. Fed. Proc. 1987, 118–126.

Yoon, W.J., Lee, N.H., Hyun, C.G., 2010. Limonene suppresses lipopolysaccharide-induced production of nitric oxide, prostaglandin E2, and pro-inflammatory cytokines in RAW 264.7 macrophages. J. Oleo Sci. 59, 415–421.

Zhang, B., Li, J.-B., Zhang, D.-M., Ding, Y., Du, G.-H., 2007. Analgesic and anti-inflammatory activities of a fraction rich in gaultherin isolated from *Gaultheria yunnanensis* (FRANCH.) REHDER. Biol. Pharm. Bull. 30, 465–469.