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Research Article

Anti-inflammatory and antinociceptive effect of *Hyptis martiusii* BENTH leaves essential oil

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ABSTRACT

Hyptis martiusii Benth. also known as "cidreira brava", has some activities verified in the literature, such as antiulcerogenic, antimicrobial and antiedematogenic. This study aimed to verify the anti-inflammatory and antinociceptive effect of the leaves essential oil. For the evaluation of the anti-inflammatory activity of OEHM (100 mg/kg/p.o.), models paw edema induced by dextran and histamine, peritonitis and vascular permeability were used. Regarding the anti-nociceptive activity of the OEHM, abdominal contortion tests by acetic acid, formalin, hot plate (50.75 and 100 mg/kg/p.o.), open field and mechanical plantar hyper-nociception (100 mg/kg/p.o.) were carried out. OEHM (100 mg/kg) showed anti-inflammatory activity, being able to remarkably deducing the paw edema induced by dextran and histamine, the total number of cell leukocytes/neutrophils in peritonitis, and exudate in vascular permeability. In antinociceptive activity, the OEHM did not promote significant effect in central nervous system in the open field assay, remarkably reduced abdominal contortions (50, 75 and 100 mg/kg), the time in the formalin assay and the mechanical hyper-nociception (100 mg/kg); however, only doses between 75 and 100 mg/kg were capable of ameliorating the reponse latency time. Regarding the probable mechanism of action, the antinociceptive activity includes the participation in the activation of opioid, TRPV1, and α2-noradrenergic systems. In short, data obtained here reveal that OEHM has anti-inflammatory and antinociceptive activity, implying that its action may be involved in the mechanism of inhibition or liberation of pro-inflammatory mediators involved in pain and inflammation.

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Abbreviations

HCDAL	Herbarium Carirense Dárdano de Andrade
i.p	intraperitoneal
g	gram
kg	kilo
mg	milligram
MPO	myeloperoxidase
OEHM	essential oil of *Hyptis martiusii* Benth; Lima
p.o	orally
s.c	subcutaneous
URCA	Regional University of Cariri
μmol	micromole

1. Introduction

Essential oils are composed of a mixture of volatile molecules of natural origin with broad applicability in cosmetics, agricultural products, and also in food and pharmaceutical industry [1]. In this context, in the Lamiaceae family, there are a plethora of species with rich content in essential oils, which are used in popular medicine as an alternative treatment for diseases [2]. For example, the genus Hyptis is considered of great economic importance since it is composed of triterpenes [3], diterpenes [4] and sesquiterpenes [5], that are responsible for interesting antimicrobial [6,7], insecticide [8], analgesic [9–11], and hypnotic-sedative and antipsychotic-like activity [12].

The species *Hyptis martiusii* Benth. is popularly known as "cidreira do campo" or "cidreira brava", and is characterized by a shrub species is found in the North, Southeast, and Northeast regions of Brazil [11]. In the literature, their insecticide [8], genotoxic [13], anti-ulcerogenic [14, 15], antiedematogenic [10], antibacterial with anti-staphylococcal [16], antifungal [6] and hypnotic-sedative and antipsychotic-like activity [12] effects have been listed.

In the face of the magnitude of studies performed to assess the anti-inflammatory effects of a multitude of compounds, it is essential to consider that the inflammatory process consists of a body's defense response against an aggressive agent, aiming to promote tissue healing or repair. Thus, it is regarded as a highly beneficial and necessary process responsible for the restoration and repair of the damaged structure [17]. This process causes alterations at biochemical, vascular, and cell levels, also promoting the activation of the immune system cells and other components present in plasma, like the complement system and coagulation factors [18], consequently leading to the emergence of the cardinal signs: heat, redness, edema, loss of function, and pain [19].

Therefore, considering the previously published studies on the antiedematogenic activity and, in addition to the prevalence of inflammatory/painful disorders in society at a worldwide level, it is worth emphasizing the importance of investigating for the validation of the medicinal use against inflammation and pain through using pharmacological models.

2. Materials and methods

The essential oil from *Hyptis martiusii* Benth leaves (OEHM) was obtained by the hydrodistillation process. The specimen voucher is deposited in the Herbarium HCDAL/URCA under register number n° 8394.

2.1. In vivo tests

2.1.1. Animals

For this study, mice *Mus musculus* specimen, male sex, weighting between 20 and 30 g were chosen randomly and maintained in the laboratory in packed in polypropylene cages before 24 h experimentation at a temperature of 22 ± 3 °C, light / dark cycle of 12 h with food (Labina, Purina ®) and water of free access. The experimental protocols are approved by the Animal Research Ethics Committee (CEUA) of the Universidade Regional do Cariri – URCA with registration 18/2012.2.

2.2. Inflammatory activity

2.2.1. Paw edema induced by intraplantar injection of dextran and histamine

The volume of the hind paws of the different groups of animals ($N = 6$) was previously measured using a plethysmometer. Animals were pretreated orally (p.o.) with 0.9% saline vehicle (0.1 mL/10 g) or 6 mg/kg promethazine (positive control) or OEHM (100 mg/kg). After 1 hour of pretreatment, the inflammatory model was induced by the administration of 1% dextran or 1% histamine (20 μl/paw) in the right hind paw and vehicle in the left paw. The inflammatory process was evaluated by measurement of the volume of the paws after 1, 2, 3, and 4 h after dextran injection. The end edema was calculated by the difference between the final and the initial volume of the paw at each time [20].

2.2.2. Peritonitis

The animals (No = 6/group) were pre-treated (p.o.) with 0.9% saline solution (0.1 mL/10 g/negative control) or 5 mg /kg dexamethasone (positive control) or 100 mg/OEHM kg. The inflammatory model was established by an intraperitoneal injection of carrageenan after one hour of treatment. After four hours, the animals were euthanized, and 3 mL of solutions of PBS heparinized was injected into the peritoneal cavity. The washed was aspired, centrifuged, and the leukocyte cells were counted in the Neubauer chamber [21].

2.2.3. Dosage of myeloperoxidase (MPO)

The levels of MPO activity were determined using the technique described by Bradley et al. (1982) [22]. For that, hydrogen peroxide (0.0005%) was used as a substratum for the MPO enzyme. By convention, the one unit of MPO was defined as the capacity of conversion of 1 μmol of hydrogen peroxide by the minute at 22 °C. So, the optical density variation was measured by spectrophotometer at 600 nm using the mixture samples with the o-dianisidine solution. The results were expressed as UMPO/μl of wash [23].

2.2.4. Vascular permeability assay

Six animals per group received pre-treatment (p.o.) with 0.9% saline (0.1 mL /10 g) or OHM 100 mg/kg, whereas native animals only received induction treatment. After one hour of treatment, the inflammatory model was established by carrageenan (solution at 1% by i.p. route), followed by application of 1 mL of solution of Evans Blue by tail vein. After four hours, the animals were euthanized, and 3 mL of solutions of PBS heparinized were injected to wash the peritoneal cavity. The washed, aspired, centrifuged for 2 min at 3500 rpm, and the supernatant was reading at 520 nm by spectroscopy[24].

2.3. Antinociceptive activity

2.3.1. Open field test

Six animals were used per group previously treated with 0.9% saline solution (0.1 mL/10 g) and OEHM 100 mg/kg, both orally, and diazepam 5 mg/ kg (i.p.). The animals were placed 30 min after the treatments in an open field made of acrylic, divided into nine previously demarcated areas, and were observed for 5 min, evaluating the total number of crossings made by the animals in the demarcated fields according to performed by Siegel (1946) methodology and validated by Archer (1973).

2.3.2. Formalin assay

Six animals per group were pre-treated (p.o.) with 0.9% saline (0.1 mL/10 g) or indomethacin 10 mg / kg (positive control) or OEHM 50, 75 and 100 mg/kg. After one hour, the nociceptive stimulus was promoted by injecting 20 μl of formalin (2.5%) into the animal's right paws. Then,

the total time (*sec*) of the animal licked, remained licking, or orbiting the injected paw ("licking-time") was recorded to evaluate the first (0–5 min) and the second (15–30 min) phase [27,28]..

2.3.3. Nociception induced by intraperitoneal injection of acetic acid

Mice were orally pretreated with 0.9% saline (0.1 mL/10 g/), indomethacin (10 mg/kg/p.o.) and OHMS (100 mg/kg/ p.o.), all orally. After one hour of treatment, the pain process was induced by an intraperitoneal injection of acetic acid (0.6%) diluted in sterile water. After pain induction, the number of cumulative abdominal contortions was counted for 30 min [29,30].

2.3.4. Hot plate assay

Mice were placed individually on a hot plate pre-programmed at 55 °C ± 0.5 °C to obtain nociceptive baseline response values. After 24 h, the animals were pre-treated with 0.9% saline solution (0.1 mL/10 g/p.o.), OEHM in the concentration of 100, 75, and 50 mg/kg/p.o. or morphine 6 mg/kg (i.p.). The hot stimulus was applied 30, 60, and 90 min after treatment. The cut-off time of 15 secs, at kept contact with the hot plate, was established to avoid paw injury [31].

2.3.5. Plantar mechanical hypernociception test

The animals ($N = 6$) were treated for 7 days with 0.9% saline (0.1 mL/ 10 g / p.o.), OEHM (100 mg / kg / p.o.), and indomethacin (10 mg/ kg / p.o.). On the 7th day of treatment, all groups were treated 45 min before the intraplantar administration of 2% (w/v) carrageenan. The intensity of hyper-nociception was evaluated by the animal's reaction, as flexion of the paw, by a mechanical stimulus produced by the gradual pressure applied by a rigid filament coupled with an Electronic Von-Frey Anesthesiometer [32]. The plantar mechanical hypernociception (degree of sensitivity to the mechanical pressure (g) stimulus) was evaluated before (zero time) and every hour until the 5th h of carrageenan injection.

2.3.6. Mustard oil-induced visceral nociception

The animals were divided into groups ($n = 6$) and treated with: 0.9% saline solution (0.1 mL/10 g/ p.o.), and OEHM 100 mg/kg (p.o.) 1.5 hr before being administered with mustard oil (0.75% in 0.9% saline; 50 µl/animal) intercolonially. For this procedure, 4 cm of cannula length was introduced through the intracolonic route for injection of mustard oil, using solid petrolatum in the perianal region to avoid local stimulation by the administration. The total number of behavior related to pain perception (licking the abdomen, crawling against the ground, contortion, and abdominal retractions) was recorded for 20 min immediately after the mustard oil injection [33].

2.3.7. The pathways involved in antinociceptive activity by OEHM

For the determination of the pain signaling pathways involved in the anti-nociceptive response of the OEHM, mice ($n = 6$) were treated with: 0.9% saline solution (0.1 mL / 10 g / p.o.) or OEHM (100 mg / kg p.o.) or morphine (5 mg / kg, sc) for the opioid-like mechanism, or clonidine (0.1 mg / kg) kg, ip) for adrenergic system, or capsazepine (3 mg / kg, sc) for capsaicin receptor involvement (TRPV1), all administered 1.5 h (p.o.) or 1 h (s.c/i.p) before receiving mustard oil (0.75% in 0.9% saline; 50 µl/animal) by intracolonic administration using the cannula with a rounded tip of 1 mm outside diameter [33–35]. The total number of behaviors related to pain (licking the abdomen, crawling against the ground, contortion and abdominal retractions) was counted for 20 min immediately after the administration of mustard oil.

The opioid system was evaluated by the administration of naloxone (2 mg/kg, i.p) 30 min before morphine (5 mg/kg, s.c), and the adrenergic system was administered yohimbine (2 mg/kg, i.p) 30 min before clonidine (0.1 mg/kg, s.c), or OEHM (100 mg / kg / p.o.). After 30 min of the administration of morphine/clonidine and 1.5 h after the administration of the OEHM, the animals were administered mustard oil (0.75% in 0.9% saline; 50 µl /animal) by intracolonic administration route. To evaluation of the involvement of the TRPV1 receptor, capsazepine (3 mg/ kg, s.c.) or OEHM (100 mg/kg/ p.o) was administered. After 1.5 h of the administration of the OEHM, the animals received mustard oil (0.75% in 0.9% saline; 50 µl / animal).

2.4. Statistical analysis

The results were expressed as mean ± standard error of the mean (S.E.M) of six observations. The area under the curve graphic (AUC) was utilized for the evaluation of the total effect. The mean difference was analyzed by ANOVA assay using the GraphPad Prism statistical software. The ANOVA one-way was followed by a post hoc test of Newman-Keuls and the ANOVA two-way by post hoc Bonferroni test. The significance was considered for $p < 0.05$.

3. Results and discussion

3.1. Inflammatory activity

3.1.1. Paw edema induced by intraplantar injection of dextran and histamine

In the model of paw edema by dextran (Fig. 1), the administration of OEHM 100 mg/kg led to a remarkable reduction (25.84 ± 3.08 µl) in the 2nd and 3rd hour of evaluation when compared to the saline group (45.98 ± 7.48 µl). Promethazine, an H1 antihistamine in the phenothiazine group, was able to inhibit edema (35.34 ± 1.93 µl) at times T1, T2, and T3.

The paw edema model by histamine demonstrated that the OEHM (100 mg/kg) markedly decreased edema (30.26 ± 3.65 µl), showing significance in the 2nd and 3rd hours of evaluation (Fig. 2) when compared to the saline group (48.10 ± 8.56 µl). Promethazine significantly inhibited edema (33.81 ± 1.92 µl) at all times, but the association between promethazine and OMEH 100 mg/kg also showed a decrease of 35.16 ± 3.18 µl at the all times.

Our previous studies showed that this essential oil reduced carrageenan-induced paw edema. This effect can be justified by the presence of essential oil constituents, such as 1,8-cineole (34.58%) as the majority compound, followed by δ-carene (21.58%). These compounds are reported in the literature data with antiedematogenic actions. [36]. Corroborating with this, the literature also shows that 1, 8-cineole inhibits edema induced by different compounds, such as carrageenan, dextran, and histamine [37].

Dextran-induced edema causes the release of histamine and serotonin by mast cells [38] which, interacting with their respective receptors on the vessel endothelium, are responsible for the vasodilation process [39]. OEHM significantly reduced the edema in both dextran and histamine, possibly suggesting interference in pathway histamine receptors. Coelho-de-Souza et al. (2021) also demonstrated that the essential oil of *H. crenata* has an anti-edematogenic effect in different models of paw edema, attributing these actions to the presence of 1, 8-cineole [40].

3.1.2. Peritonitis

OEHM (100 mg/kg) and dexamethasone (5 mg/kg) reduced the total number of cell leukocytes (A) (2025 ± 69.5 and 1700 ± 87.6, respectively) and neutrophils (B) (1086 ± 27.7 and 904 ± 22.8, respectively) in comparison to the saline group (Fig. 3).

In the peritonitis assay, the inflammatory process is caused by the intraperitoneal administration of carrageenan, which promotes the release of cytokines and the migration of cells such as neutrophils [41]. The cytokines increase the expression of the enzyme as nitric oxide synthase and inducible COX-2 enzymes, increasing the effect of nitric oxide and eicosanoid pathway [42]. In view of the results, it is suggested that the OEHM inhibits the migration of granulocyte cells such as leukocytes and neutrophils to the peritoneal cavity, corroborating the study by Paulino and collaborators (2008) . This effect possibly occurs by the

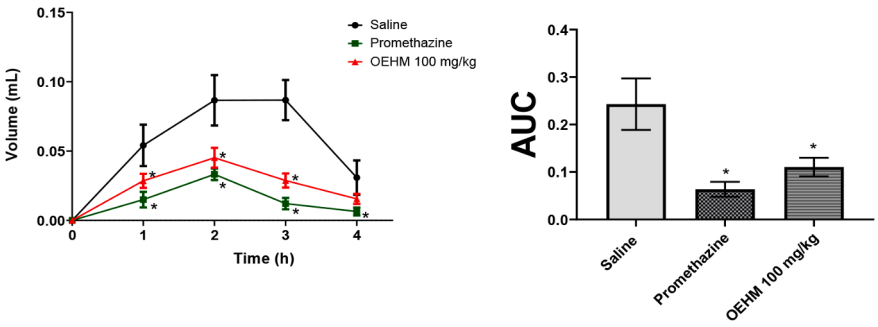

Fig. 1. Antiedematogenic effect of OEHM 100 mg/kg on paw edema induced by 1% dextran. Values expressed as mean ± S.E.M. of 6 animals. * = $p < 0.05$ vs saline by ANOVA following Bonferroni test.

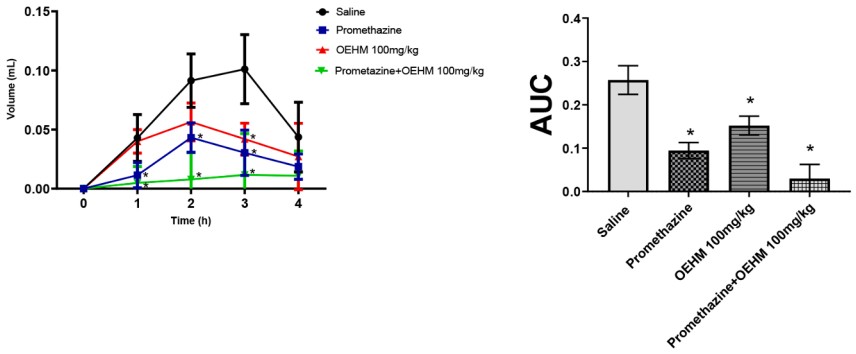

Fig. 2. Effect of OEHM 100 mg/kg on edema induced by histamine in mice, Values expressed as mean ± S.E.M. of 6 animals. * = $p < 0.05$ vs saline by ANOVA following Bonferroni test.

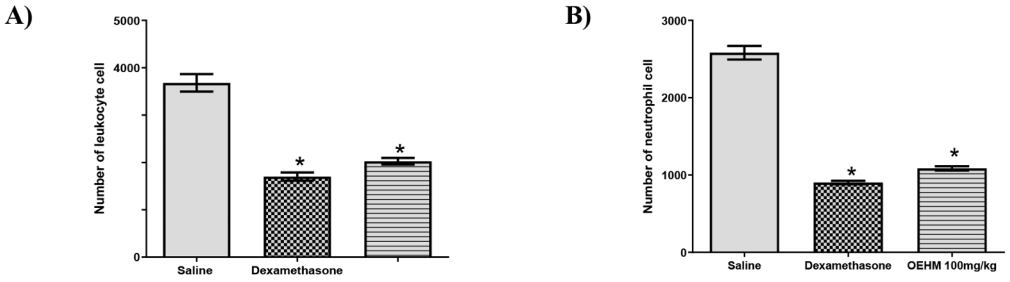

Fig. 3. Effect of the administration of OEHM 100 mg / kg on the migration of leukocytes (A) and neutrophils (B) in carrageenan-induced peritonitis, Values expressed as mean ± S.E.M. of 6 animals. * = $p < 0.05$ vs. saline; by ANOVA following Student Newman Keuls Test.

reduction of vasodilation of the capillaries of the peritoneal membrane, which can be caused by mediators, such as prostaglandins E2 [43]. Thus, confirming our study, the other species of the genus, such as *Hyptis umbrosa*, inhibited the leukocyte migration in the peritoneal cavity induced by carrageenan [44].

Other species of this genus, namely the essential oil of *Hyptis crenata* presented similar results in the dextran-induced edema assay by reducing edema at all times of evaluation, promoting the reduction of the migration of inflammatory cells (leukocytes and neutrophils) into the peritoneal cavity caused by peritonitis [45], these species having the major component as 1,8-cineole (34%), a percentage similar used in our study. Then, considering all results obtained, it is suggested that 1, 8-cineole can be possibly the compound responsible for the anti-edematogenic and anti-inflammatory activity [37].

3.1.3. Measurement of myeloperoxidase (MPO)

The OEHM (100 mg/kg) did not show a significant capacity to inhibit the activity of the myeloperoxidase enzyme (MPO) after the 4th hour of inflammation induced by carrageenan (Fig. 4). However, this action

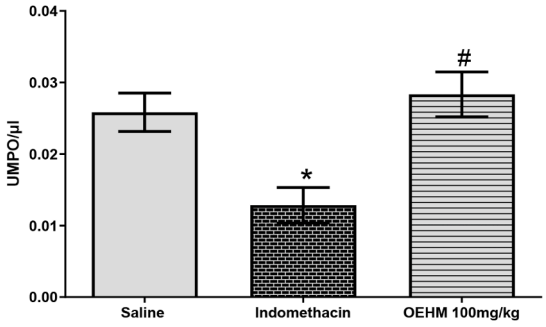

Fig. 4. Effect of OEHM 100 mg/kg on myeloperoxidase activity in carrageenan-induced inflammation, Values expressed as mean ± S.E.M. of 6 animals. * = $p < 0.05$ vs. saline; # = $p < 0.05$ vs. indomethacin by ANOVA following Student Newman Keuls Test.

differs in that it reduces leukocyte migration. Such an event could be justified by the presence of other granulocyte cells that are also involved in the inflammatory process.

In the pathophysiology of the inflammatory process, lymphocytes, macrophages, and monocytes are important contributors to the generation of pro-inflammatory cytokines that influence the edema and infiltration of neutrophils into tissue. Thus, macrophages and neutrophils play a complex role at sites of tissue injuries, with emphasis on the action of phagocyte oxidase and myeloperoxidase (MPO), which is found predominantly in neutrophils, monocytes, and tissue macrophages, being promptly released as a contribution to the defense of the innate immune system [22].

3.1.4. Vascular permeability

When injected intravenously, the Evans blue presents a strong affinity for albumin [24] as a possibility of a promissory method for evaluating vascular permeability [46]. The treatment with OEHM at a dose of 100 mg/kg (0.26 ± 0.01 UA) significantly reduced the extravasation of exudate decurrent of increased vascular permeability compared with saline (0.34 ± 0.009 UA). The naive group received no treatment or induction of the inflammatory process (0.10 ± 0.01UA) (Fig. 5).

The results obtained suggest that OEHM has an anti-inflammatory potential with a reduction of vascular permeability, probably causing interference in the vasodilation of capillaries, corroborating with other the data already presented that show the decrease of the cell migration into the peritoneal cavity as well as inhibition of the formation of paw edema by dextran and histamine.

3.2. Antinociceptive activity

3.2.1. Open field test

The OEHM 100 mg/kg did not significantly change the exploratory activity in the number of crossings (55.25±1.35), whereas, diazepam (5 mg/kg) showed a significant reduction ($p < 0.001$) of exploratory action (35.63±0.99) in comparison with saline group (58.63±1.88) (Fig. 6).

Alterations in the exploratory activity of the animal can be the consequence of drugs that promote the interference in the action of the central nervous system (CNS), as depressure drugs that cause muscle relaxants effect and promote reductions of the response to the pain reactions. However, when evaluating nociceptive parameters, it is necessary to draw attention to drugs that can reduce CNS activity which can cause a false positive result in nociceptive effect measurements, such as licking and paw removal locomotion, the exploratory ability of the animal, among others [26]. Given the demonstrated results, no observed reduction of the animals' exploratory activity in the open field assay. So, this absence of central activity of OEHM is an important indication that it does not cause a depressant effect on the CNS, suggesting the hypothesis of the independence of the antinociceptive activity concerning the central effect.

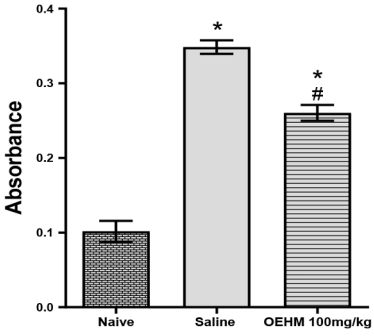

Fig. 5. Effect of OEHM 100 mg/kg in vascular permeability evaluated by Evans blue in peritonitis model. Values expressed as mean ± S.E.M. of meansure of absorbance per animals ($n = 6$). * = $p < 0.05$ vs naive, # = $p < 0.05$ vs saline.; by ANOVA following Student Newman Keuls Test.

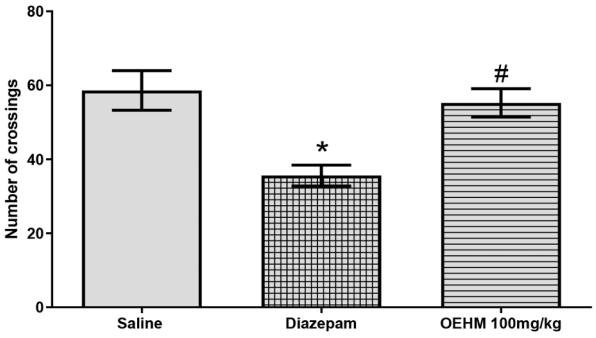

Fig. 6. Effect of OEHM on the exploratory activity of mice in the open field. Values expressed as mean ± S.E.M. of 6 animals. * = $p < 0.05$ vs. saline; # = $p < 0.05$ vs. diazepam by ANOVA following Student Newman Keuls Test.

3.2.2. Formalin test

The administration of OEHM (100 mg/kg) significantly reduced (1st phase: 37.83 ± 1.72 s; 2nd phase:18.83 ± 6.52 s) licking time of the paw injected with formalin in both phases compared to the saline group (first phase: 74.17 ± 3.18 s; second phase: 86.83 ± 8.57 s). Morphine, a significant opioid analgesic, was able to decrease paw licking time in both phases significantly (1st phase: 23.33 ± 0.42 s; 2nd phase: 28.5 ± 3.89 s) (Fig. 7).

This test is a classic experimental model for evaluating the antinociceptive effects of substances, consisting of two phases involving different mechanisms [27,28,31]. The first phase (0–5 min) is characterized by a response of the neurogenic pain resulting from stimulation of nociceptors of sensory fibers, and the second phase (15–30 min) is described by a pain of inflammatory origin associated with inflammatory mediators, like histamine, prostaglandins, serotonin and bradykinin [47], or involving of response by a central nervous process [48,49].

Some studies corroborate our results that also show the anti-inflammatory and antinociceptive effects and have reported the chemical composition with the presence of a major constituent, 1,8-cineole, demonstrating a significant antinociceptive response in both phases in the formalin test [50]. Other research by Franco et al. (2011) describe that the essential oil from *H. fruticosa*, which presents a similar chemical composition by the significant presence of 1,8-cineole and α-pinene, also shows antinociceptive potential through the formalin assay [51]. Raymundo et al. (2011) corroborate by stating that *Hyptis pectinata* essential oil inhibits formalin-induced nociceptive response in both phases [52].

3.2.3. Viceral pain induced by acetic acid

In Fig. 8, indomethacin (10.57 ± 1.25) and OEHM at doses of 50 (23.5 ± 1.19), 75 (23.43 ± 3.03), 100 mg/kg (15.14 ± 0.79) led to a significant reduction in writhing number in comparison with the saline (33.29 ± 1.12).

The acetic acid-induced writhing model is a pre-clinical trial used to evaluate the drug's effect on central or peripheral actions since the acetic acid causes the increase of sensitization of peripheral afferent sensory endings as the response to pro-inflammatory product release from mast cells and macrophages [53]. This model induces the release of mediators in the inflammatory process, such as some cytokines, prostaglandins, histamine, and serotonin [54]. Some studies present in the literature data corroborate our results and, Menezes et al. (2007) describe that the essential oil of *H. fruticosa* [55], of which the predominant compound is 1,8-cineole, as well as, *H. pectinata* [52] showed a decrease in the number of abdominal contortions, suggesting that the oil may have a peripheral analgesic action.

In another study, it is noteworthy that the major constituent of the OEHM (1,8-cineole), a significant reduction in the number of abdominal

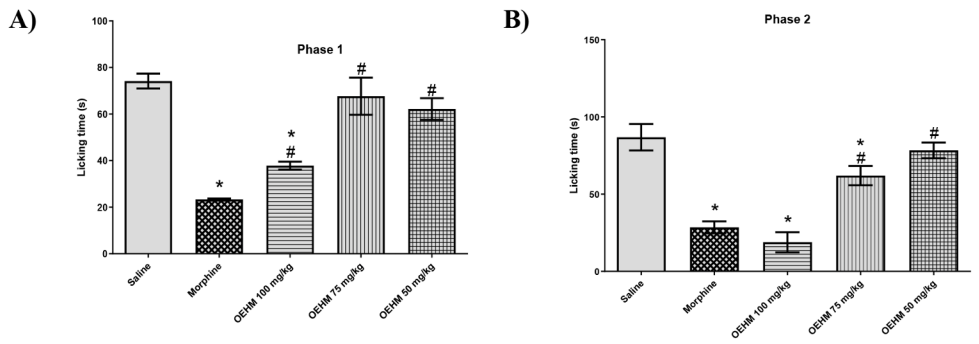

Fig. 7. Antinociceptive action of OEHM (50, 75 and 100 mg/kg) on right hind paw licking time by formalin assay: A) Neurogenic phase (phase 1) and B) Inflammatory phase (second phase). Values expressed as mean ± S.E.M. for right hind paw licking time at 6 animals. * = $p < 0.05$ vs. saline; # = $p < 0.05$ vs. morphine ANOVA following Bonferroni test.

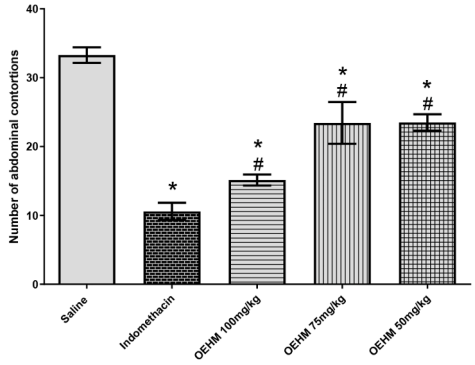

Fig. 8. Effect of OEHM 50, 75, and 100 mg/kg in the number of writhing in visceral pain caused by acetic acid. Values expressed as mean ± S.E.M. of the abdominal writhing number. * = $p < 0.05$ vs. Saline and # = $p < 0.05$ vs. indomethacin. ANOVA following Student Newman Keuls test.

contortions induced by acetic acid [50] and this action is attributed to the inhibition of mediators such as prostaglandins, cytokines, and GABAergic mediators [56]. Therefore, in view of the results described, it is suggested that the OEHM has a peripheral antinociceptive effect, possibly associated with the 1,8-cineole antinociceptive activity.

3.2.4. *Hot plate test*

Both Morphine and OEHM (100 mg/kg) increased the animal latency time on the hot plate at all observation intervals, but OEHM significantly at 30 and 60 min (5.75 ± 1.25 s and 6.08 ± 1.71 s, respectively), and the morphine group at 60 min (8.83 ± 1.71 s), all compared to the saline group (Fig. 9).

The thermal stimulus of the hot plate induces a response in the animal, characterized by jumping or licking the paw, which is associated with central neurotransmission mediated by the activation of nociceptors, such as fibers C and Aδ [31,57]. Corroborating our study, the essential oil of *H. pectinata* showed antinociceptive potential, demonstrating a possible analgesic activity of central origin by increasing latency time in the hot plate assay [52,58]. Similar to our study, Santos et al.(2007), the aqueous extract of *H. suaveolens* increased the time on the hot plate and reduced the contortions by acetic acid and the licking time in formalin [59]. Because of this, we can suggest that the OEHM demonstrates a possible antinociceptive effect with central involvement by presenting results similar to morphine and activity of peripheral origin by interfering in the inflammatory process, as demonstrated in the formalin and abdominal contortion tests.

3.2.5. *Plantar mechanical hyper-nociception*

In Fig. 10, consecutive treatment for seven days with OEHM (100 mg/kg) or indomethacin resulted in a significant reduction of the pain threshold compared with the saline group [25].

The intraplantar administration of carrageenan promotes release of cytokines and sympathomimetic amines and, formation of eicosanoid derivatives [60] that are responsible for an increase in sensitization of nociceptors [61]. This process can be evaluted by Von Frey method by measuring hypernociception phenomena [62,63]. The effect of OEHM (100 mg/kg) on plantar mechanical hyper-nociception test corroborates with other results evaluated systemic inflammation models, such as paw edema and peritonitis induced by carrageenan, which in both tests, were promoted the reduction of the inflammatory process reducing edema and recruiting of inflammatory cells such as leukocytes. Therefore, it is important to point out that the possible antinociceptive activity of OEHM may be closely associated or not with the production of mediators since, in these models, several mediators are released that promote inflammation and, consequently, pain.

3.2.6. *Mustard oil-induced visceral nociception*

In Fig. 11, OEHM (100 mg/kg) significantly reduced (9.5 ± 0.67) the total number of visceral nociception behavior (abdomen licking, dragging against the ground, abdominal contortions, and retractions) in comparison with the saline group (25.83±0.94).

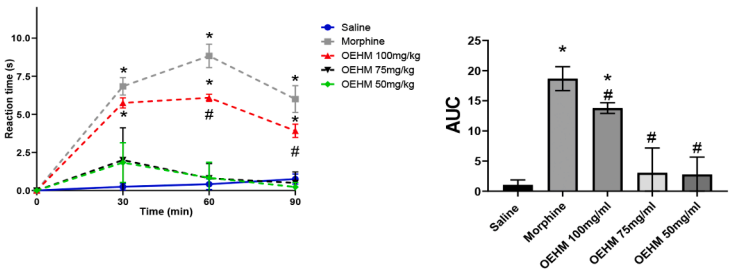

Fig. 9. Effect of OEHM 50, 75 and 100 mg/kg on thermal nociceptive stimulus (hot plate) induced in mice, Values represent the mean ± S.E.M. (standard error of the mean) of reaction time to thermal stimulus. * = $p < 0.05$ vs. Saline and # = $p < 0.05$ vs morphine. ANOVA following Bonferroni test.

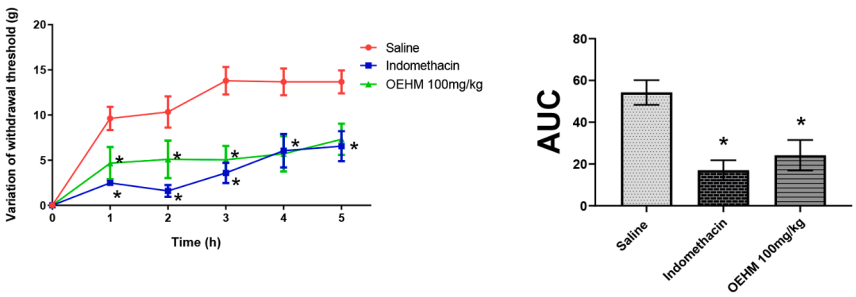

Fig. 10. Effect of OEHM 100 mg/kg on carrageenan-induced plantar mechanical hyper-nociception. Values represent the mean ± S.E.M. (standard error of the mean) for threshold of paw withdrawal in grams (intensity of hypernociception) for groups of 6 animals. * = $p < 0.05$ vs. saline by ANOVA following Bonferroni test.

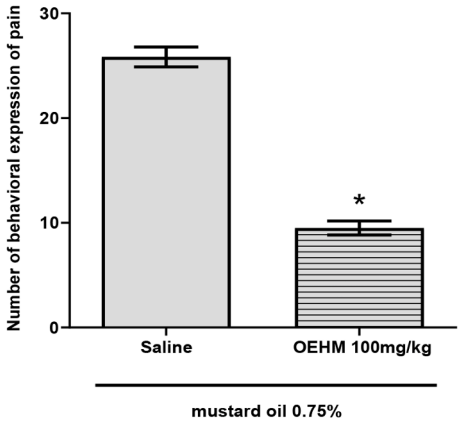

Fig. 11. Antinociceptive effect of OEHM 100 mg/kg in the mustard oil-induced visceral nociception model, Values represent the mean ± S.E.M. (standard error of the mean) for the total number behaviors expression of visceral pain exhibited by animals. * = $p < 0.05$ vs. saline. ANOVA, following Student Newman Keuls test.

Mustard oil is a potent neuronal activator that promotes increased sensitivity to noxious stimuli within minutes after its application, causing sensations of allodynia and hyperalgesia. The applications of this substance in the skin promote activation of nerve endings and hypersensitivity to thermal and mechanical stimuli [64]. The Allyl-isothiocyanate, present in this oil, causes pungency, and the lachrymatory effect of AITC mediated through the TRPA1 and TRPV1 ion channels act, as well as stimulates the production of inflammatory mediators such as prostaglandins and bradykinin [65]. The behavior of abdominal contortion can be explained due to the wide variety of membrane receptors, such as vanilloid (TRPV1), which are present in the viscera and are sensitive to chemical stimuli that involve sensory signaling from the central to the peripheral nervous system [66]. However, even in the face of the described results corroborating each other in the possible antinociceptive activity of OEHM, the importance of investigating the possible pathways involved in the response is suggested.

3.2.7. Pathways involved in the antinociceptive response

Regarding opioid receptor involvement, both treatment OEHM 100 mg/kg and morphine were promoted a significant inhibition (9.67±1.05 and 4.17±0.31, respectively) compared to the saline group (26.8 ± 2.36). The use of pre-treatment with naloxone associated with both promoted a reversion of the antinociceptive effect (Fig. 12A) significantly.

In Fig. 12B, it's observed that capsaicin and OEHM promoted significant inhibition (7 ± 0.77 and 8.28±1.01 respectively) compared with the saline group. However, the use of Capsazepine, a selective antagonist for TRPV1 receptors, enables a reversion of this effect then; showing evidence that the antinociceptive effect of OEHM can be associated with the involvement of TRPV1 receptors.

Fig. 12C evaluates the influence of α2 noradrenergic receptor participation; both clonidine and OEHM demonstrated a significant reduction of antinociceptive response (8.16±0.60 and 9.50±0.67, respectively) compared with the saline group (25.83±0.94). However, pre-treatment with yohimbine reversed these actions for OEHM and clonidine (21.67±2.76 and 19.83±2.56, respectively), indicating a significant involvement of α2 noradrenergic receptor in the antinociception.

Capsaicin is an agonist of the TRPV1 vanilloid receptor [67]. This receptor is an important component in the functional regulation of sensory nerves [68] and inhibition of this target produces antihyperalgesia effects in neuropathic and inflammatory pain models [69]. The action of capsaicin (trans-8-methyl-N-vanylyl-6-nonenamide) on TPRV receptors can depolarize C or A-δ fibers by opening ion channels generating an influx of calcium in the nerve fiber [70]. Capsazepine (exogenous substance) inhibits pain induced in inflammatory processes related to the TRPV1 receptor [71]. Thus, the presented results suggest that OEHM promotes interference in the TRPV1 receptor since capsazepine reverses the antihyperalgesic effect.

The literature shows that morphine strongly affects the opioid receptor; however, the subtype κ-opioid receptor presents an expressive role in the modulation of visceral nociception. Other studies claim that endogenous opioids can suppress the inflammatory process, aiding in the reduction of hyperalgesia [72]. Peripherally, it has been associated with the capacity of μ-opioid receptor agonists to cause the inhibition of adenylate cyclase enzyme in primary afferent neurons by inflammatory mediators such as serotonin and PGE2. That δ- and κ-opioid receptor agonists act by inhibiting the secretion of pro-inflammatory substances by sympathetic neurons [73]. So, the present study results suggest the possible interference in the opioid system by the action of OEHM since naloxone, a non-selective antagonist for opioid receptors, significantly reversed the effect of morphine and OEHM.

Thus, the antinociceptive effect of OEHM presents similarly to that observed in the morphine group; this can be justified through the release of endogenous opioids and the interaction between specific receptors or directly with the opioids. Furthermore, it is worth noting that this action may also be associated with the anti-inflammatory effect described here through the significant interference on dextran/histamine-induced paw edema, peritonitis, myeloperoxidase dosage, and vascular permeability assays.

Clonidine is an α2 adrenergic receptor agonist [74] and exhibits important antinociceptive properties to various types of noxious stimuli (pressure, temperature, and chemical agents) [75]. Activation of α2 receptors by descending noradrenergic pathways exerts an important inhibitory regulatory effect in modulating acute pain of somatic or visceral origin [76]. In modulating pain of visceral origin, activation of α2 receptors acts via the G protein pathway, inhibiting adenylate cyclase, promoting $K+$ efflux, and suppressing Ca+2 currents, preventing the continued release of substance P and glutamate by nerve

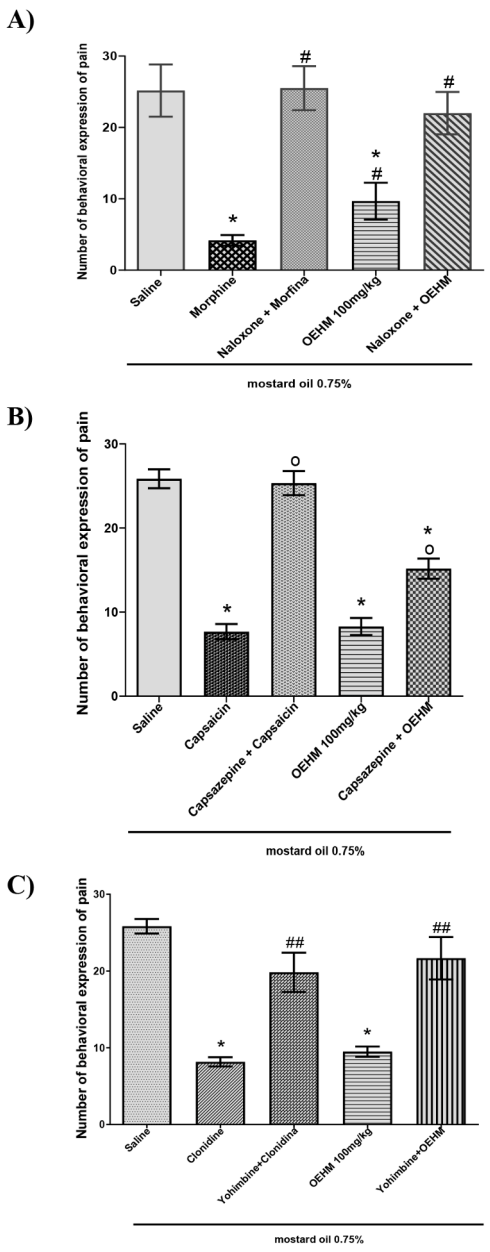

Fig. 12. Evaluation of involvement of different systems in the antinociceptive effect of OEHM. A) involvement of opioid receptor B) involvement of TPRV receptors and C) involvement α2 adrenergic receptor. Values represent the mean ± S.E.M. for the total number behaviors expression of visceral pain exhibited by animals. * = $p < 0.05$ vs saline; # = $p < 0.05$ vs morphine; *o* = $p < 0.05$ vs capsaicin; ## = $p < 0.05$ vs clonidine. ANOVA, Student Newman Keuls test.

terminals [77]. Therefore, it suggests that the antinociceptive effect of OEHM has participated in the adrenergic pathway since yohimbine (an α2 receptor antagonist) promotes the reversion of its action.

4. Conclusion

Hyptis martiusii leaves-derived essential oil exerted a marked anti-inflammatory and antinociceptive activity. Its action may be involved in the mechanism of inhibition or release of pro-inflammatory mediators involved in pain and inflammation. Based on the antinociceptive activity, it is possible to conclude that there is a potential interference of chemical compounds of this essential oil in the transient, opioid, and adrenergic pathways. Therefore, the results presented here provide new knowledge regarding the pharmacological potential of this specie, with essential contributing information that can be used in the scientific validation of the effective and safe use of "cidreira brava" in popular herbal medicine, highlighting the importance of its conservation.

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Ethical approval

All experiment were performed following the current rules and bioethical guidelines. The all experimental protocols were approved under number 18/2012.2 by Commission for Experimentation and Use of Animals (CEUA) of the Regional University of Cariri URCA.

Consent for publication

Not applicable.

CRediT authorship contribution statement

Andreza G.R. Barbosa: Formal analysis, Investigation. **Cícera D.M. O. Tintino:** Formal analysis, Investigation. **Renata T. Pessoa:** Formal analysis, Investigation. **Luiz J. de Lacerda Neto:** Methodology. **Anita O.B.P.B. Martins:** Methodology. **Maria R.C. de Oliveira:** Methodology. **Henrique D.M. Coutinho:** Conceptualization, Writing – original draft, Funding acquisition. **Natália Cruz-Martins:** Writing – original draft. **Lucindo J. Quintans Junior:** Writing – review & editing, Funding acquisition. **Polrat Wilairatana:** Funding acquisition, Writing – original draft. **Irwin R.A. de Menezes:** Conceptualization, Writing – original draft.

Declaration of Competing Interest

The authors declare that they have no known competing financialinterestsor personal relationships that could have appeared to influence the work reported in this paper.

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