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Effects of the *Hyptis martiusii* Benth. leaf essential oil and 1,8-cineole (eucalyptol) on the central nervous system of mice

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ABSTRACT

The aim of this study was to characterize the central effects of the *Hyptis martiusii* leaf essential oil (OEHM) and 1,8-cineole (eucalyptol) using behavioral animal models. Gas chromatography coupled to mass spectrometry (GC/MS) was used to characterize the chemical compounds present in the OEHM. For the behavioral tests, female Swiss mice treated with the OEHM (25, 50, 100 and 200 mg/kg, i.p.) and 1,8-cineole (50 mg/kg, i.p.) were used and subjected to the following tests: open field, elevated cross maze, rotarod, sodium pentobarbital- or ethyl ether-induced sleep time, pentylenetetrazol-induced convulsions, haloperidol-induced catalepsy, and ketamine-induced hyperkinesia. GC/MS analysis identified 20 constituents with the majority of them being monoterpenes and sesquiterpenes, with eucalyptol (1,8-cineol), the major sample compound (25.93%), standing out. The results showed the OEHM (25, 50 100 and 200 mg/kg, i.p.) and its major compound (50 mg/kg, i.p.) reduced animal motility in the open field test, increased pentobarbital- and ethyl ether-induced sleep time, as well as death latency in the pentylenetetrazole-induced convulsion model. However, the tested compounds were devoid of anxiolytic-like and myorelaxant activity. In addition, the OEHM (100 and 200 mg/kg, i.p.) and 1,8-cineole (50 mg/kg, i.p.) potentiated haloperidol-induced catalepsy and reduced ketamine-induced hyperkinesia. Taken together, the results suggest the OEHM has important hypnotic-sedative and antipsychotic-like effects, which appear to be due to the monoterpene 1,8-cineole, the major compound identified in the essential oil.

1. Introduction

The *Hyptis* genus is known worldwide for being rich in species with great economic and ethnopharmacological importance, where the population uses species from this genus for medicinal purposes to treat colds, flus, fevers, asthma and behavioral disorders such as anxiety and depression due to their antiseptic, anti-infectious, anti-fungal, anti-bacterial and anti-inflammatory properties. Pharmacological investigations report the biological potential of this genus, especially as antibacterial, antifungal, antiviral and antiulcerogenic agents (Falcão and Menezes, 2003; Diniz et al., 2013).

Chemical analyzes of the *Hyptis* genus indicate the presence of important bioactive compounds, where terpenes (monoterpenes, diterpenes and triterpenes) are the main class, followed by flavonoids, lactones, lignans and fatty acids (Falcão and Menezes, 2003; Falcão et al., 2003; Misra et al., 1981, 1983; Din et al., 1988; Mukherjee et al.,

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1984; Botrel et al., 2009).

Some *Hyptis* species are reported with pharmacological activity at the level of the central nervous system (CNS). *Hyptis pectinata* Poit. is described as having antidepressant activity (Bueno et al., 2006), while *Hyptis spicigera* Lam. has presented anticonvulsant activity (Bum et al., 2009), and *Hyptis suaveolens* Poit. (Santos et al., 2007), *Hyptis fruticosa* Salmz. ex Benth (Silva et al., 2006) and *Hyptis pectinata* (Paixão et al., 2013) present an important central antinociceptive profile.

Hyptis martiusii Benth. (Lamiaceae), a native and endemic species in Brazil, popularly known as "cidreira brava" or "cidreira-do-campo" is a small shrub found in the northeast, southeast and central-west regions of Brazil (Flora do Brasil, 2018; Harley et al., 2015). This species is still scarcely studied and the few reports found in the literature present the following biological activities: antiulcerogenic (Caldas et al., 2014), antimicrobial (Coutinho et al., 2008, 2009), larvicidal (Costa et al., 2005), insecticidal (Araújo et al., 2003) and antiproliferative (Costa-Lotufo et al., 2004).

Thus, given the absence of studies addressing the behavioral effects of this species, and the presence of reports pointing to the *Hyptis* genus as an important source of compounds endowed with activity over the central nervous system, this study sought to perform a phytochemical analysis and to characterize the central effects of the *Hyptis martiusii* leaf essential oil and 1,8-cineole (eucalyptol) using behavioral animal models.

2. Material and methods

2.1. Plant material

Leaves from *Hyptis martiusii* Benth were collected in the city of Crato, Brazil, in March 2014 with a voucher specimen (#10.185) being deposited at the Herbarium Caririense Dárdano de Andrade-Lima of the Regional University of Cariri (URCA).

2.2. Preparation of the Hyptis martiusii essential oil (EOHM)

Oil extraction was performed using 250 g of fresh leaves, which were placed in a 5 L glass flask filled with 1.5 L of water and boiled for a period of 2 h. After the boiling period, the essential oil was extracted from the plant and condensed to form a heterogeneous mixture with water. The mixture consisting of water and oil was collected in a modified Clevenger-type apparatus (Gottlieb and Magalhães, 1960) and then separated, dried with anhydrous sodium sulfate (Na_2SO_4) and filtered, yielding 0.72% of EOHM.

2.3. Animals

Female Swiss mice (*Mus musculus*) weighting 25–30 g were housed in polypropylene cages under controlled temperature conditions (22 ± 2 °C) with a 12 h light/dark cycle, with free access to water and food (Labina, Purina, Brazil). The mice were allowed to acclimatize to laboratory conditions for at least 24 h before testing. The experimental use of these animals was approved by the ethical committee of the Regional University of Cariri (nº 00213/2013.1.).

2.4. GC/MS essential oil chemical analysis

The chemical composition analysis was performed using a gas chromatograph coupled to a mass spectrometer (GC/MS) in a SHIMADZU apparatus with a QP5050A mass selective detector, operating under an ionization energy of 70 eV. The DB-5HT (30 m × 0.25 mm internal diameter) capillary column was used alongside the following specifications: injector temperature of 270 °C and 290 °C for the detector, with helium as the carrier gas (1.7 mL/min.); linear velocity of 47.3 cm/s; total flow 24 mL/min; carrier flow 24 mL/min; 107.8 kPa pressure; and a column heating temperature program of 60 °C (2 min) - 180 °C (1 min) at 4 °C/min, followed by 180–260 °C at 10 °C/min (10 min). The identity of each compound was determined by comparing its retention index relative to those of C_8-C_{20} n-alkanes (Fluka Analytical, 1.0 mL Alkane Standard Solution) with retention indices reported in the literature, as well as by visual comparison of their mass spectra with those from the spectrometer database and those reported in the literature (Adams, 1991).

2.5. Drugs

All drugs were diluted/dissolved in saline and injected intraperitoneally (i.p.) in a total volume of 0.1 mL/10 g. The EOHM and 1,8-cineole were emulsified in saline with Tween 80 (0.5%). The control group received the vehicle (Tween 80–0.5% in saline). Pentobarbital sodium and pentylenetetrazole were purchased from Sigma Chemical Co. (USA). Haloperidol and ketamine were purchased from Cristália Farma (Brazil). Diazepam was purchased from União Química (Brazil). Ethyl ether was purchased from Merk.

2.6. Acute toxicity test

Groups of mice (n = 4) were individually given doses of 10, 50, 100, 200, 500, 1000, 2000 or 5000 mg/kg (OEHM - intraperitoneally). Afterwards, the animals were observed for EOHM effects and lethality during 14 days (OECD, 2009). The median lethal dose (LD_{50}) was determined through the probit method using the mortality percentage and the logarithm of the dose followed by linear regression (Litchfield and Wilcoxon, 1949).

2.7. Behavioral experiments

2.7.1. Elevated plus-maze test

Vehicle (Tween 80–0.5%), diazepam (1 mg/kg), EOHM (25, 50, 100, 200 mg/kg) and 1,8-cineole 50 mg/kg (CIN 50), were administered in mice (n = 9) intraperitoneally. After 30 min, each animal was placed individually on the central platform of the apparatus, with its head turned towards one of the closed arms. The behavioral parameters observed were: the number of entries and the time spent (in seconds) in the open and closed arms, during a period of 5 min (Pellow et al., 1985). Afterwards, the same animals were subjected to the open field and rotarod tests as follows.

2.7.2. Open field test

Groups of animals (n = 9) received vehicle (Tween 80–0.5%), diazepam (1 mg/kg), EOHM (25, 50, 100, 200 mg/kg) and 1,8-cineole 50 mg/kg (CIN 50) intraperitoneally (i.p.). After 30 min, each animal was placed in the center of the apparatus and observed for 5 min. The behavioral parameters observed were: number of crossings (NC – number of squares crossed by the animal, with its four paws), grooming (NG – stereotyped behavior) and rearing (NR – vertical exploratory activity) (Archer, 1973).

2.7.3. Rotarod test

In this test, the animals were pre-selected in a training session 24 h prior to the test based on their capacity to stay on the rotating bar (at 16 rpm) for 3 min. Groups of the pre-selected animals (n = 9) were treated with vehicle (Tween 80–0.5%), diazepam (5 mg/kg), EOHM (25, 50, 100, 200 mg/kg) and 1,8-cineole 50 mg/kg (CIN 50) i.p.; 30 min later, the animals were placed on the rotating bar to evaluate the number of falls (NF) and the time of permanence (TP). For each animal, NF (up to three falls) and TP were registered for 1 min (Carlini and Burgos, 1979; Dunham and Miya, 1957).

2.7.4. Pentobarbital-induced sleeping time test

The pentobarbital-induced sleeping time test was carried out according to a previous report (Carlini, 1973). Mice (n = 9) were pre-

treated with vehicle (Tween 80–0.5%), diazepam (2 mg/kg) and EOHM (100 and 200 mg/kg) i.p. After 30 min, animals received a single pentobarbital sodium (40 mg/kg, i.p.) injection. Each mouse was then observed for the time taken to induce sleep (sleep latency; the interval between the administration of sodium pentobarbital and the loss of the righting reflex) and the duration of the sleeping time (interval between the loss and recovery of the righting reflex) for a period of time of up to 3 h.

2.7.5. Ethyl ether-induced sleeping time test

The ethyl ether-induced sleeping time test was carried out in mice according to a previous report (Lapa et al., 2008). Mice (n = 9) were pre-treated with vehicle (Tween 80–0.5%), diazepam (2 mg/kg) and EOHM (100 and 200 mg/kg) i.p. After 30 min, mice were individually inserted into glass cylinders containing a cotton ball suspended at 20 cm from the floor, weighing 6 g and moistened with 5.0 mL of ethyl ether. Each mouse was observed for the time taken to induce sleep (sleep latency; the interval between the administration of sodium pentobarbital and the loss of the righting reflex) and the duration of the sleeping time (interval between the loss and recovery of the righting reflex) for a period of time of up to 3 h.

2.7.6. Pentylenetetrazole-induced seizure test

The PTZ-induced seizure test was carried out as described by Loscher et al. (1991). Mice (n = 9) were pre-treated with vehicle (Tween 80–0.5%), diazepam (2 mg/kg), EOHM (100, 200 and 400 mg/kg) and 1,8-cineole 50 mg/kg (CIN 50) i.p. Thirty minutes later, all groups received a single injection of PTZ (80 mg/kg, i.p.) and the following parameters were observed: latency to the first tonic-clonic seizure and death latency (both in seconds). The total time of observation (cut off time) was 30 min.

2.7.7. Haloperidol-induced catalepsy test

Mice (n = 9) were intraperitoneally treated with EOHM (100 and 200 mg/kg) and 1,8-cineole 50 mg/kg (CIN 50), alone or in association with haloperidol (0.1 mg/kg, i.p.). Two extra groups were treated with haloperidol alone (0.1 and 5 mg/kg, i.p.) as a positive control group for cataleptic behavior. After 30 min, the time of immobility of the animal in the bar was registered during a period of 5 min. The evaluation of this effect (muscle stiffness) was observed when treated mice were placed with their front paws resting on a horizontal bar 5 cm high, so that it remained in a vertical position. If the animal left the position imposed before the determined time, the process was repeated once again, with up to three attempts, according to the protocol adapted from Carlini (1973).

2.7.8. Ketamine-induced hyperkinesia test

Mice (n = 9) were intraperitoneally treated with vehicle (Tween 80–0.5%), haloperidol (0.2 mg/kg), EOHM (25, 100, 200 mg/kg) and 1,8-cineole 50 mg/kg (CIN 50), alone or in association with ketamine (20 mg/kg). An extra group was treated with ketamine alone (20 mg/kg) as a positive control group for hyperkinesia behavior. Immediately after ketamine administration, the animals were placed in the open field apparatus and the number of crossings were observed for a period of 20 min.

2.8. Statistical analysis

The results were analyzed using an analysis of variance (ANOVA) followed by Student–Newman–Keuls test or *t*-test (unpaired) and represented as the mean ± standard deviation (SD). The results were considered significant when $P < 0.05$.

Table 1
Chemical composition of the *Hyptis martiusii* Benth. leaf essential oil.

Compounds	RT (min.)	Retention Index[a]	Kovats Retention Index[b]	(%)
α-pinene	12.50	944	939	2.50
β-pinene	14.90	994	1002	1.30
β-ocimene	16.84	1050	1058	23.40
p-cimene	17.62	1026	1024	2.50
Limonene	17.90	1196	1180	5.30
1,8-cineole	18.10	1183	1185	25.93
Camphor	25.04	1146	1143	3.88
β-caryophyllene	41.11	1421	1412	0.81
Bicyclogermacrene	45.89	1500	1510	3.15
δ-cadinene	46.65	1523	1526	1.79
Valencene	47.17	1496	1495	1.42
Palustrol	47.70	1568	1550	1.39
Spathulenol	47.86	1581	1576	2.15
Caryophyllene oxide	47.98	1581	1572	6.93
Guaiol	48.10	1601	1595	2.10
β-eudesmol	48.23	1654	1649	1.62
Ledol	48.28	1565	1549	1.68
Torreiol	48.55	1645	1633	1.28
Aromadendrene	48.70	1441	1438	2.53
Viridiflorol	48.91	1580	1587	3.63
TOTAL	–	–	–	93.99

RT = retention time.; RI.
[a] Retention indices calculated from retention times in relation to those of a series of C_8–C_{20} n-alkanes in a DB-5HT column; RI.
[b] Retention indices from the literature (Adams R.P. 2007).

3. Results

3.1. Phytochemical analysis

The essential oil yield obtained through hydrodistillation was of 0.72% with a density of 1.0 g/mL. GC/MS analysis identified 20 constituents with their majority being monoterpenes and sesquiterpenes (93.99%), with eucalyptol (1,8-cineole) standing out as the major sample compound (25.93%) (Table 1).

3.2. Elevated plus-maze

Treatment with the OEHM did not change the number of entries into open branches compared to the control group (Fig. 1a). However, the OEHM (25, 50, 100 and 200 mg/kg) and 1,8-cineole (CIN 50 mg/kg) significantly reduced the number of entries into closed branches by 27.68%, 17.62%, 26.85%, 37.82% and 25.19%, respectively, compared to the control group (Fig. 1b). On the other hand, statistical differences were not observed for permanence time in the open and closed branches compared to the control group (Fig. 1c and d).

3.3. Open field

The OEHM (25, 50, 100 and 200 mg/kg) and 1,8-cineole (CIN 50 mg/kg) reduced all behavioral parameters evaluated in the open field test. Animal motility was significantly reduced by 19.20%, 16.57%, 32.11%, 30.38% and 38.80%, respectively, when compared to the control group (Fig. 2a). Rearing behavior was also significantly reduced, when compared to the control group, by 37.52%, 56.28%, 60.41%, 77.11% and 47.84%, respectively (Fig. 2b). Lastly, a significant reduction in grooming by 31.51%, 36.72%, 57.81%, 63.03% and 68.48%, respectively (Fig. 2c), also occurred.

3.4. Rotarod

The OEHM and 1,8-cineole did not cause alterations in the motor coordination of mice submitted to the rotarod test (Table 2).

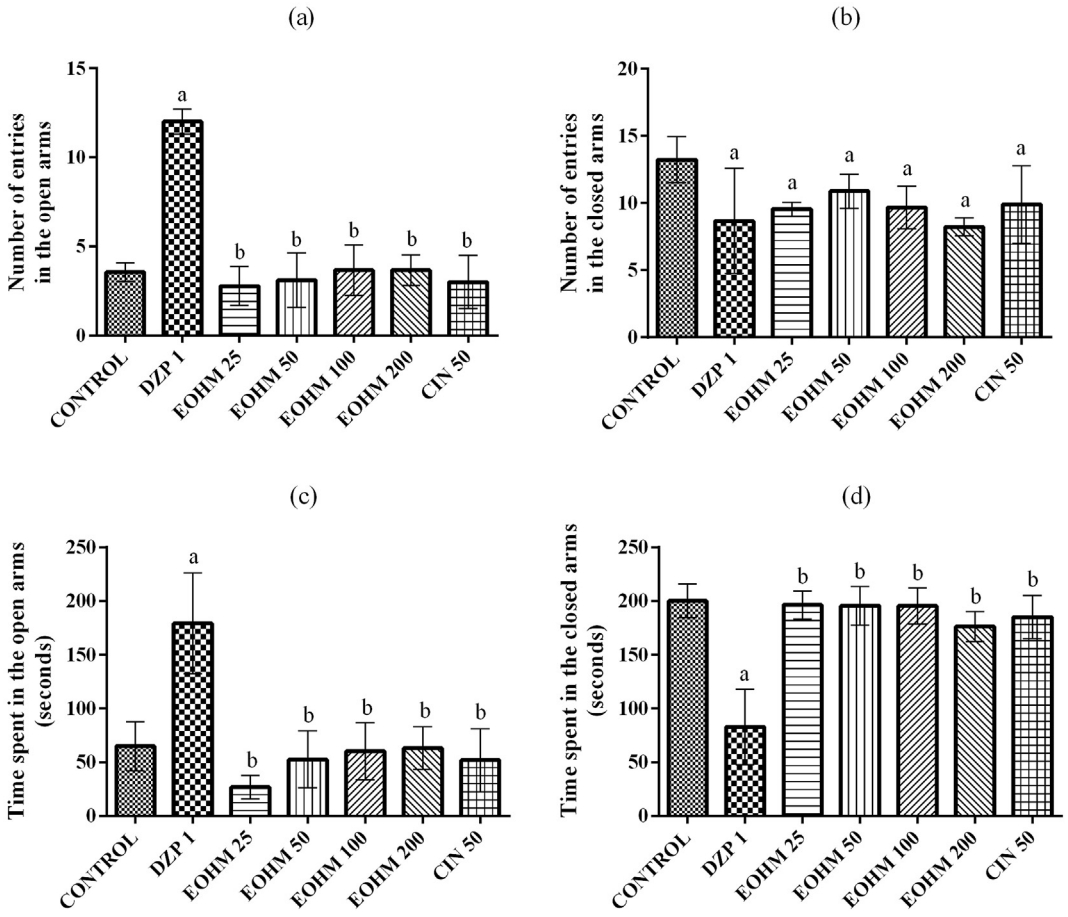

Fig. 1. Effect of the *Hyptis martiusii* Benth. leaf essential and 1,8-cineole on the number of entries in the open (a) and closed arms (b), and the time spent in the open (c) and closed arms (d) in the elevated plus-maze test (n = 9). The results are presented as the mean ± standard deviation (SD), analyzed by ANOVA followed by Student–Newman–Keuls test or *t*-test (unpaired). Significant values: "a" and "b" vs CONTROL and DZP 1, respectively, when $P < 0.05$. DZP 1 = Diazepam 1 mg/kg; EOHM = *Hyptis martiusii* Essential Oil; CIN 50 = 1,8-cineole 50 mg/kg.

3.5. Pentobarbital-induced sleeping time

The OEHM (100 and 200 mg/kg) and 1,8-cineole (CIN 50 mg/kg) reduced sleep induction latency by 18.06%, 20.67% and 50.65%, respectively, compared to the control group (Fig. 3a). However, only the OEHM 200 (50.2%) and CIN 50 (59.3%) groups increased the duration of postural reflex loss (Fig. 3b).

3.6. Ethyl ether-induced sleeping time

Only the OEHM at 200 mg/kg was observed to significantly reduce sleep latency (37.5%) and significantly increase the duration of postural reflex loss (duration of sleep; 53.8%) compared to the control group (Fig. 4a and b).

3.7. Pentylenetetrazole-induced seizures

Treatment with the OEHM did not alter the time taken for the appearance of the first tonic-clonic seizure (Fig. 5a). On the other hand, death latency was significantly increased in groups treated with the OEHM (100, 200 and 400 mg/kg) and 1,8-cineole (CIN 50 mg/kg) by 57.6%, 70.3%, 68.9% and 70.5%, respectively, compared to the control group (Fig. 5b).

3.8. Haloperidol-induced catalepsy

Groups treated with the OEHM (100 and 200 mg/kg) and 1,8-cineole (CIN 50 mg/kg) in association with HALO 0.1 increased the immobility time in the bar by 74.3%, 86.1% and 86.4% %, respectively, when compared to the control group (HALO 0.1). No statistical difference was observed between the OEHM 200 and CIN 50 groups. Potentiation is confirmed by the 89.9%, 87.7% and 81.6% increases, respectively, in the OEHM (100 and 200 mg/kg) and 1,8-cineole groups associated with haloperidol compared to groups who received these substances alone. For comparison purposes, the HALO 5.0 group showed a significant increase in animal immobility time of 86% compared to the HALO 0.1 group (Fig. 6).

3.9. Ketamine-induced hyperkinesia

Groups treated with the OEHM (100 and 200 mg/kg) and 1,8-cineole (50 mg/kg CIN) partially reversed hyperkinesia by 42.63%, 42.80% and 50%, respectively, when compared to the isolated ketamine group (KET 20) (Fig. 7).

4. Discussion

Essential oils and their constituents have been extensively studied where several effects are reported for neurological disorders and disturbances (Blanco et al., 2009; Passos et al., 2009; Li et al., 2012). It is known that essential oils, and the terpenes present in their constitution, have anxiolytic (De Sousa et al., 2015), antidepressant (Bueno et al., 2006), analgesic (Almeida et al., 2013) and anticonvulsive (Almeida et al., 2011) properties. This variety in pharmacological effect is

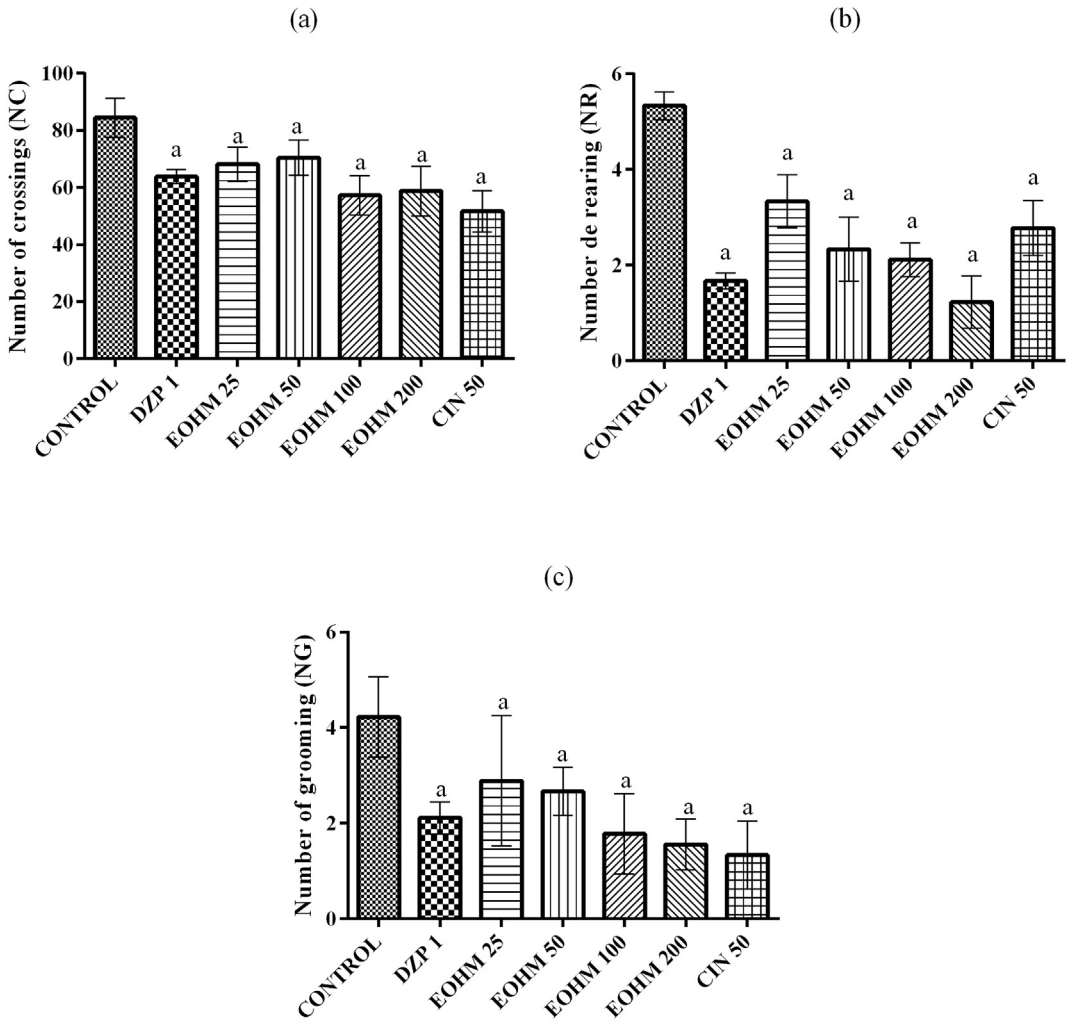

Fig. 2. Effect of the *Hyptis martiusii* Benth. leaf essential oil and 1,8-cineole on the NC (a), NR (b) and NG (c) in the open field test (n = 9). The results are presented as the mean ± standard deviation (SD), analyzed by ANOVA followed by Student–Newman–Keuls test or *t*-test (unpaired). Significant values: "a" vs CONTROL, when $P < 0.05$. DZP 1 = Diazepam 1 mg/kg; EOHM = *Hyptis martiusii* Essential Oil; CIN 50 = 1,8-cineole 50 mg/kg.

Table 2
Effect of the *Hyptis martiusii* Benth. leaf essential and 1,8-cineole in the rotarod test.

GROUPS (n = 9)	NF	TP
CONTROL (a)	0.0 ± 0.0	60 ± 0.0
DZP 5 (b)	1.4 ± 0.5[a]	32.4 ± 10.6[a]
EOHM 25 (c)	0.0 ± 0.0[b]	60 ± 0.0[b]
EOHM 50 (d)	0.0 ± 0.0[b]	60 ± 0.0[b]
EOHM 100 (e)	0.0 ± 0.0[b]	60 ± 0.0[b]
EOHM 200 (f)	0.0 ± 0.0[b]	60 ± 0.0[b]
CIN 50 (g)	0.1 ± 0.3[b]	59.6 ± 1.0[b]

All of the results were expressed as the mean ± standard deviation (SD), analyzed by ANOVA followed by Student-Newman-Keuls (post hoc). Significant values: "a" and "b" vs CONTROL and DZP 5, respectively, when $P < 0.05$. DZP 5 = Diazepam 5 mg/kg; EOHM = *Hyptis martiusii* Essential Oil; CIN 50 = 1,8-cineole 50 mg/kg; NF = Number of Falls; TP = Time of Permanence (seconds).

justified by the great structural diversity of essential oil constituents, with terpenes being among the most investigated and cited compounds with pharmacological activities at the level of the central nervous system (Passos et al., 2009).

Chemical analysis of the *Hyptis martiusii* Benth. leaf essential oil expressed high concentrations of compounds belonging to the terpene class, including 1,8-cineole as the major monoterpene.

Previous studies with species from the *Hyptis* genus have reported the presence of 1,8-cineole (eucalyptol). *H. crenata* Pohl was characterized by the presence of eucalyptol, *α*-pinene and *β*-caryophyllene, among others, to which its antiulcerogenic potential (Diniz et al., 2013) is attributed. *H. suaveolen* (L.) is described as having antifungal activity where its phytochemical profile reported the presence of eucalyptol, *β*-pinene, (+)-carene, *trans-β*-caryophyllene and germacrene (Moreira et al., 2010). Lastly, *H. spicigera* Lam. presented *α*-pinene, *β*-pinene and cineole as its main phytoconstituents (Takayama et al., 2011).

Following chemical characterization of the *Hyptis martiusii* leaf essential oil and the determination of 1,8-cineole as the major metabolite, standard classical experiments were carried out to evaluate the central profile of these substances and to correlate these effects with the presence of eucalyptol.

In the present study, the results suggest the OEHM presented central depressant activity. All OEHM and 1,8-cineole doses showed a decrease in the parameters analyzed in the open field test. According to Lacerda (2006), increases in the number of crosses, rearing and grooming are considered indices of locomotor and exploratory activity, as well as a stimulant effect. Thus, a reduction in these three parameters is indicative of compounds with central depressant effects (Carlini and Mendes, 2011).

Based on spontaneous exploratory activity and natural mice aversion to open environments, the number of entries and the length of stay

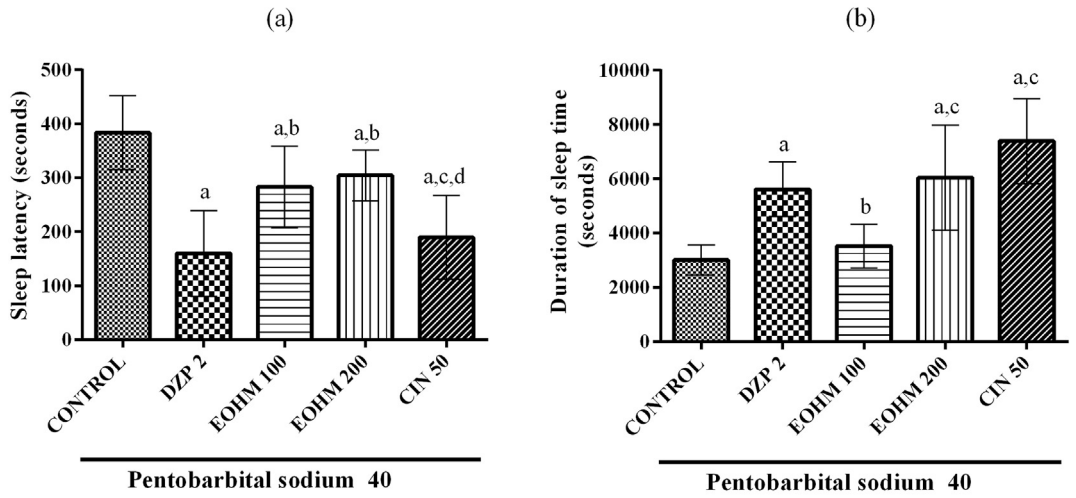

Fig. 3. Effect of the *Hyptis martiusii* Benth. leaf essential and 1,8-cineole on sleep latency (a) and duration of sleep time (b) in the pentobarbital-induced sleeping time test (n = 9). The results are presented as the mean ± standard deviation (SD), analyzed by ANOVA followed by Student-Newman-Keuls (post hoc) or *t*-test (unpaired). Significant values: "a", "b", "c" and "d" vs CONTROL, DZP 2, EOHM 100 and EOHM 200, respectively, when $P < 0.05$. DZP 2 = Diazepam 2 mg/kg; EOHM = *Hyptis martiusii* Essential Oil; CIN 50 = 1,8-cineole 50 mg/kg.

in the open branches of the elevated cross maze test are used as anxiolytic-like effect indicators (Lister, 1987, 1990; Pellow et al., 1985). Thus, substances which stimulate animals to enter and remain for longer periods of time in the open branches, indicate they are endowed with an anxiolytic-like effect (Almeida et al., 1999). However, this behavior was not observed in the present study, suggesting the OEHM and its major component seem to be devoid of anxiolytic-like activity since when placed in the elevated plus-maze, the animals showed signs of intense fear (File, 1993; Almeida, 2006).

Interestingly, although the results indicate the OEHM and 1,8-cineole are central activity depressants, the rotarod test showed no myorelaxant effect, indicating these compounds do not alter animal motor coordination. These data are important since they reveal the oil or its major compound are devoid of harmful motor performance effects, where this characteristic is of great importance when it is desired that pharmacological treatment does not cause motor coordination impairment. The loss of motor coordination is a characteristic of several neurological disorders and one of the most common adverse effects associated with psychotropic drugs (Massaquoi and Hallett, 1998; Nunes and Bastos, 2016).

Based on a predictive central depressant effect evidenced in the open field test, the study was directed to evaluate a possible hypnotic-sedative effect. For this, sleep-potentiation models induced by sodium pentobarbital or ethyl ether were used (Carlini, 1973; Lapa et al., 2008).

Drugs from the barbiturate class act by increasing the inhibitory action of the GABAergic system and it is known that anxiolytic, hypnotic and antiepileptic drugs may prolong sleep time induced by pentobarbital (Ha et al., 2006). Compounds with a sedative characteristic reduce the latency to reflex loss and significantly increase sleep duration in animals (Oliveira, 2012).

The OEHM at the 200 mg/kg dose, and 1,8-cineole potentiated the pentobarbital hypnotic effect. These results suggest the possible hypnotic-sedative effect of the oil is associated with the presence of eucalyptol in its constitution. This result corroborates with the study by Freire et al. (2006), in which the *Ocimun gratissimun* L. essential oil, which presents 1,8-cineole as the main compound, also presented the effect in question.

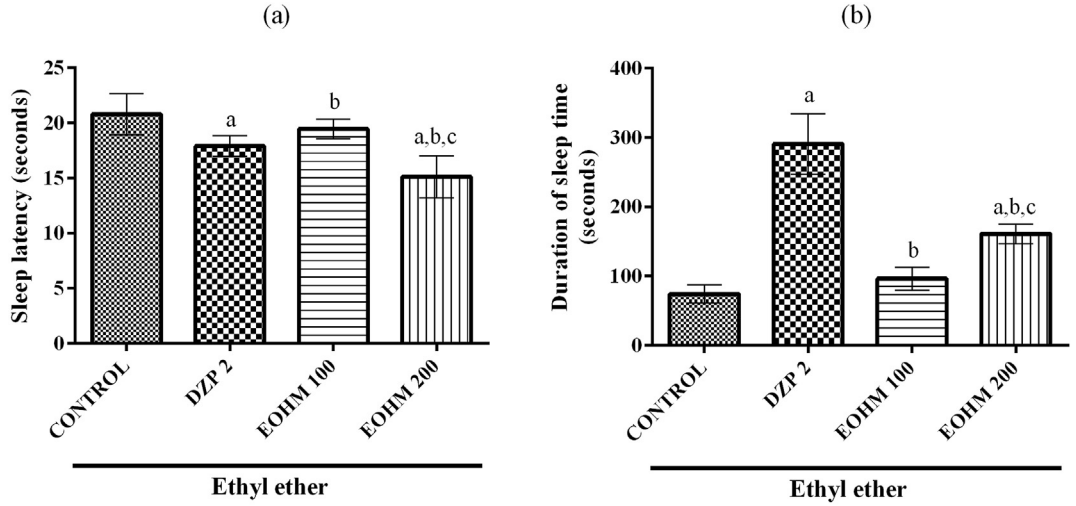

Fig. 4. Effect of the *Hyptis martiusii* Benth. leaf essential and 1,8-cineole on sleep latency (a) and duration of sleep time (b) in the ethyl ether-induced sleeping time test (n = 9). The results are presented as the mean ± standard deviation (SD), analyzed by ANOVA followed by Student-Newman-Keuls (post hoc) or *t*-test (unpaired). Significant values: "a", "b" and "c" vs CONTROL, DZP 2 and EOHM 100, respectively, when $P < 0.05$. DZP 2 = Diazepam 2 mg/kg; EOHM = *Hyptis martiusii* Essential Oil.

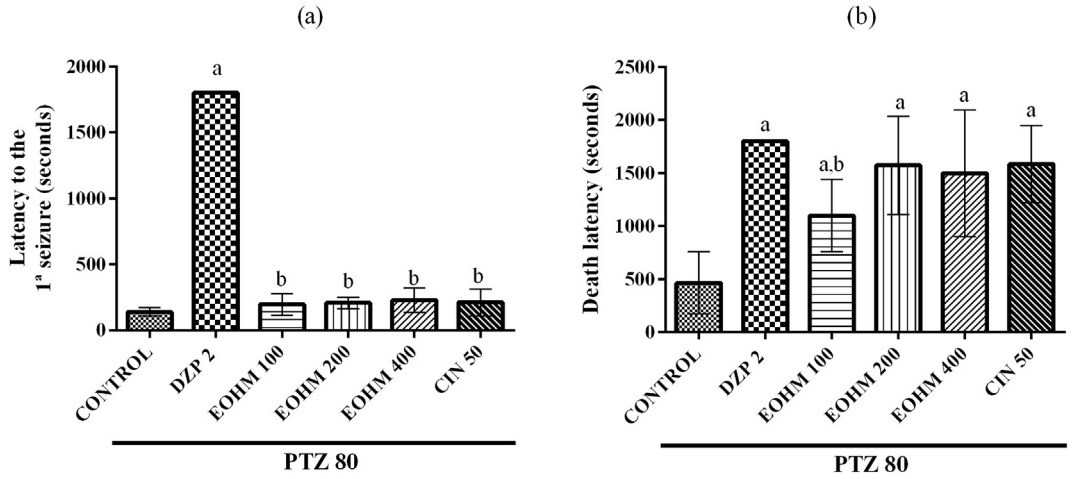

Fig. 5. Effect of the *Hyptis martiusii* Benth. leaf essential and 1,8-cineole on the latency to the 1st seizure (a) and death latency (b) in the pentylenetetrazole-induced seizure test (n = 9). The results are presented as the mean ± standard deviation (SD), analyzed by ANOVA followed by Student-Newman-Keuls (post hoc) or *t*-test (unpaired). Significant values: "a" and "b" vs CONTROL and DZP 2, respectively, when $P < 0.05$. DZP 2 = Diazepam 2 mg/kg; EOHM = *Hyptis martiusii* Essential Oil.

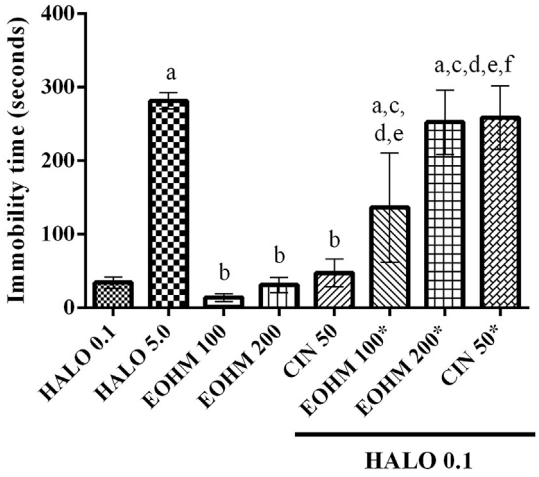

Fig. 6. Effect of the *Hyptis martiusii* Benth. leaf essential and 1,8-cineole on immobility time in the haloperidol-induced catalepsy test (n = 9). The results are presented as the mean ± standard deviation (SD), analyzed by ANOVA followed by Student-Newman-Keuls (post hoc) or *t*-test (unpaired). Significant values: "a", "b", "c", "d", "e" and "f" vs HALO 0.1, HALO 5, EOHM 100, EOHM 200, CIN 50 and EOHM 100*, respectively, when $P < 0.05$. HALO = Haloperidol 0.1 and 5 mg/kg; EOHM = *Hyptis martiusii* Essential Oil; CIN 50 = 1,8-cineole 50 mg/kg.

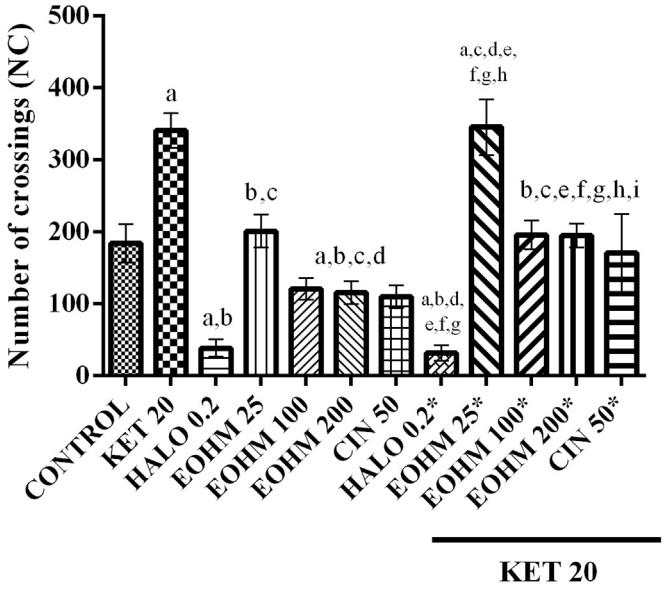

Fig. 7. Effect of the *Hyptis martiusii* Benth. leaf essential and 1,8-cineole on the number of crossings in the ketamine-induced hyperkinesia test (n = 9). The results are presented as the mean ± standard deviation (SD), analyzed by ANOVA followed by Student-Newman-Keuls (post hoc) or *t*-test (unpaired). Significant values: "a", "b", "c", "d", "e", "f", "g", "h" and "i" vs CONTROL, KET 20, HALO 0.2, EOHM 25, EOHM 100, EOHM 200, CIN 50, HALO 0.2* and EOHM 25*, respectively, when $P < 0.05$. KET 20 = Ketamine 20 mg/kg; HALO = Haloperidol 0.2 mg/kg; EOHM = *Hyptis martiusii* Essential Oil; CIN 50 = 1,8-cineole 50 mg/kg.

It has been reported that compounds administered intraperitoneally are absorbed primarily through the hepatic-portal circulation, and consequently undergo first-pass metabolism (Lukas et al., 1971), a hypothesis which could justify the absence of effects at lower doses (25 and 100 mg/kg), where barbiturates are important P-450 enzymatic complex inducers, one of the main systems associated with the metabolism of xenobiotics in the liver (Katzung and Trevor, 2017).

Thus, to rule out the possibility of increased OEHM hepatic metabolism by pentobarbital, the ethyl ether sleep-induced model was performed. In this model, only the 200 mg/kg OEHM dose showed a hypnotic effect by reducing latency to reflex loss and increasing ethyl ether-induced sleep duration, suggesting that pentobarbital does not influence OEHM hepatic metabolism and hence its hypnotic effect.

Subsequently, a model which could evaluate the OEHM and 1,8-cineole anticonvulsive profile was developed. Pentylenetetrazole (PTZ) is a strong CNS stimulant due to its $GABA_A$ receptor inhibitory capacity, which induces extensive excitotoxic processes mediated by glutamate and changes in the cerebral antioxidant system, which are mainly manifested as generalized tonic-clonic seizures (Lasoń et al., 2013; Armijo et al., 2002; Singh et al., 2002). In this analysis, the oil presented a protective effect by prolonging death latency of the animals, which may be justified by the presence of 1,8-cineole, which also significantly increased the same parameter. Terpenoids constitute a vast group of secondary metabolites with actions on the CNS, including anticonvulsant activity, among others (Passos et al., 2009). Almeida et al. (2011) described thirty essential oil chemical constituents with anti-convulsive properties, including 1,8-cineole (eucalyptol), present in *Ocimum basilicum* L. leaves, which increased the latency for seizure development and death latency in tests using pentylenetetrazole and picrotoxin; such effects, according to the authors, were reversed by flumazenil, indicating possible modulation of the compound in the

GABAergic system. Additionally, *Hyptis spicigera* protected 87.5% of mice against PTZ-induced seizures at a 160 mg/kg dose in a previous study (Bum et al., 2009). These data suggest the OEHM has a neuroprotective effect in the PTZ chemically induced convulsions model, which seems to depend on its major compound (1,8-cineole).

According to Pellow et al. (1985), drugs lacking an anxiolytic effect, however which possess a sedative effect may present a possible neuroleptic (antipsychotic) profile. The previously described profile was seen over the previously discussed results, which led to the investigation of a possible antipsychotic-like effect of the OEHM and its major component.

Initially, the possibility of a haloperidol-induced catalepsy potentiation, when associated with the OEHM and CIN, was evaluated. According to Carlini (1973), this is an experimental model with the capacity to cause muscle stiffness in animals, an effect present in typical antipsychotics used in the treatment of schizophrenia. The results showed that mice pretreated with the OEHM and eucalyptol, when associated with haloperidol, showed increased catatonia (muscle stiffness). Araújo et al. (2009) reported the *Alpinia zerumbet* essential oil, which has 1,8-cineole in its composition, was also able to potentiate cataleptic behavior in animals submitted to the same experimental model.

Lastly, to support findings associated with a possible OEHM antipsychotic-simile profile, hyperkinesia induced by the ketamine model was used. Ketamine is a drug which at appropriate amounts acts to competitively antagonize the NMDA receptor (Tsai and Coyle, 2002). Among the experimental models, a decrease in NMDA receptor function is what enables psychotic scenarios that most closely resemble schizophrenia (Krystal et al., 1994; Newcomer et al., 1999). Thus, a reduction in animal motility increase in this experimental model is predictive of a drug with neuroleptic potential.

According to the results, ketamine provoked increased motility in the animals, however, haloperidol, a dopaminergic antagonist, caused a decrease in animal locomotor activity. The same was observed with the OEHM at the 200 mg/kg dose and 1,8-cineole, which partially decreased the locomotor activity induced by ketamine. Taken together, these data suggest these effects may be due to a eucalyptol-mediated modulation of glutamatergic and dopaminergic neurotransmission. The *Alpinia zerumbet* essential oil, a species characterized by the presence of high 1,8-cineole concentrations, has been described with an antipsychotic activity by reducing schizophrenic symptoms (Araújo et al., 2009; Galdino, 2013).

In summary, the results suggest the OEHM has important hypnotic-sedative and antipsychotic-like effects, which appear to be due to the monoterpene 1,8-cineole, possibly through modulation of dopaminergic and glutamatergic systems.

Declaration of competing interest

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

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