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Anxiolytic-like effect of the monoterpene 1,4-cineole in mice

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ABSTRACT

Recent studies have shown that some monoterpenes exert anxiolytic- and depressant-like actions, however, these effects from monoterpene 1,4-cineole are still unknown. This work aimed to study the effects of 1,4-cineole in classic animal models for depression- and anxiety-like behavior, specifically the elevated plus maze (EPM), hole board, open field, pentobarbital sleeping time, forced swimming, tail suspension and rota rod tests. 1,4-Cineole was administered orally to mice (100, 200 and 400 mg/kg), while diazepam (1 or 2 mg/kg) and imipramine (10 or 30 mg/kg) were used as standard drugs. 1,4-Cineole (400 mg/kg) modified all parameters observed in the EPM, while no significant variation was observed on general motor activity in the open-field test. In the hole-board assay, 1,4-cineole induced increase on the number of head dips. Forced swimming and tail suspension tests showed that cineole (200 and/or 400 mg/kg) was able to promote significant increase on the immobility time, while a decreased sleep latency was observed (200 and 400 mg/kg) on the pentobarbital sleeping time. Cineole had no effect on the motor coordination of animals in the rota rod test. The results suggest that 1,4-cineole presents potential anxiolytic-like action consistent with possible general depression of the CNS.

1. Introduction

Essential oils are natural products that contribute to the perception of natural flavors and fragrances from plants (Lis-Balchin and Hart, 1999). Nevertheless, recent studies have showed that natural compounds isolated from several essential oils exhibit a variety of biologic properties, such as analgesic (Amaral et al., 2007), anticonvulsant (Almeida et al., 2003), anti-inflamatory (Almeida et al., 2001), anxiolytic (Silva et al., 2007) and gastro-protective activities (Paula et al., 2006). These effects are frequently attributed to monoterpenes, which are the major constituent of essential oils.

Monoterpene cineoles are commonly found as components of essential oils from several aromatic plants such as *Laurus nobilis* L., *Salvia* spp., *Eucalytus* spp., *Xanthoxylum rhetsa* D.C., *Cunila spicata* and *Artemisia* spp. (Joanne et al., 2000). 1,8-Cineole, also known as eucalyptol or cajeputol (Magalhães et al., 2003), is a monoterpene ether, present in many plant essential oils. It has been reported to alter neural firing in certain areas of the olfactory lobe, as well as to have hypotensive and smooth muscle relaxant (Nikitin and Balaban, 2000; Hummel et al., 2003), anti-inflammatory (Lahlou et al., 2002), and antinociceptive activities (Santos and Rao, 2000). The anti-inflammatory activity of 1,8-cineole is attributed to modulation of the release of mediators from nerve terminals (Khalil et al., 2004).

Natural monoterpene analogs of 1,8-cineole, such as 1,4-cineole, are minor components of some of the same plant extracts (Joanne et al., 2000). 1,4-Cineole is a widely distributed monoterpene ether, which is one of the flavor constituents of lime juice. Its metabolism has been described and metabolites have been isolated from the urine of rabbits (Asakawa et al., 1988). Previous studies have demonstrated that 1,4-cineole is a potent phytotoxin (Romagni et al., 2000) and acts as a precursor for microbial hydroxylation. (Rosazza et al., 1987). Despite a large body of previous studies have demonstrated that some monoterpenes show anxiety-like behavior consistent with general depression of the central nervous system (CNS) (Sousa et al., 2005; Silva et al., 2007), as far as we know, similar reports from monoterpene 1,4-cineole are still unknown in literature. These observations led us to investigate the effects of 1,4-cineole on classic animal models for depression- and anxiety-like behavior.

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2. Materials and methods

2.1. Animals

Male Swiss mice (20–30 g) were used in each experiment and animals were maintained at a controlled temperature (25 ± 1 °C) with a 12 h dark/light cycle with free access to water and food. For the complete study, a total of 367 mice were used. Animals were treated in accordance with the current law and the NIH Guide for Care and Use of Laboratory Animals. The study was performed under the consent and surveillance of the Committee of Ethics in Animal Research, Department of Physiology and Pharmacology, Faculty of Medicine, Federal University of Ceará, Ceará, Brazil.

2.2. Drugs and doses

1,4-Cineole (Sigma-USA) was emulsified with 0.2% Tween 80 (Sigma-USA) and dissolved in distilled water. Animals were treated with the substance at doses of 100, 200 and 400 mg/kg, orally, 60 min before the experiments. Controls received vehicle (saline with 0.2% Tween 80) at the same volume (10 ml/kg) administered by the same route as the treated groups.

Diazepam (DZP) 1 or 2 mg/kg (União Química/Brazil) and Imipramine (Imip) 10 or 30 mg/kg (Geigy), used as standards, were intraperitoneally injected after dissolution in distilled water. It is well known that benzodiazepines act as anxiolytics (at low doses) and also produce sedation and myorelaxant effect at higher doses (Novas et al., 1988). Thereby, our group has used diazepam at 1 mg/kg in EPM and hole-board tests as standard drug for anxiolytic effect, as well as diazepam 2 mg/kg in open field and rota rod tests as standard drug for sedative and miorelaxant effects, respectively. Imipramine 10 or 30 mg/kg was used as standard drug for antidepressive effect in both forced swimming and tail suspension tests in accordance to sensibility from each model (Porsolt et al., 1977; Steru et al., 1985). Flumazenil (FLU), a recognized competitive antagonist at the central benzodiazepine receptor, was intraperitoneally injected after dissolution in distilled water 15 min before the treatment with cineole to elucidate a possible action mechanism $GABA_A$/benzodiazepine related.

2.3. Experimental protocol

The animals were tested during the light period and observed in a closed room at constant temperature (25 ± 1 °C) which was poorly illuminated with a 15-V red light, except in the forced swimming test which was illuminated with normal light. All tests were performed on different days with distinct groups of animals.

2.4. Elevated plus maze test (EPM)

The elevated plus maze test for mice (Lister, 1987) consisted of two perpendicular open arms (30 × 5 cm) and two closed arms (30 × 5 × 25 cm) also in perpendicular position. The open and closed arms were connected by a central platform (5 × 5 cm).

The platform and the lateral walls of the closed arms were made of transparent acrylic and the floor of black acrylic. The maze was 45 cm above the floor. After treatment, the animal was placed at the center of the plus maze with its nose in the direction of one of the closed arms, and observed for 5 min, according to the following parameters: number of entries in the open and closed arms, and time of permanence in each of them. The time of permanence measures the time spent by the animal in the open and closed arms. Anxiolytic compounds reduce the animal's aversion to the open arms and promote the exploration thereof. The parameters observed were: percentages of entries into open arms (PEOA), number of entries in the open arms (NEOA), time of permanence in open arms (TPOA) and percentage of time of permanence in the open arms (PTOA).

To this test, the animals were divided into eight groups of 10–15 animals each. The different groups were treated with: saline (control), cineole (100, 200 and 400 mg/kg), diazepam 1 mg/kg, FLU (2.5 mg/kg) + cineole (400 mg/kg), FLU (2.5 mg/kg) + diazepam (1 mg/kg) and FLU (2.5 mg/kg).

2.5. Open-field test

The open-field area was made of acrylic (transparent walls and black floor, 30 × 30 × 15 cm) divided into nine squares of equal area. This apparatus was used to evaluate the exploratory activity of the animal for 5 min (Archer, 1973). The observed parameters were as follows: number of squares crossed (with the four paws) and number of groomings and rearings. The animals were divided into five groups of 10–15 animals each. The different groups were treated with: saline (control), cineole (100, 200 and 400 mg/kg) and Diazepam 2 mg/kg.

2.6. Hole-board test

The hole-board test for exploratory behavior in mice was used as described previously by Clark et al. (1971). The apparatus used was an Ugo Basile of 60 × 30 cm with 16 evenly spaced holes with built-in infrared sensors. In brief, adult male mice were randomly divided into five groups with 8 mice per group. Three groups received graded doses of 1,4-cineole (100, 200 and 400 mg/kg, p.o.). One group received DZP (1 mg/kg, i.p.) as standard and the remaining group (control) received saline. Thirty minutes after the administration of DZP and 60 min after the administration of 1,4-cineole, the number of head dips into the holes was counted for each animal for 5 min.

2.7. Forced swimming test

For these experiments the tank size was 22 cm in diameter and 40 cm in height (Porsolt et al., 1978). The tank had a rounded lid and contained 20 cm deep fresh water at 25 °C. During the exposure, mice were placed in the tank and left there for 5 min during which immobility time was registered. A mouse was considered immobile when it remained floating in the water, without struggling, making only very slight movements necessary to keep its head above water.

The animals were divided into five groups with 10–13 animals per group. The different groups were treated with: saline (control), cineole (100, 200 and 400 mg/kg) and Imipramine 10 mg/kg.

2.8. Tail suspension test

The tail suspension test has been described by Steru et al. (1985). Male Swiss mice were housed in plastic cages witha 12 h light cycle with food and water freely available. Animals were transported from the housing room to the testing area in their own cages and allowed to adapt to the new environment for 1 h before testing. For the test, the animals were divided into five groups with 8 animals per group. The different groups were treated with: saline (control), cineole (100, 200 and 400 mg/kg) and Imipramine 30 mg/kg, in accordance to the described method (Steru et al., 1985). They were suspended on the edge of a shelf 58 cm above a table top by adhesive tape placed approximately 1 cm from the tip of the tail. The duration of immobility is recorded for a period of 6 min.

2.9. Pentobarbital sleeping time

Sixty minutes after oral administration of 1,4-cineole at all doses or vehicle, all groups received sodium pentobarbital (40 mg/kg, i.p.). The time since the injection up to the loss of the righting reflex was recorded as sleep latency and the time elapsed between the loss and voluntary recovery of the righting reflex was recorded as sleeping time (Rolland et al., 1991; Wambebe, 1985).

For the test, the animals were divided into five groups with 8–15 animals per group. The different groups were treated with: saline (control), cineole (100, 200 and 400 mg/kg) and Diazepam 1 mg/kg. Sixty minutes after administration of treatments, all groups received sodium pentobarbital (40 mg/kg, i.p.).

2.10. Rota rod

Animals were selected for the rota rod test before the pharmacological test. Mice, 8 per group, were divided in five groups and treated with: saline (control), cineole (100, 200 and 400 mg/kg) and Diazepam 2 mg/kg. Sixty minutes after oral administration of treatments, mice were placed with the four paws on a 2.5 cm diameter bar, 25 cm above the floor and the time of permanence on the bar was measured for 1 min, for each animal. The rotating speed was of 12 rpm (Dunham and Miya, 1957).

2.11. Statistical analyses

All results are presented as mean ± S.E.M.. Data were analyzed by ANOVA followed by Student–Newman–Keuls's *post hoc* test. Results were considered significant at $p<0.05$

3. Results

3.1. Elevated plus maze test (EPM)

In this test (Fig. 1), similar to diazepam 1 mg/kg (DZP-1), only 1,4-cineole 400 mg/kg (Cin 400) significantly increased all the parameters observed when compared to control (C): number of entries in the open arms (NEOA) [$F(7,75)=10.39$, $p<0.001$], percentage of entries into open arms (PEOA) [$F(7,75)=7.03$, $p<0.05$], time of permanence in the open arms (TPOA) [$F(7,75)=7.64$, $p<0.01$] and percentage of time in the open arms (PTOA) [$F(7,75)=9.82$, $p<0.01$]. Flumazenil (FLU), a benzodiazepine antagonist, alone, did not alter the parameters observed. However, as expected, the pretreatment with this antagonist prevented the anxiolytic effect of diazepam, but did not alter the anxiolytic effect of Cin 400.

3.2. Open-field test

Fig. 2 shows that 1,4-cineole (Cin 100, 200 and 400 mg/kg) did not alter the number of crossings, rearings and groomings, as compared to control group (C). The animals treated with diazepam 2 mg/kg (DZP-2) showed a significant decrease in all the parameters analyzed: crossing [$F(4,40)=6.67$, $p<0.01$], rearing [$F(4,40)=4.01$, $p<0.05$] and grooming [$F(4,40)=3.78$, $p<0.05$)] as compared to the control group.

3.3. Hole-board test

Similar to diazepam 1 mg/kg (DZP-1), 1,4-cineole at dose of 400 mg/kg (Fig. 3) increased significantly the number of head dips [$F(4,41)=6.22$, $p<0.05$], as compared to control.

3.4. Forced swimming test

In this test (Fig. 4), 1,4-cineole at doses 200 (Cin 200) and 400 mg/kg (Cin 400), induced a significant increase in the immobility time of mice,

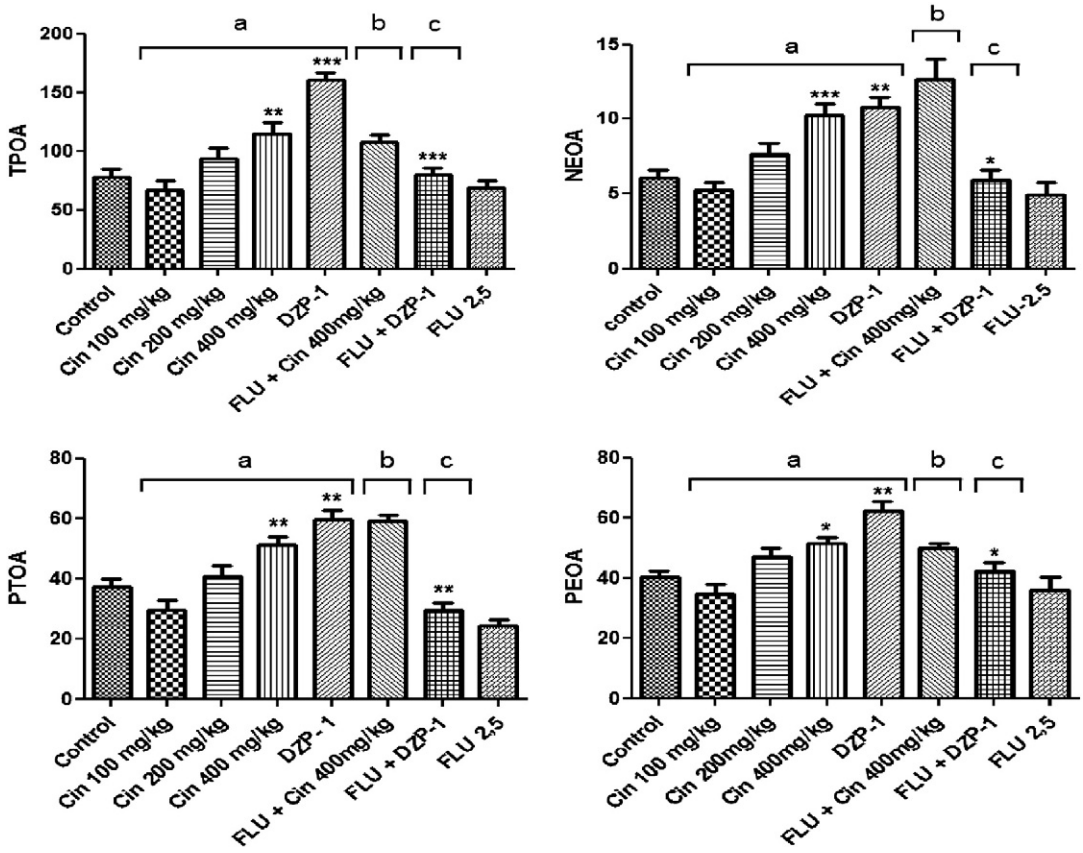

Fig. 1. Plus maze test of groups of mice which received vehicle, 1,4-cineole (Cin 100, 200 and 400 mg/kg), diazepam (DZP 1 mg/kg) or flumazenil (FLU 2.5 mg/kg). The parameters analyzed were: NEOA: number of entries into open arms; PEOA: percentage of entries into open arms; TPOA: time of permanence in the open arms; and PTOA: percentage of time in the open arms. The results are presented as means ± S.E.M. The letter a means a significant difference when compared with control; the letter b means a significant difference when compared with 1,4-cineole 400 mg/kg (Cin 400 mg/kg); and the letter c means a significant difference when compared with DZP 1 mg/kg (*$p<0.05$, **$p<0.01$, ***$p<0.001$). ANOVA and Student–Newman–Keuls's as the *post hoc* test.

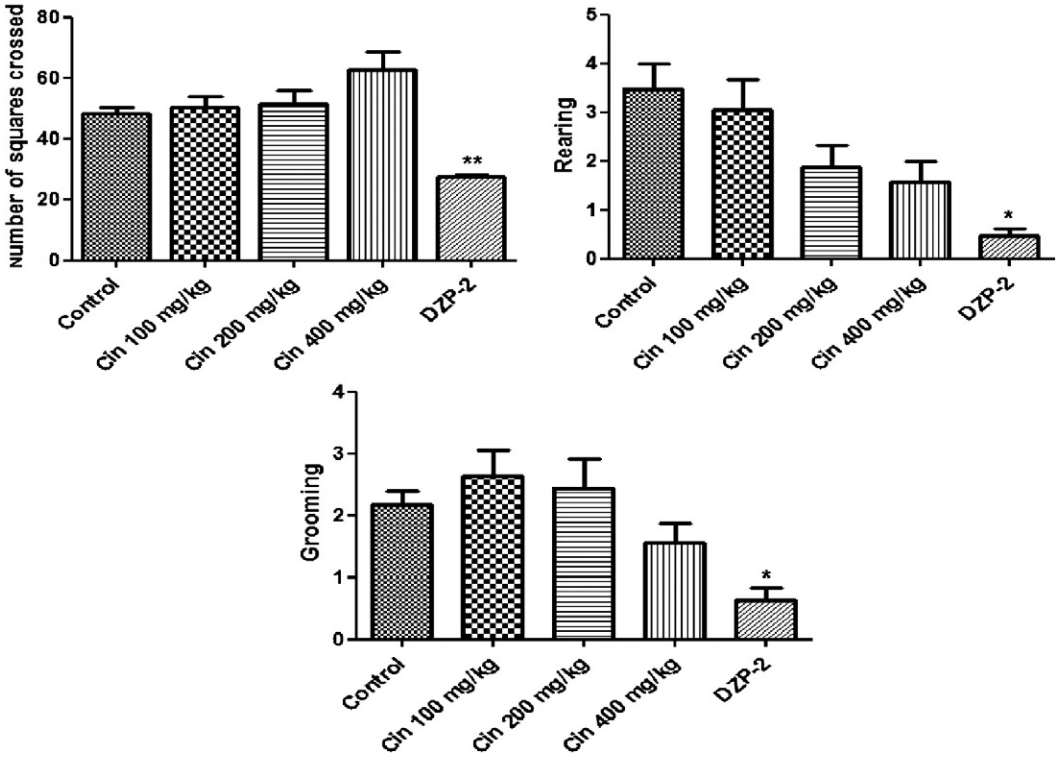

Fig. 2. Open-field test of groups of mice which received vehicle, 1,4-cineole (Cin 100, 200 and 400 mg/kg), and diazepam (DZP 2 mg/kg). The parameters analyzed were: number of squares crossed; grooming; and rearing. The results are presented as means ± S.E.M. Significant difference compared with control (*$p<0.05$, **$p<0.01$). ANOVA and Student–Newman–Keuls's as the *post hoc* test.

as compared to control (C). On the other hand, treatment with imipramine 10 mg/kg (Imip) decreased the immobility time [$F(4,54) = 37.49$, $p<0.001$].

3.5. Tail suspension test

Fig. 5 shows that, similar to those results observed in forced swimming test, 1,4-cineole 400 mg/kg (Cin 400) significantly increased the immobility time in animals, while imipramine (Imip 30 mg/kg) produced opposite effect, as compared to the control group [$F(4,50) = 33.29$, $p<0.001$].

3.6. Pentobarbital induced sleeping time

The absolute values of sleep latency and duration of sleep are showed in Fig. 6. In this test, 1,4-cineole at doses of 200 (Cin 200) and 400 mg/kg (Cin 400), 60 min before injection of pentobarbital and diazepam (DZP-1), induced a significant decrease in the sleep latency [$F(4,50) = 15.47$, $p<0.01$], when compared to control. Only DZP increased the duration of sleep [$F(4,50) = 13.32$, $p<0.001$] compared to control.

3.7. Rota rod

No change was observed in the rota rod test (Fig. 7) at 12 rpm after treatment with 1,4-cineole 100, 200 and 400 mg/kg as compared to control, while diazepam 2 mg/kg (DZP-2), as expected, decreased the

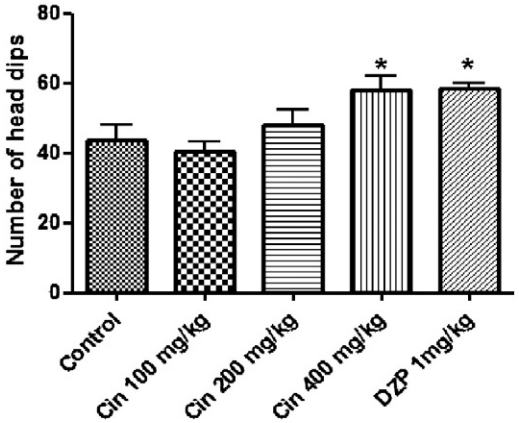

Fig. 3. Hole-board test of groups of mice which received vehicle, 1,4-cineole (Cin 100, 200 and 400 mg/kg), and diazepam (DZP 1 mg/kg). Number of head dips. The results are presented as means ± S.E.M. Significant difference compared with control (*$p<0.05$). ANOVA and Student–Newman–Keuls's as the *post hoc* test.

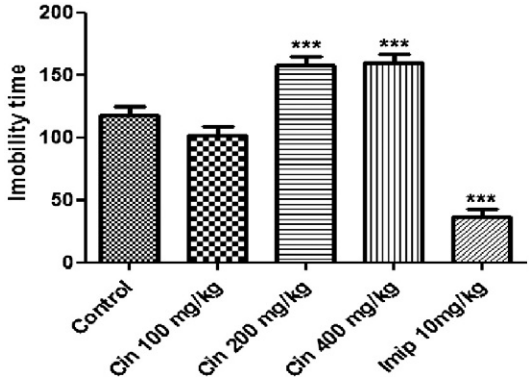

Fig. 4. Forced swimming test of groups of mice which received vehicle, 1,4-cineole (Cin 100, 200 and 400 mg/kg), and imipramine (Imip 10 mg/kg). The parameter analyzed was the immobility time. The results are presented as means ± S.E.M. Significant difference compared with control (***$p<0.001$). ANOVA and Student–Newman–Keuls's as the *post hoc* test.

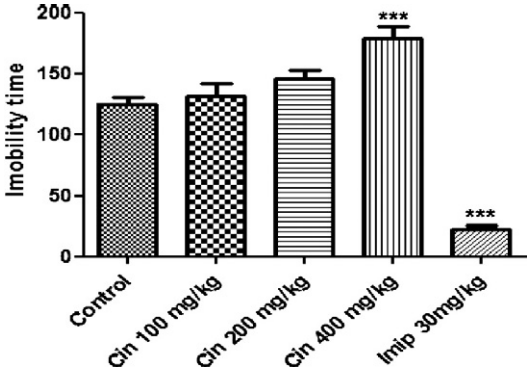

Fig. 5. Tail suspension test of groups of mice which received vehicle, 1,4-cineole (Cin 100, 200 and 400 mg/kg), and imipramine (Imip 30 mg/kg). The parameter analyzed was the immobility time. The results are presented as means ± S.E.M. Significant difference compared with control (***$p<0.001$). ANOVA and Student–Newman–Keuls's as the *post hoc* test.

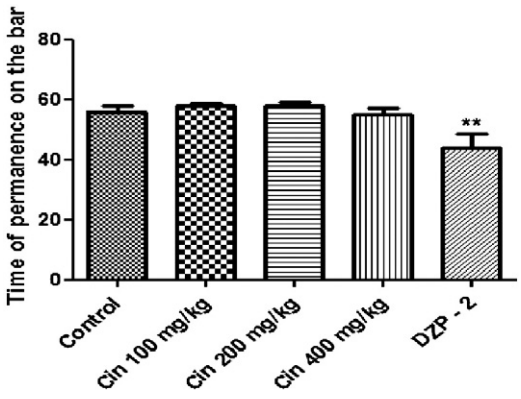

Fig. 7. Rota rod test of groups of mice which received vehicle, 1,4-cineole (Cin 100, 200 and 400 mg/kg), and diazepam (DZP 2 mg/kg). The parameter analyzed was the time of permanence on the bar. The results are presented as means ± S.E.M. Significant difference compared with control (**$p<0.01$). ANOVA and Student–Newman–Keuls's as the *post hoc* test.

parameter time of permanence of animals on the bar [$F(4, 50) = 5.72$, $F(4,50) = 33.29$, $p<0.001$], when compared to control.

4. Discussion

In the present work, the behavior effects of 1,4-cineole, a monoterpene ether, were investigated on classic animal models of CNS actions, such as EPM, open field, hole board, forced swimming, tail suspension, barbiturate-induced sleeping time and rota rod tests. Animal models of anxiety and depression are typically based on exposure of animals to a stressful condition (a potential or actual threatening situation) and a specific test for measuring behavioral and physiological responses (Palanza, 2001). The EPM test is considered one of the most widely validated tests for assaying new benzodiazepine-like anxiolytic agents (Pellow et al., 1985; Ohl, 2003). This test is based on the observation that rodents tend to avoid elevated areas and, therefore avoidance of the open arms in EPM test is interpreted as anxiety behavior (Ohl, 2003). Our results showed that 1,4-cineole at dose of 400 mg/kg was able to increase significantly all the parameters (PEOA, NEOA, PTOA and TPOA) in the EPM test, as compared to the control group. Similar results were also observed with the diazepam treated group at a recognized anxiolytic dose (1 mg/kg), suggesting an anxiolytic-like effect from 1,4-cineole.

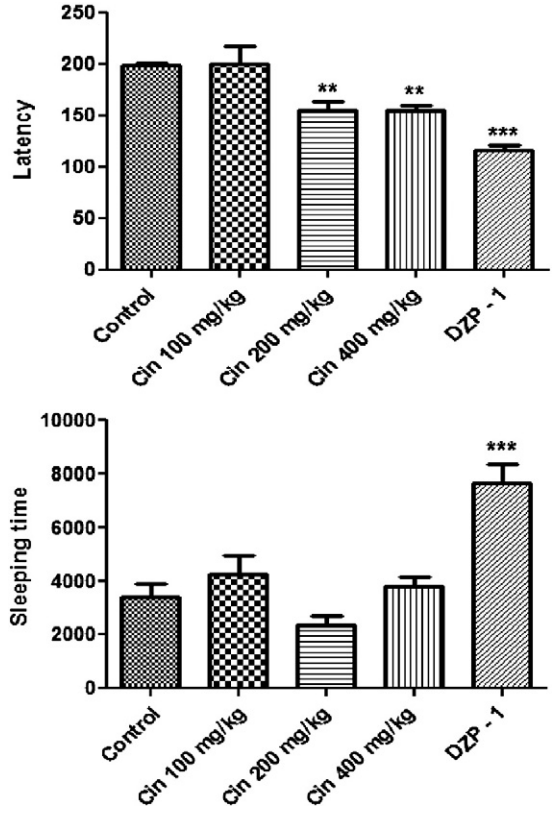

Fig. 6. Pentobarbital sleeping time test of groups of mice which received vehicle, 1,4-cineole (Cin100, 200 and 400 mg/kg), and diazepam (DZP 1 mg/kg). The parameters analyzed were the latency time and the sleeping time. The results are presented as means ± S.E.M. Significant difference compared with control (**$p<0.01$, ***$p<0.001$). ANOVA and Student–Newman–Keuls's as the *post hoc* test.

In order to elucidate the mechanism of the anxiolytic effect of 1,4-cineole, we decided to use flumazenil, an antagonist of benzodiazepine drugs. Our results showed that the effect of 1,4-cineole was not reversed by the administration of flumazenil. On the other hand, the effect of diazepam was antagonized by flumazenil. This indicates that the anxiolytic effect of 1,4-cineole does not involve benzodiazepine receptors.

In order to further corroborate the anxiolytic activity observed in the EPM test, we also used the hole-board test, in which exploration is also gradually inhibited by anxiety (Crawley, 1985). Similar to EPM, this test is also useful for modeling anxiety and anxiolytic agents have been shown to increase the number of head dips (Takeda et al., 1998). Our results showed that 1,4-cineole at dose of 400 mg/kg significantly increased the number of head dips, indicating an anxiolytic-like effect.

The tests described above are only predictive of a narrow spectrum of behavioral patterns. Consequently, additional tests are recommended to control for possible confounding factors, e.g. locomotor activity (Ohl et al., 2001; Escorihuela et al., 1999). The open-field test allows measurements by which it is possible to evaluate autonomic effects of drugs and general activity of animals (Novas et al., 1988). This test utilizes behavioral changes in rodents exposed to a novel environment and has been used to detect anxiogenic and anxiolytic activity under identical situations (Bhattacharya and Satyan, 1997), although some studies also report a lack of sensitivity for anxiety-modulation from this model (Saudou et al., 1994).

Several studies have reported that anxiolytic dose of diazepam facilitates exploratory behavior expressed as increased head dips and locomotion in the EPM and open field (Bhattacharya and Mitra, 1991; Ramanathan et al., 1998). Our findings showed that the animals treated with 1,4-cineole 100 and 200 mg/kg did not induce changes in locomotion of mice in the open-field arena, while dose of 400 mg/kg showed an evident increase in this parameter, although this change was not significant. Also, diazepam at an accepted sedative dose (2 mg/kg) decreased the locomotion of mice in the arena, showing a significant sedative effect. Taken together, our results observed in EPM, hole board

and open-field tests suggest that 1,4-cineole 400 mg/kg has anxiolytic-like effects without inducing significant sedative action in animals.

Considering the above, we decided, in addition, to investigate the activity of 1,4-cineole in depressed animal models. On the basis of the clinical association of depressive episodes and stressful life events, many of the animal models for the evaluation of antidepressant drug activity assess stress-precipitated behaviors (Sousa et al., 2004). The two most widely used animal models for antidepressant screening drugs are the forced swimming (Porsolt et al., 1978) and tail suspension (Steru et al., 1985) tests. Briefly, when mice are forced to swim or are hung upside down by the tail in an inescapable situation, they tend to become immobile after initial vigorous activity. Moreover, substances that decrease immobility often have antidepressant properties in humans. This immobility has been described as a symptom of "behavioral despair," and both tests have been suggested as animal models of human depression, although this is open to considerable argument (Karolewiaz and Paul, 2001). In the present study, a significant increase in immobility time was observed in both forced swimming (200 and 400 mg/kg) and tail suspension (400 mg/kg) tests, indicating depressant activity from 1,4-cineole, opposite to the effects promoted by imipramine, a classical antidepressant drug (Porsolt et al., 1977; Willner, 1990).

A deficit in motor coordination would very likely affect performance in the forced swimming and tail suspension tests. In this way, we aimed to investigate the effects of 1,4-cineole in the rota rod test, a classical animal model used to evaluate peripheral neuromuscular blockage. Our findings showed that 1,4-cineole (100, 200 and 400 mg/kg), different from diazepam (2 mg/kg), had no significant effect on the motor coordination of the animals in this test. Thus, the observed increase in the immobility time probably is not related to peripheral neuromuscular blockage, but may involve neurons that control central depressant activity (Adzu et al., 2002; Silva et al., 2007).

Pentobarbital sleeping time test was used to confirm or not the possible depressive-like effects observed with 1,4-cineole in tests above. Decrease in sleep latency and increase in sleeping time are classically related to central nervous system (CNS) depressant drugs (Willianson et al., 1996). The present results showed that 1,4-cineole (at all doses tested) significantly decrease the sleep latency, but did not change the duration of the sodium pentobarbital-induced hypnotic effect, suggesting a possibly sedative effect. However, this effect was not corroborated in the open-field test in a significant way, suggesting that 1,4-cineole possibly was able to potentiate the pentobarbital effects showing its depressive potential, but it may not be able to induce sedative effect itself in the doses tested. Nevertheless, it is possible hypothesize that a higher dose of cineole is able to induce significant sedative effects.

In summary, the present study showed that acute administration of 1,4-cineole presented anxiolytic-like effects in the elevated plus maze and hole-board tests and this effect was not related to benzodiazepine receptors. This effect was also devoid of significant sedative effect as assessed by the open-field test. Parameters observed in the forced swimming, tail suspension and pentobarbital sleeping time tests support the idea that 1,4-cineole possibly presents depressor activity on the central nervous system. These findings indicate that 1,4-cineole present potential anxiolytic activity, however this effect requires further studies in order to investigate the dose–response relationship considering also its CNS depressor potential. Also, studies are required in order to elucidate the exact mechanism involved in the cineole actions, as well as to investigate the toxicity from this monoterpene.

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References

Adzu B, Amos S, Muazzam I, Inyang US, Gamaniel KS. Neuropharmacological screening of Diospyros mespiliformis in mice. J Ethnopharmacol 2002;83:139–43.
Almeida RN, Navarro DS, Barbosa-Filho JM. Plants with central analgesic activity. Phytomed 2001;8:310–22.
Almeida RN, Motta SC, Leite JR. Óleos Essenciais com atividades anticonvulsivantes. Bol Latinoam Caribe Plantas Med Aromat 2003;2:3–6.
Amaral JF, Silva MIG, Aquino Neto MR, Teixeira Neto PF, Moura BA, Melo CTV, et al. Antinociceptive effect of the monoterpene R-(+)-limonene in mice. Biol Pharm Bull 2007;30:1217–20.
Archer J. Tests for emotionality in rats and mice: a review. Anim Behav 1973;21: 205–35.
Asakawa Y, Toyota M, Ishida T. Biotransformation of 1, 4-cineole, a monoterpene ether. Xenobiotica 1988;18:1129–34.
Bhattacharya SK, Mitra SK. Anxiolytic activity of *Panax gingeng* roots: an experimental study. J Ethnopharmacol 1991;34:87–92.
Bhattacharya SK, Satyan KS. Experimental methods for evaluation psychotropic agents in rodents: I-Anti-anxiety agents. Indian J Exp Biol 1997;35:565–75.
Clark G, Koster AG, Person DW. Exploratory behavior in chronic disulfoton poisoning in mice. Psychopharmacol 1971;20:169–71.
Crawley JN. Exploratory behavior models of anxiety in mice. Neurosci Behav Rev 1985;9:37–44.
Dunham NW, Miya TS. A note on a simple apparatus for detecting neurological deficits in rats and mice. J Am Pharm Assoc 1957;46:208–9.
Escorihuela RM, Fernandez-Teruel A, Gil L, Aguilar R, Tobena A, Driscoll P. Inbred Roman high- and low-avoidance rats: differences in anxiety, novelty-seeking, and shuttlebox behavior. Physiol Behav 1999;67:19–26.
Hummel T, Futschik T, Frasnelli J, Huttenbrink KB. Effects of olfactory function, age, and gender on trigeminally mediated sensations: a study based on the lateralization of chemosensory stimuli. Toxicol Lett 2003;140–141:273–80.
Joanne GR, Stephen OD, Franck ED. Inhibition of plant asparagine synthetase by monoterpene cineoles. Plant Physiol 2000;123:725–32.
Karolewiaz B, Paul IA. Group housing of mice increases immobility and antidepressant sensitivity in the forced swim and tail suspension tests. Eur J Pharmacol 2001;415: 197–201.
Khalil Z, Pearce AL, Satkunanathan N, Storer E, Finlay-Jones JJ, Hart PH. Regulation of wheal and flare by tea tree oil: complementary human rodent studies. J Invest Dermatol 2004;123:683–90.
Lahlou S, Figuereido AF, Magalhães PJC, Leal-Cardoso JH. Cardiovascular effects of 1,8-cineole, a terpenoid oxide present in many plant essential oils, in normotensive rats. Can J Physiol Pharmacol 2002;80:1125–30.
Lis-Balchin M, Hart S. Studies on the mode of action of the essential oil of lavender (*Lavandula angustifolia* P. Miller). Phytother Res 1999;13:540–2.
Lister RG. The use of a plus-maze to measure anxiety in the mouse. Psychopharmacol 1987;92:180–5.
Magalhães PJC, Lahlou S, Vasconcelos-dos-Santos MA, Pradines TL, Leal-Cardoso JH. Myorelaxant effects of the essential oil of *Croton nepetaefolius* on the contractile activity of the guinea-pig tracheal smooth muscle. Planta Med 2003;69:874–7.
Nikitin ES, Balaban PM. Optical recording of odor-evoked responses in olfactory brain of the naïve and adversity trained terrestrial snails. Learn Mem 2000;7:422–32.
Novas ML, Wolfman C, Medina JH, De Robertis E. Proconvulsant and anxiogenic effects of n-butyl-β-carboline-3-carboxilate on endogenous benzodiazepine binding inhibitor from brain. Pharmacol Biochem Behav 1988;30:331–6.
Ohl F. Testing for anxiety. Clin Neurosci Res 2003;3:233–8.
Ohl F, Toschi N, Wigger A, Henniger MSH, Landgraf R. Dimensions of emotionality in an animal model of inborn hyperanxiety. Behav Neurosci 2001;115:429–36.
Palanza P. Animal models of anxiety and depression: how are females different? Neurosci Biobehav Rev 2001;25:219–33.
Paula AC, Toma W, Gracioso JS, Hiruma-Lima CA, Carneiro EM, Souza Brito AR. The gastroprotective effect of the essential oil of *Croton cajucara* is different in normal rats than in malnourished rats. Br J Nutr 2006;96:310–5.
Pellow S, Chopin P, File SE, Briley M. Validation of open: closed arm entries in an elevated plus maze as a measure of anxiety in the rat. J Neurosci Methods 1985;14: 149–67.
Porsolt RD, Bertin A, Jalfre M. Behavioral despair in mice: a primary screening test for antidepressants. Arch Int Pharmacodyn Thér 1977;36:229–327.
Porsolt RD, Anton G, Blavet N, Jafre M. Behavioral despair in rats: a new model sensitive to antidepressant treatments. Eur J Pharmacol 1978;47:339–79.
Ramanathan M, Jaiswal AK, Bhattacharya SK. Differential effects of diazepam on anxiety in streptozotocin induced diabetic and non-diabetic rats. Psychopharmacology 1998;135:361–7.
Rolland A, Fleurentin J, Lanhers MC, Younos C, Misslin R, Mortier F, et al. Behavioural effects of the American traditional plant *Eschscholzia californica*: sedative and anxiolytic properties. Planta Med 1991;57:212–6.
Romagni JG, Duke SO, Dayan FE. Inhibition of plant asparagine synthetase by monoterpene cineoles. Plant Physiol 2000;123:725–32.
Rosazza JPN, Steffens JJ, Sariaslani FS, Goswami A, Beale JM, Reeg S, et al. Microbial hydroxylation of 1,4-cineole. Appl Environ Microbiol 1987;53:2482–6.
Santos FA, Rao VS. Anti-inflammatory and antinociceptive effects of 1,8-cineole a terpenoid oxide present in many plant essential oils. Phytother Res 2000;14:240–4.
Saudou F, Ait Amara D, Dierich A, LeMeur M, Ramboz S, Segu L, et al. Enhanced aggresive behavior in mice lacking 5-HT1b receptor. Science 1994;265:1875–8.
Silva MI, de Aquino Neto MR, Teixeira Neto PF, Moura BA, do Amaral JF, de Sousa DP, et al. Central nervous system activity of acute administration of isopulegol in mice. Pharmacol Biochem Behav 2007;88:141–7.

Sousa FCF, Melo CTV, Monteiro AP, Lima VTM, Gutierrez SJC, Pereira BA, et al. Antianxiety and antidepressant effects of riparin III from *Aniba riparia* (Nees) Mez (Lauraceae) in mice. Pharmacol Biochem Behav 2004;78:27–33.

Sousa FCF, Pereira BA, Lima VT, Lacerda CDG, Barbosa-filho JM, et al. Central nervous system activity of Yangambin from *Ocotea duckei* Vattimo (Lauraceae) in mice. Phytother Res 2005;19:282–6.

Steru L, Chermat R, Thierry B, Simon P. Tail suspension test: a new method for screening antidepressants in mice. Psychopharmacol 1985;85:367–70.

Takeda H, Tsuji M, Matsumiya T. Changes in head-dipping behavior in the hole-board test reflect the anxiogenic and/or anxiolytic state in mice. Eur J Pharmacol 1998;350:21–9.

Wambebe C. Influence of some agents that affect 5-hydroxy-tryptamine metabolism and receptors on nitrazepam-induced sleep in mice. Braz J Pharmacol 1985;84:185–91.

Willianson E, Okpako D, Evans FJ. Selection, preparation and pharmacological evaluation of plant material. Chichester: Wiley; 1996.

Willner P. Animals model of depression: an overview. Pharmacol Ther 1990;45:425–55.