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Stimulative and Sedative Effects of Essential Oils upon Inhalation in Mice

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This study investigated the stimulative or sedative effects of inhaling fragrant essential oils (EOs) by using a forced swimming test (FST) with mice. This behavioral test is commonly used to measure the effects of antidepressant drugs. The inhalation by mice of EOs, such as ginger oil ($p<0.05$), thyme oil ($p<0.05$), peppermint oil ($p<0.05$), and cypress oil ($p<0.01$) resulted in 5% to 22% reduction of immobility. The same results were achieved when over-agitation was artificially induced in the mice by an intraperitoneal injection of caffeine (a psycho-stimulant). In contrast, inhalation of some EOs by the mice resulted in increased immobility. To evaluate more correctly the sedative effects of EOs, the immobility of over-agitated mice induced with caffeine was ascertained after the inhalation of various EOs. Inhalation of lavender oil ($p<0.01$) and hyssop oil ($p<0.01$) increased the immobile state in mice that were treated with caffeine. The results of this study indicate that the inhalation of essential oils may induce stimulative or sedative effects in mice.

Key words: Essential oil, Sedative effect, Stimulative effect, Forced swimming test, Fragrance, Mice

INTRODUCTION

A variety of plant-derived essential oils (EOs) have traditionally been used in the treatment of numerous mental disorders. Presently, aromatherapy has spread worldwide, despite that these therapeutic treatments have been lacking a scientific basis for the effectiveness of EOs.

It has been reported that inhalation of fragrances has induced sedative or stimulative effects on humans. However, experimental references under standardized conditions to prove or disprove the effects of fragrant compounds by way of inhalation are rarely found in scientific literary journals. Only a few papers have reported on these properties for some fragrant compounds and on their effects upon humans and animals. The effects of a fragrance on brain function have been studied by using alpha and theta activity in the electro encephalogram (EEG, Lorig and Schwarts, 1988) or by using the contingent negative variation (CNV, Manley, 1993). These studies have shown that some fragrances exert stimulant or inhibitory effects on brain function. Moreover, it has been reported that inhalation of essential oils modulates sympathetic activity in normal adults, which is represented by low frequency amplitude of the systolic blood pressure (Haze *et al.*, 2002).

Alternatively, there have been several studies using behavioral methods on animals for testing that EOs or fragrant compounds possess stimulative or sedative effects. Inhalation and oral administration of rosemary oil increased locomotor activity in mice (Kovar *et al.*, 1987). A series of fragrant compounds and essential oils induced sedative effects upon inhalation, which was tested by assessing the motility of the mice (Buchbauer *et al.*, 1993). A similar sedative result was achieved in mice by assessing their accumulative spontaneous motor activity, when they inhaled cedrol, the main component of cedar wood oil (Kagawa *et al.*, 2003). In addition, intraperitoneal administration in mice of peppermint oil increased an ambulation-promoting effect (Umezu *et al.*, 2001). However, those experimental procedures on animals were not suitable for the primary screening of fragrant compounds and EOs. What is needed for the primary screening of

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fragrant compounds and EOs is a standardized experimental procedure that produces more rapid results and is less expensive.

The aim of this study was to screen and quantify a series of EOs for their sedative or stimulative effects by using a forced swimming test (FST), and we also wanted to prove or disprove the effectiveness of the FST as a standardized experimental procedure. The FST evokes a characteristic behavioral immobility by exposing rodents to a swimming session within a confined cylinder. Because antidepressant drugs reduce the duration of immobility in the FST, the FST has become the most extensively used test for measuring the behavioral effects of antidepressant drugs (Porsolt *et al.*, 1977a, 1977b; Redrobe and Bourin, 1996; Lucki *et al.*, 2001). Porsolt *et al.* (1977b) confirmed that all major classes of antidepressants and also the psycho-stimulants (d-amphetamine and caffeine) reduced immobility. Moreover, it has been reported that inhalation of fragrant compounds and EOs had the same stimulative effects as caffeine on the motility of mice (Buchbauer *et al.*, 1993).

In an attempt to search for the stimulative or sedative effects of the inhalation of essential oils and fragrant compounds, this study has investigated the behavior of mice by using the forced swimming test.

MATERIALS AND METHODS

Materials

As test samples, this study used 21 EOs: basil oil, bergamot oil, black pepper oil, cedar wood oil, cinnamon bark oil, clary sage oil, clove bud oil, eucalyptus oil, fennel oil, ginger oil, grapefruit oil, hyssop oil, lavender oil, lemon oil, patchouli oil, peppermint oil, pine oil, rosemary oil, tea tree oil, thyme oil, and cypress oil (Mane, Grass, France). These EOs are widely used as fragrances in the cosmetics and in the food industries. One hour before the test, a 0.5 mL of 0.9% saline solution of caffeine was injected intraperitoneally (ip) into each mouse. The dosage of caffeine was 10 mg/kg per animal.

Animals

The subjects were 8- to 9-week-old female ICR mice that were housed in groups of four per cage (cage size: 20.0×26.0×13.0 cm) for at least 3 weeks prior to testing. The housing environment was under a controlled temperature of approximately 24°C, and 50% humidity. At 07:00 h each day, the lights were turned on for a 12 h light/dark cycle. Food and water were freely available. All subjects were used only once for testing purposes.

Forced swimming test

All experimental sessions were conducted between 13:00 and 16:00 hours. Swimming sessions were conducted by placing each mouse in an individual glass cylinder (30 cm tall×15 cm in diameter) filled with water at 25°C to a depth of 15 cm. The depth was deep enough so that the mouse could not support itself by placing its paws on the base of the cylinder. The procedure was essentially similar to that described by Porsolt *et al.* (1977b). The duration of immobility of the mouse was ascertained after the inhalation of the essential oil to evaluate the stimulative effect compared with the effect of caffeine. This study ascertained the immobility of over-agitated mice by injecting caffeine after the inhalation of a fragrance to evaluate its sedative effect.

Fragrance inhalation

The smelling cage contained a piece of 2×10 cm filter paper that was used to absorb and hold the volatile EOs. Ten minutes before the test, the animals were put into the smelling cage to inhale the EOs. The cage was made airtight with a transparent plastic case. After preinhalation of the EOs for 10 minutes, the swimming sessions were carried out. The mouse was placed in an individual glass cylinder that was also made airtight with a transparent plastic cover. A 2×10 cm filter paper was used for the inhalation of the EOs during the swimming test. The EOs were refreshed between the experiments when using a different subject.

Behavioral scoring

A six minute test period duration was employed. The duration of immobility was scored during the last 5 minutes of the test period. A mouse was judged to be immobile when it was making only those movements that were necessary to keep its head above water. The water was changed between subjects.

Statistical analysis

All values obtained are represented as the mean ± SE, and the number of animals is represented as n. For analysis of significance of the differences, Student's t-test was used. P values <0.05 were considered as significant.

RESULTS AND DISCUSSION

Some essential oils are believed to be effective for the treatment of nervous disorders and mental fatigue (Tisserand, 1993), and they might possess a similar action as psycho-stimulants. It has been reported that an intraperitoneal administration of peppermint oil and its constituent elements to mice increased their ambulatory activity (Umezu *et al.*, 2001). A similar result has been reported that caffeine also increased ambulatory activity in mice (Kuribara, 1994). Also, Porsolt *et al.* (1977b) report-

Table I. Effects of the inhalation of fragrance on the duration of the immobility in mice

Duration of Immobility Mean ± S.E [sec] (n)				
Test Group	Odorant	Control Group	% of Control	t-Test
251.00 ± 3.11 (4)	Peppermint	266.50 ± 4.97 (4)	−5.82%	p<0.05
217.50 ± 18.82 (4)	Thyme	282.00 ± 3.46 (4)	−22.87%	p<0.05
235.75 ± 8.50 (4)	Ginger	265.75 ± 5.66 (4)	−11.29%	p<0.05
236.00 ± 6.03 (4)	Cypress	283.75 ± 1.75 (4)	−16.83%	p<0.01
285.75 ± 3.73 (4)	Teatree	280.00 ± 8.42 (4)	+2.05%	ns
255.5 ± 10.21 (4)	Rosemary	243.5 ± 15.47 (4)	+4.93%	ns
276.50 ± 4.92 (4)	Pepper	266.00 ± 7.81 (4)	+3.94%	ns
256.75 ± 14.59 (4)	Neroli	261.25 ± 16.72 (4)	−1.72%	ns
256.75 ± 9.94 (4)	Lemon	248.75 ± 7.00 (4)	+3.21%	ns
265.00 ± 16.20 (4)	Bergamot	250.25 ± 5.39 (4)	+5.89%	ns
266.00 ± 6.86 (4)	Pine	253.00 ± 6.48 (4)	+5.14%	ns
279.25 ± 8.93 (4)	Eucalyptus	272.25 ± 11.49 (4)	+2.57%	ns
276.22 ± 14.05 (4)	Basil	262.50 ± 5.20 (4)	+5.22%	ns
271.25 ± 5.15 (4)	Clove	277.75 ± 5.66 (4)	−2.34%	ns
268.50 ± 14.20 (4)	Cedarwood	269.00 ± 9.48 (4)	0.00%	ns
279.75 ± 4.75 (4)	Lavender	254.50 ± 7.98 (4)	+9.92%	p<0.05
277.75 ± 4.64 (4)	Cinnamon	259.67 ± 7.57 (4)	+6.96%	p<0.05
275.67 ± 5.90 (4)	Clarysage	238.00 ± 6.43 (4)	+15.82%	p<0.05
297.50 ± 1.50 (4)	Fennel	283.50 ± 1.26 (4)	+4.94%	p<0.01
300.00 ± 0.00 (4)	Hyssop	271.00 ± 3.49 (4)	+10.70%	p<0.01
267.75 ± 2.87 (4)	Grapefruit	257.00 ± 2.00 (4)	+4.18%	p<0.05
228.08 ± 3.55 (13)	Caffeine	269.75 ± 6.43 (8)	−15.45%	p<0.01

The data are represented as means ± S.E. [sec] from all animals. The number of animals in each group is indicated in parentheses. Caffeine (10 mg/kg) was injected 0.5 mL per animal intraperitoneally 1 hour before the test.

ed that d-amphetamine and caffeine (psycho-stimulants) reduced the duration of immobility in the FST, but minor tranquilizers and major tranquilizers did not. Based on these studies, this study selected the forced swimming test as the test to screen and quantify a series of EOs on their sedative or stimulative effects. To the best of our knowledge, there is no previous study of EOs on the FST in mice.

The duration of immobility was calculated to demonstrate potent stimulative effects after inhalation of EOs. As listed in Table I, ten essential oils have been proven to increase or decrease the duration of immobility in mice. The inhalation of ginger oil, thyme oil, peppermint oil and cypress oil resulted in significantly decreasing the duration of immobility. The effects of the inhalation of those fragrances were −11.29%, −22.87%, −5.82%, and −16.83 %, respectively. Injecting 10 mg/kg of a 0.9% saline solution of caffeine into mice, decreased the duration of immobility by −15.45%. Thyme oil showed the most

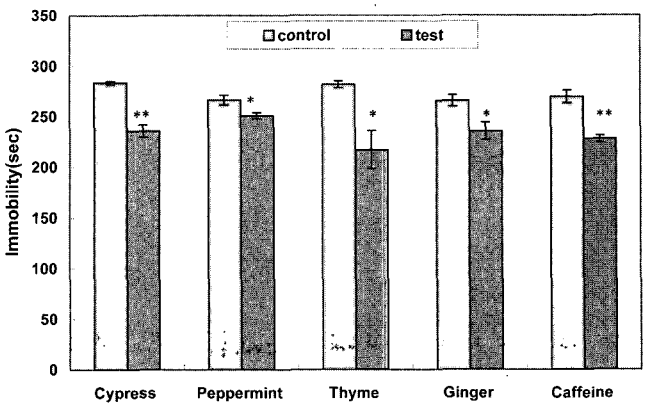

Fig. 1. Stimulative effects induced by inhalation of EOs. The data are represented as mean ± S.E. [sec] from all animals. *p<0.05, **p<0.01. Caffeine (10 mg/kg) was injected 0.5 mL per animal intraperitoneally 1 hour before the test.

stimulative effects, even more than the caffeine treated group. Fig. 1 shows the decrease of the duration of immobility by inhaling the fragrance of the EOs and the intraperitoneal injection of caffeine.

In contrast, inhalation of some essential oils resulted in the increase of immobility in mice. To more correctly evaluate the sedative effects of essential oils, the immobility of over-agitation in mice was ascertained by injecting caffeine. Inhalation of lavender oil and hyssop oil increased the duration of immobility of caffeine treated mice (Table II). Caffeine induced a decrease of 15.45% in the duration of immobility in mice. However, when the caffeine-treated mice were exposed to lavender and hyssop oils, the duration of immobility decreased 3.12% and 3.32% with each other, which was similar to that of the caffeine-untreated mice. All the results are shown in Fig. 2. This suggests that lavender and hyssop oil have

Table II. Effects of the inhalation of a fragrance on the duration of immobility in caffeine treated mice

Duration of Immobility after Caffeine Mean ± S.E. [sec] (n)				
Test Group	Odorant	Control Group	% of Control	t-Test
228.08 ± 3.55 (13)	Caffeine	269.75 ± 6.43 (8)	−15.45%	p<0.01
261.33 ± 1.76 (4)	Lavender	269.75 ± 6.43 (8)	−3.12%	p<0.01
260.80 ± 7.71 (5)	Hyssop	269.75 ± 6.43 (8)	−3.32%	p<0.01
235.38 ± 6.30 (8)	Clarysage	269.75 ± 6.43 (8)	−12.74%	ns
227.00 ± 7.70 (7)	Cinnamon	269.75 ± 6.43 (8)	−15.85%	ns
240.25 ± 11.87 (8)	Fennel	269.75 ± 6.43 (8)	−10.94%	ns
221.30 ± 8.56 (10)	Grapefruit	269.75 ± 6.43 (8)	−17.96%	ns

The data are represented as mean ± S.E. [sec] from all animals. The number of animals in each group is indicated in parentheses. Caffeine (10 mg/kg) was injected 0.5 mL per animal intraperitoneally 1 hour before the test.

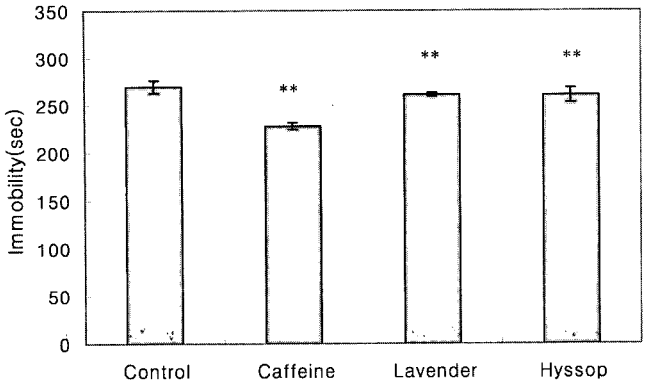

Fig. 2. Sedative effects induced by inhalation of EOs. The data are represented as mean ± S.E. [sec] from all animals. **p<0.01. Caffeine (10 mg/kg) was injected 0.5 mL per animal intraperitoneally 1 hour before the test.

sedative effects.

A similar result has been reported that lavender oil decreased the motility of mice after a 1 hour inhalation of the fragrance and it was able to compensate for the caffeine-induced over-agitation (Buchbauer *et al.*, 1993). Buchbauer *et al.* (1993) also reported that linalool and linalyl acetate, which are the main component ingredients of lavender oil, had the same effects as lavender oil. Moreover, they reported that thymol, the main component ingredient of thyme oil, increased motility in mice. The present study revealed a decrease in the immobility of the mice being tested for the effects of thyme oil. This suggests that the stimulative effects of thyme oil may be due to the thymol. In this connection, the data of Umezu *et al.* (2001) are of interest. They reported that intraperitoneal administration of natural peppermint oil and several of the main component ingredients caused a significant dosage dependent increase in ambulatory activity in mice. This is similar to our result for peppermint oil. Moreover, intravenous administration of those main component ingredients increased ambulatory activity more quickly than intraperitoneal administration. Kovar *et al.* (1987) reported on the inhalation or the oral administration of rosemary oil. As the blood level of 1,8-cineol, one of the constituents of rosemary oil, increased, it resulted in the increased locomotor activity of mice.

Based on these studies, one possible mechanism of stimulative and sedative effects of inhaling essential oils can be suggested in that the constituent elements of EOs produce their effect by acting on the central nervous system. When mice inhale the EOs, its constituent elements were absorbed into the blood stream. They pass through the blood-brain barrier, producing their effects on the nerves in the brain. This is similar to what is known about the effect of psychoactive drugs.

However, the study of Tsuchiya *et al.* (1991) showed another possible mechanism of how the inhalation of a fragrance works on the central nervous system. They reported that there were clear differences in the effects of the inhalation of an odorant. The sleep time of animals was induced by using pentobarbital. A zinc sulfate treatment was administered to create anosmic animals, while the olfactory animals were left intact. The pentobarbital sleep time was effected only by the odorant inhalation in the olfactory animals. The olfactory neurons have relatively direct connections to the hypothalamus, the cerebral limbic system and other central nervous areas. Thus, the inhalation of fragrances of various essential oils having stimulative or sedative effects can elicit memories or emotions that induce behavioral changes. However, more studies will be necessary to determine which mechanisms mediate the phenomena described in this paper.

In summation, based on the results of this study, it can be concluded that the forced swimming test is a useful experimental procedure in mice for the primary screening of essential oils in evaluating their stimulative or sedative effects. Using this experimental procedure, certain EOs have shown significant effectiveness in decreasing the duration of immobility in mice. Other essential oils decreased the duration of an induced over-agitation by the intraperitoneal application of caffeine. This result supports an aroma therapeutic hypothesis in that essential oils possess pharmacological effects on brain functions and it also suggests the possibility that those essential oils may be utilized as a mild regulator of nervous disorders in the cosmetics field.

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