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# Ambulation-promoting effect of peppermint oil and identification of its active constituents

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**Abstract**

Various plant-derived essential oils (EOs) have traditionally been used in the treatment of mental disorders, despite a lack of scientific evidence. In a previous study, we demonstrated that certain EOs possess behavioral effects, a finding that supports our original hypotheses that EOs possess psychoactive actions. The present study was conducted in order to obtain further evidence to support our hypothesis. Peppermint oil, a type of EO, is believed to be effective for treating mental fatigue. When the oil was administered intraperitoneally to ICR mice, the ambulatory activity of mice increased dramatically. We identified α-pinene, β-pinene, (*R*)-(+)-limonene, 1,8-cineol, isomenthone, menthone, menthol, (*R*)-(+)-pulegone, menthyl acetate and caryophyllene as constituent elements of peppermint oil by GC–MS analysis. We then examined the effect of each constituent element of peppermint oil on ambulatory activity in mice. Intraperitoneal administration of 1,8-cineol, menthone, isomenthone, menthol, (*R*)-(+)-pulegone, menthyl acetate and caryophyllene significantly increased ambulatory activity in mice, suggesting that these chemicals are the behaviorally active elements of peppermint oil. Intravenous administration of these substances to mice induced a significant increase in ambulatory activity at much lower doses. The present study provides further evidence demonstrating that EOs possess pharmacological actions on behavior. In addition, our finding revealed that the action of peppermint oil comes from its constituent elements. 

*Keywords:* Peppermint oil; Constituent elements; Ambulatory activity; Mice

## 1. Introduction

Various plant-derived essential oils (EOs) have traditionally been used in the treatment of a variety of mental disorders. The medicinal use of EOs began in the ancient Egyptian Era, and has continued ever since. The ''aromatherapy'' movement (Tisserand, 1993) has spread worldwide, despite the lack of scientific basis for the effectiveness of EOs. On the other hand, the long history of EOs in therapy suggests that they may indeed be effective. The odor of EOs is believed to be important for their effectiveness in treating various illnesses. However, we believed that it is unlikely that odor of EOs is sufficiently potent to treat illnesses because adaptation to odorant occurs quickly (Kurahashi and Menini, 1998). Therefore, the authors hypothesized that EOs possess pharmacological effects on brain function (i.e. psychoactive actions). This hypothesis was supported by our previous studies, in which certain types of EOs such as rose oil and lavender oil were shown to produce an antianxiety-like effect in animal experiments (Umezu, 1999, 2000). The present study attempted to gather further evidence to support our hypothesis.

Peppermint is a very popular herb that has been used in a variety of different ways. It possesses a unique and pleasant flavor, a fact that is likely the reason for its widespread use. Moreover, peppermint and its EO are also believed to be effective in the treatment of nervous disorders and mental fatigue (Tisserand, 1993), suggesting that they may exert some psychoactive actions. However, there is no scientific evidence to support this claim. Thus, the authors examined the effects of EO extracted from peppermint plant on animal behavior. The oil was expected to possess an excitatory effect given its use in the treatment of mental fatigue. Therefore, we examined the effect of peppermint oil on ambulatory activity in mice due to the

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fact that drugs that cause mental excitation (i.e. psychostimulants) are known to increase ambulatory activity in mice (Kuribara, 1994a,b, 1997; Kuribara and Tadokoro, 1983, 1984; Kuribara et al., 1992a).

Generally, the amounts of EOs used for aromatherapy are much larger than those used for adding flavor to foods and beverages. The textbook of aromatherapy (Tisserand, 1993) tells that two to five drops of EOs are enough for therapeutic use of them. However, there is no standard for the unit ''drop'', and the amount of a drop varies case by case (Olleveant et al., 1999). Therefore, the doses of EOs that have been used in aromatherapy are not known exactly, but almost homeopathic doses used by aromatherapists in their massage oil mixes in the UK ad USA rarely exceed 0.5 ml (ca. 500 mg) per massage. Some EOs are already used as orthodox medicines in Europe and USA. The use of peppermint oil and some components of EOs such as pinene, limonene, camphene and borneol given orally can cure certain internal ailments such as gallstones or ureteric stones. The doses of them sometimes exceed 45 ml/day in France and Germany (Balchin, 1997). In another case, 10 g of peppermint is reported to be enough to reduce experimentally induced pain in human subjects (Buckle, 1999). There is a case study (Burkhard, 1999) in which some EOs such as sage, eucalyptus, pine and thyme caused epileptic convulsion. The people used the EOs for the treatment of fatigue and respiratory disease, as usually made in Europe. This case study suggests that the doses of EOs usually used for medicinal purpose are enough to produce effects on brain functions. In addition, it is notable that already-known psychoactive drugs generally exhibit their apparent behavioral effects in animals at larger doses than the doses at which they produce psychoactive actions in human subjects. Thus, we examined effects of 100–800 mg/kg of peppermint oil in mice in this study.

EOs contain numerous different chemicals. Thus, we reasoned that if certain EOs possess behavioral actions, this effect must be due to one or more of its constituent elements. We initially observed an apparent effect of peppermint oil on ambulatory activity in mice, and thus further analyzed the tested peppermint oil using GC–MS, followed by behavioral tests on each constituent element to identify the behaviorally active elements of peppermint oil.

## 2. Methods

### 2.1. Animals

Male ICR mice (Clea Japan, Tokyo) were used for the behavioral experiment. The mice were 7–10 weeks old, weighed between 32 and 40 g and were housed in Plexiglas cages (10 mice/cage) with stainless-steel mesh tops and excelsior bedding (Clea Japan). Commercial solid food (Clea Japan) and tap water were available ad libitum. The animals were housed in a room artificially illuminated by fluorescent lamps on a 12L:12D schedule (light period: 07:00–19:00 hours), with the room temperature maintained at $25 \pm 1^{\circ}C$.

All experiments in this study were performed in accordance with the Ethics Committee for Experimental Animals of the National Institute for Environmental Studies, Japan.

### 2.2. Chemicals

We used natural peppermint oil extracted from *Mentha piperita* (Tisserand, 1993). The sample was produced by Maggie Tisserand (Brighton, UK).

The chemicals used as authentic standard substances in this study were α-pinene, β-pinene, (*R*)-(+)-limonene, 1,8-cineol, menthol and (*R*)-(+)-pulegone (purchased from Nacalai Tesque, Kyoto) and menthone, isomenthone, menthyl acetate and caryophyllene (gift from Nippon Terpene Chemical, Hiroshima). The EO of peppermint was diluted with olive oil (Wako, Osaka). Menthol was initially mixed with a small amount of Tween 80 (Nacalai Tesque), and then suspended in physiological saline (0.9% NaCl solution). Other chemicals were diluted with olive oil in the case of intraperitoneal administration. In the case of intravenous administration, all chemicals were mixed with a small amount of Tween 80 (final concentration was ca. 2% v/v) and then suspended in saline. These suspensions were sonicated well before injection. All injection volumes were 1 ml/100 g body weight regardless of dosage.

### 2.3. Experimental procedure

#### 2.3.1. Experiment 1. Effect of peppermint oil on ambulatory activity in mice

Mice were placed individually in activity cages, and after an adaptation of 30 min, olive oil (vehicle) or 100, 200, 400 or 800 mg/kg of peppermint oil was administered intraperitoneally. Following the injection, ambulatory activity was measured continuously for 2 h.

Ambulatory activity, a type of spontaneous motor activity in mice, was measured using a tilting-type ambulometer consisting of 10 bucket-like Plexiglas activity cages 20 cm in diameter (SAM-10, O'hara and Co., Tokyo) (Umezu et al., 1998). The details of this apparatus have been reported elsewhere (Hirabayashi et al., 1978).

#### 2.3.2. Experiment 2. Identification of constituent elements of peppermint oil

Peppermint oil tested in Experiment 1 was analyzed using GC–MS in order to identify its constituent elements.

Computerized GC–MS analysis was carried out on JMS 700 (JEOL) with HP 5890 II gas chromatograph (Hewlett-Packard). The GC was fitted with a fused silica capillary column (HP-5: 0.25 mm i.d. × 30 m) coated with 5% phenyl methyl silicone. The column temperature was

increased from 40°C to 250°C in 5°C/min increments. Peppermint oil and authentic standard substances were diluted with *n*-hexane, then injected into the GC in splitless mode. The injection temperature and separator temperature were 250°C and 260°C, respectively. Helium was used as the carrier gas and the flow rate was 1.0 ml/min. Mass spectrum analysis was performed in the electron impact ionization (EI) mode.

### *2.3.3. Experiment 3. Effects of intraperitoneal administration of constituent elements of peppermint oil on ambulatory activity in mice*

Animals were placed individually in activity cages, and after an adaptation of 30 min, vehicle or a chemical identified as a constituent element of peppermint oil were administered intraperitoneally. Following the injection, ambulatory activity was measured continuously for 1–2 h. The doses administered were α-pinene (200–1600 mg/kg), β-pinene (200–1600 mg/kg), (*R*)-(+)-limonene (200–3200 mg/kg), 1,8-cineol (200–1600 mg/kg), menthone (100–800 mg/kg), isomenthone (50–800 mg/kg), menthol (50–200 mg/kg), (*R*)-(+)-pulegone (100–800 mg/kg), menthyl acetate (200–1600 mg/kg) and caryophyllene (100–6400 mg/kg).

### *2.3.4. Experiment 4. Effects of intravenous administration of constituent elements of peppermint oil on ambulatory activity in mice*

Based on the hypothesis that constituent elements of peppermint oil produced their effects via peripheral stimulation, their effects on ambulatory activity were examined via intravenous administration. A suspension of one of the constituent elements was injected into the caudal vein of mice after 30-min adaptation to the activity cages, and ambulatory activity was then measured for 30 min. The doses administered were 1,8-cineol (10–40 mg/kg), menthol (10–40 mg/kg), menthone (10–20 mg/kg), isomenthone (20–40 mg/kg), (*R*)-(+)-pulegone (20–40 mg/kg) and menthyl acetate (20–40 mg/kg), respectively.

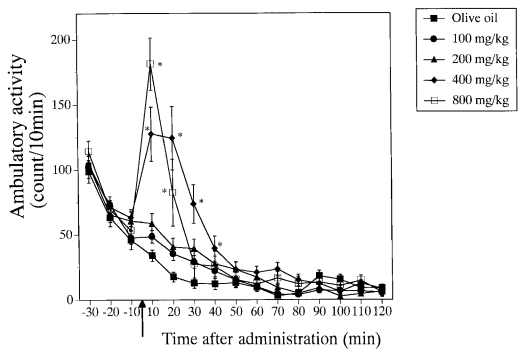


Fig. 1. Changes in ambulatory activity in mice after administration of natural peppermint oil. Symbols show mean values of ambulatory activity for each 10-min period, and vertical lines denote standard error of the mean (S.E.M.). Ambulatory activity after administration was analyzed by repeated-measures ANOVA, followed by Fisher's PLSD test (* $P<0.05$ compared to control (vehicle administration) values). The number of animals used for each dose was 20 ($N=20$).

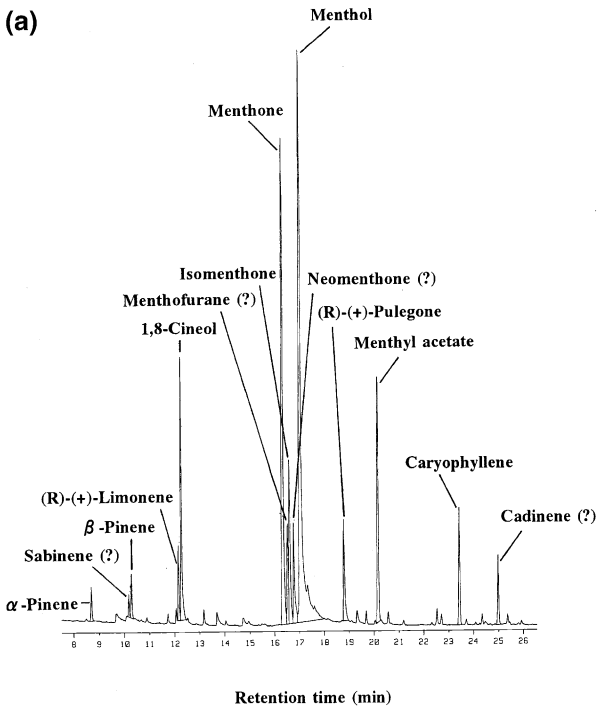


Fig. 2. Total ion chromatogram of peppermint oil obtained by GC–MS analysis (a). Mass spectra of each peak were analyzed and compared with the results of authentic standard substances obtained using the same GC–MS under the same conditions as for peppermint oil analysis. Following this procedure, chemicals at each peak were determined as shown in the figure. (?) indicates chemicals that were not confirmed due to the lack of their authentic standard substances. Mass spectra of these chemicals are shown in (b).

### *2.4. Statistical analyses*

Ambulatory activity after administration of peppermint oil or one of the constituent elements was first examined by repeated-measures analysis of variance (ANOVA), followed by Fisher's PLSD tests for each 10-min measurement. Five percent was established as the significant level.

## 3. Results

### *3.1. Experiment 1. Effect of peppermint oil on ambulatory activity in mice*

As shown in Fig. 1, peppermint oil at 400 mg/kg and higher dramatically increased the ambulatory activity of the mice. Repeated-measures ANOVA revealed that doses [$F(4,95)=7.56$, $P<.01$], time course [$F(11,1045)=72.67$, $P<.01$] and their interaction [$F(44,1045)=9.73$, $P<.01$] were statistically significant. Fisher's PLSD test for each 10-min measurement showed that ambulatory activity levels at 10–40 min after administration of 400 mg/kg were significantly higher than those in the control (vehicle treated)

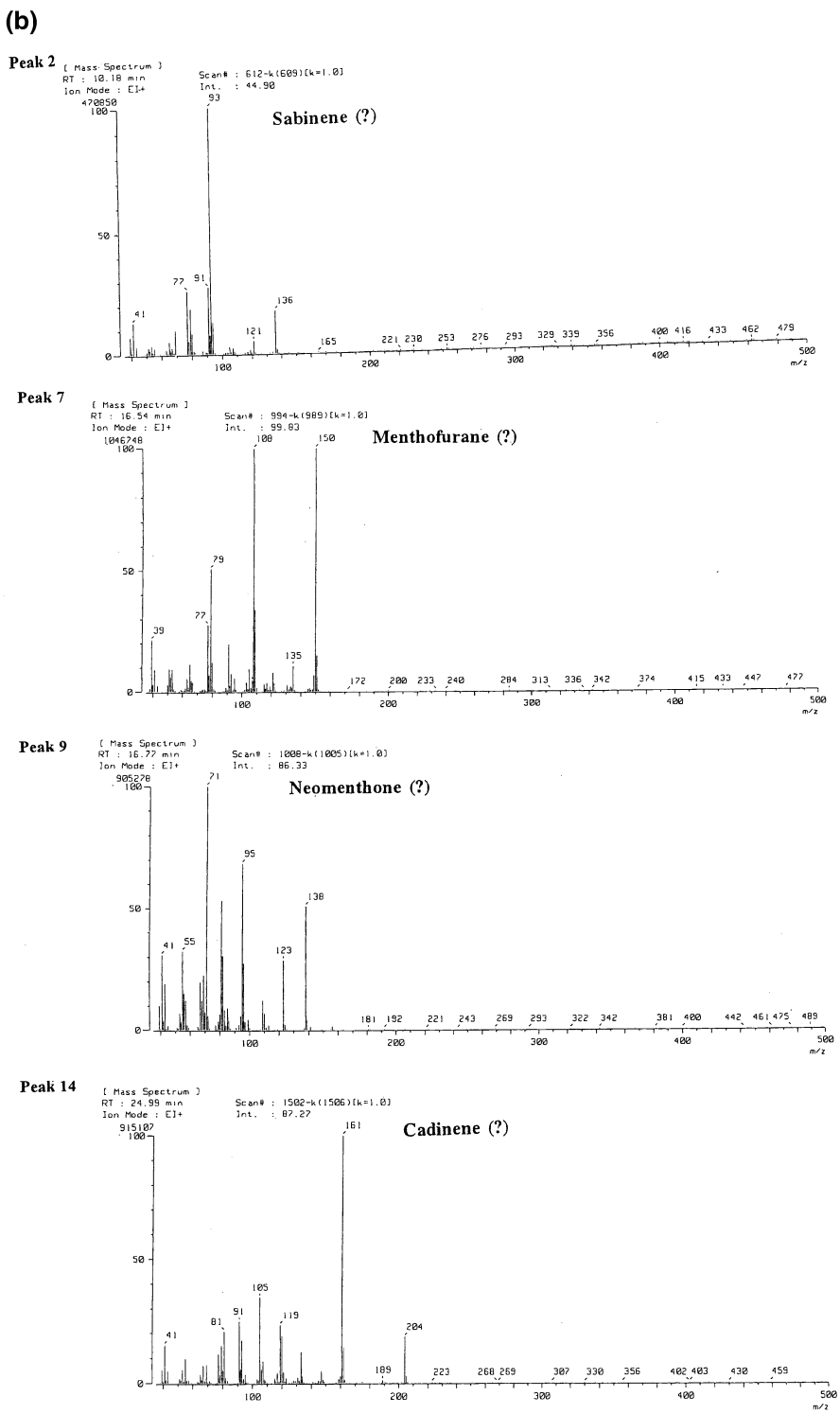


Fig. 2 (*continued*)

group at the same time point. Similarly, ambulatory activity levels at 10–20 min after administration of 800 mg/kg were significantly higher than those in the control group (Fig. 1). The effect of 800 mg/kg on the ambulatory activity seemed to disappear quickly. At that time, the mice exhibited ataxia, which was recoverable.

### *3.2. Experiment 2. Identification of constituent elements of peppermint oil*

Peppermint oil tested in Experiment 1 was analyzed using GC–MS. Total ion chromatography revealed 14 large peaks (Fig. 2(a)). The mass spectrum of each peak

was analyzed, followed by a search of the library of mass spectra of known chemicals. We then determined candidates for each constituent element of peppermint oil by comparing the known and observed spectra. Next, authentic standard substances for each candidate were analyzed using GC–MS under the same conditions as in the peppermint oil analysis and the results for the two analyses were compared. When the retention time on the total ion chromatogram and the mass spectrum pattern of each authentic standard substances were identical to one peak of peppermint oil, we concluded that the peppermint oil peak was that of the authentic standard substance. Following this procedure, the constituent elements of peppermint oil were identified Fig. 2(a). Sabinene, menthofurane, neomenthone and cadinene were likely also present in peppermint oil. However, it was impossible to confirm whether these substances were indeed constituent elements of peppermint oil because authentic standard substances for them were not available. The mass spectra of them are shown in Fig. 2(b).

The content of each constituent element was α-pinene (0.88%), sabinene (?) (0.50%), β-pinene (1.19%), (*R*)-(+)-limonene (1.98%), 1,8-cineol (7.58%), menthone (20.01%), menthofurane (?) (3.56%), isomenthone (6.16%), neomenthone (?) (3.79%), menthol (36.98%), (*R*)-(+)-pulegone (3.2%), menthyl acetate (8.43%), caryophyllene (3.67%) and cadinene (?) (2.05%), respectively.

### *3.3. Experiment 3. Effects of intraperitoneal administration of constituent elements of peppermint oil on ambulatory activity in mice*

The effects of each constituent element of peppermint oil determined in Experiment 2 on ambulatory activity in mice were examined in this experiment in order to behaviorally determine the active element(s) of peppermint oil.

All results are shown in Fig. 3. α-Pinene, β-pinene and (*R*)-(+)-limonene did not produce any apparent effect on ambulatory activity. In contrast, 1,8-cineol [dose: $F(4,95)=5.97$, $P<.01$; time course: $F(5,475)=139.57$, $P<.01$; interaction: $F(20,475)=14.77$, $P<.01$], menthone [dose: $F(4,95)=7.38$, $P<.01$; time course: $F(5,475)=9.56$, $P<.01$; interaction: $F(20,475)=1.53$, $P>.05$], isomenthone[dose: $F(5,111)=16.85$, $P<.01$; time course: $F(11,1221)=71.45$, $P<.01$; interaction: $F(55,1221)=5.54$, $P<.05$], menthol [dose: $F(3,72)=17.10$, $P<.01$; time course: $F(11,792)=80.03$, $P<.01$; interaction: $F(33,792)=14.11$, $P<.01$], (*R*)-(+)-pulegone [dose: $F(4,93)=7.96$, $P<.01$; time course: $F(11,1023)=69.94$, $P<.01$; interaction: $F(44,1023)=4.18$, $P<.01$], menthyl acetate [dose: $F(4,94)=11.37$, $P<.01$; time course: $F(11,1034)=9.58$, $P<.01$; interaction: $F(44,1034)=1.38$, $P>.05$] andcaryophyllene [dose: $F(7,121)=5.38$, $P<.01$; time course: $F(5,605)=88.66$, $P<.01$; interaction: $F(35,605)=2.00$,

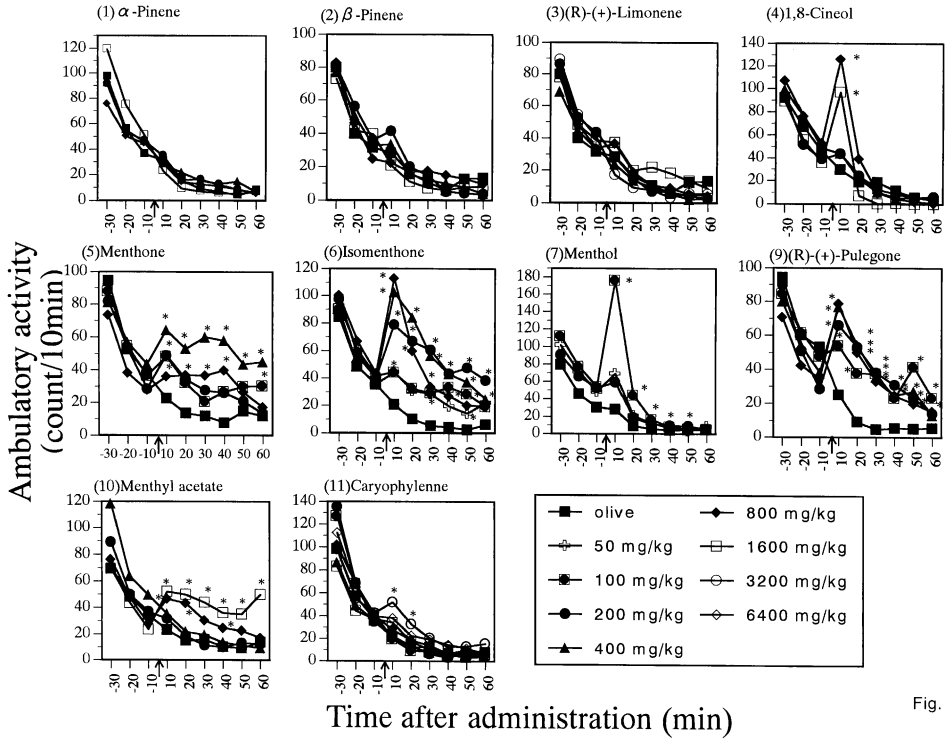


Fig.

Fig. 3. Changes in ambulatory activity in ICR mice after administration of (1) α-pinene ($N=20$), (2) β-pinene ($N=15-20$), (3) (*R*)-(+)-limonene ($N=19-20$), (4) 1,8-cineol ($N=20$), (5) menthone ($N=20$), (6) isomenthone ($N=19-20$), (7) menthol ($N=18-20$), (8) (*R*)-(+)-pulegone ($N=18-20$), (9) menthyl acetate ($N=20$) and (10) caryophyllene ($N=19-20$), which were identified as constituent elements of peppermint oil by GC–MS analysis. All data are shown as in Fig. 1.

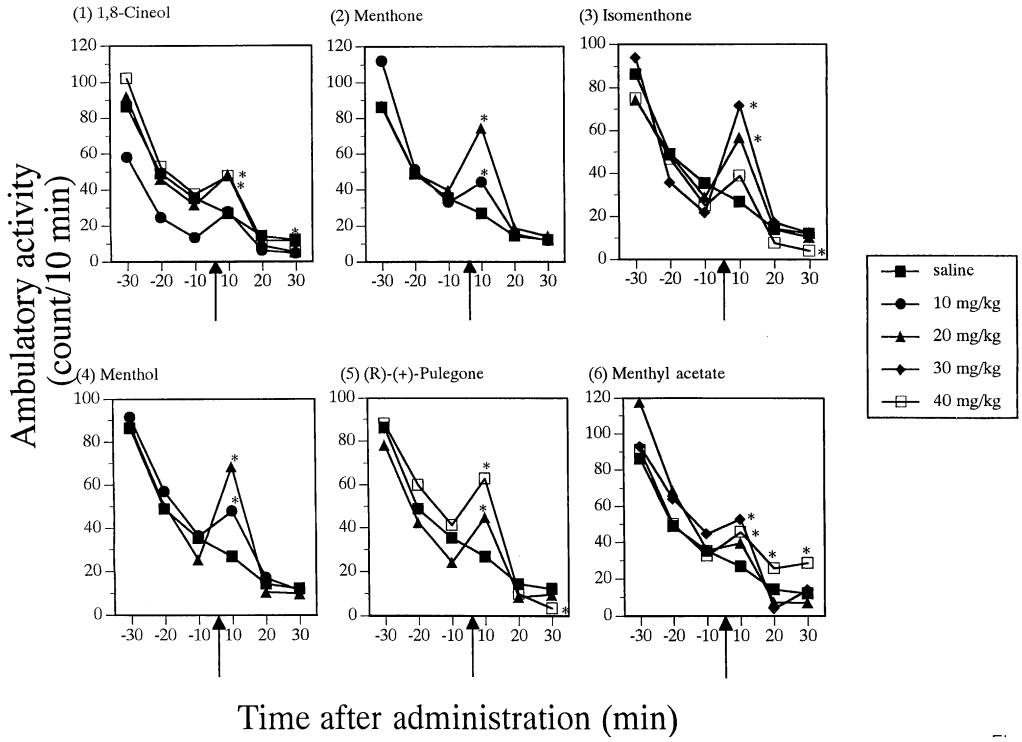


Fig. 4. Changes in ambulatory activity in ICR mice after intravenous administration of (1) 1,8-cineol ($N=9-47$), (2) menthone ($N=12-47$), (3) isomenthone ($N=9-47$), (4) menthol ($N=12-47$), (5) (*R*)-(+)-pulegone ($N=9-47$) and (6) menthyl acetate ($N=14-47$). All data are shown as in Fig. 1.

$P<.01$] significantly increased ambulatory activity in a dose-dependent manner (Fig. 3), as did peppermint oil.

Generally, these active constituent elements did not produce writhing, tremor or convulsion, stereotyped behaviors and catalepsy. At higher doses, these chemicals caused ataxia, as well as peppermint oil.

### *3.4. Experiment 4. Effects of intravenous administration of constituent elements of peppermint oil on ambulatory activity in mice*

Experiment 3 revealed that 1,8-cineol, menthone, isomenthone, menthol, (*R*)-(+)-pulegone, menthyl acetate and caryophyllene promote ambulation. We initially reasoned that these chemicals likely produce their effect via peripheral stimulation. Thus, we examined the effects of intravenous administration of these chemicals on ambulatory activity.

The results are shown in Fig. 4. When the effects of 1,8-cineol and (*R*)-(+)-pulegone on ambulatory activity were analyzed by repeated-measures ANOVA, the effects of doses were not statistically significant [1,8-cineol: dose: $F(3,94)=2.21$, $P>.05$; time course: $F(2,188)=110.19$, $P<.01$; interaction: $F(6,188)=8.38$, $P<.01$; (*R*)-(+)-pulegone: dose: $F(2,62)=2.49$, $P>.05$; time course: $F(2,124)=80.04$, $P<.01$; interaction: $F(4,124)=19.94$, $P<.01$]. However, Fisher's PLSD test revealed that effects at 20 and 40 mg/kg of 1,8-cineol and (*R*)-(+)-pulegone were statistically significant (Fig. 4(1),(5)). Menthone [dose: $F(2,68)=10.88$, $P<.01$; time course: $F(2,136)=93.23$, $P<.01$; interaction: $F(4,16)=23.13$, $P<.01$], isomenthone [dose: $F(3,75)=5.61$, $P<.01$; time course: $F(2,150)=75.54$, $P<.01$; interaction: $F(6,150)=10.08$, $P<.01$], menthol [dose: $F(2,68)=4.50$, $P<.05$; time course: $F(2,136)=67.92$, $P<.01$; nteraction: $F(4,136)=15.99$, $P<.01$], menthyl acetate [dose: $F(3,85)=3.95$, $P<.05$; time course: $F(2,170)=32.51$, $P<.01$; interaction: $F(6,170)=2.38$, $P<.05$] significantly increased ambulatory activity by intravenous administration (Fig. 4(2)–4),(6)). Fisher's PSLD test on these four substances confirmed that they produced significant effects on ambulatory activity in mice.

## 4. Discussion

The use of EOs in the treatment of various illnesses over the centuries suggests that these EOs might indeed have some degree of efficacy. The odor of EOs is believed to be important for their effectiveness in treating various illnesses. In fact, the odor of a given EO has been shown to change mood (Balchin, 1997). However, we believe it is unlikely that the odor of EOs is sufficiently potent to treat an illnesses because adaptation to odorant occurs quickly (Kurahashi and Menini, 1998). Therefore, we hypothesized that EOs exert a pharmacological action, a hypothesis supported by results from our laboratory (Umezu, 1999, 2000), in which different EOs, such as rose oil and lavender oil, produced anticonflict

effects in mice, which, in turn, suggests that they possess antianxiety effects. The present study attempted to gather further evidence to support our hypothesis.

Peppermint and its EO are believed to be effective in the treatment of nervous disorders and mental fatigue (Tisserand, 1993), suggesting that they may exert some psychoactive actions. However, to our knowledge, there is no scientific evidence to support this claim. Thus, we examined the effects of peppermint oil on behavior in mice. The specific hypothesis used to test for such pharmacological actions was guided by reports that it may be effective in the treatment of mental fatigue (Tisserand, 1993), suggesting that the oil might possess a similar action to psychostimulants. Thus, we examined the effect of peppermint oil on ambulatory activity in mice, given that drugs that cause mental excitation such as methamphetamine (Kuribara, 1997), cocaine (Kuribara, 1994b), caffeine (Kuribara, 1994a) and scopolamine (Kuribara and Tadokoro, 1983, 1984; Kuribara et al., 1992a) generally increase ambulatory activity in mice.

The present study revealed that intraperitoneal administration of natural peppermint oil, which is used for medicinal purposes in aromatherapy, caused a significant dose-dependent increase in ambulatory activity. This result demonstrated for the first time that peppermint oil produces an apparent effect on behavior in mice. There is a report on effects of EOs on spontaneous motor activity in rodent (Buchbauer et al., 1993). Generally, spontaneous motor activity decreased by EOs. To our knowledge, there is no previous study on the ambulation-promoting effect of EOs in mice. Peppermint oil produced the ambulation-promoting effect at 800 mg/kg, however, the effect at the dose seemed to disappear more quickly than at 400 mg/kg. At this time point, the animals exhibited ataxia. This is not unusual because it is known that some kinds of psychoactive drugs such as MK-801 produce the ambulation-promoting effect and ataxia in mice (Kuribara et al., 1992b).

Peppermint oil contains numerous different chemicals (Masada, 1975), and the behavioral effect observed in the present study was likely due to one or more of its constituent elements, the majority of which have been identified. However, it is also known that the type and content of constituent elements of EOs differ depending upon climate, the farm where the plants have been grown and the plant species. Therefore, we attempted to precisely identify the constituent elements of peppermint oil used in this study. GC–MS analysis is a standard method to examine the constituent elements of herb oils. Total ion chromatography revealed 14 large peaks, with the total percent area of these 14 peaks on the chromatogram being over 99%. Thus, these chemicals were determined to be the major constituent elements of peppermint oil.

Based upon mass spectrometry of each peak, the constituent elements of the oil were identified as α-pinene, β-pinene, (*R*)-(+)-limonene, 1,8-cineol, isomenthone, menthone, menthol, (*R*)-(+)-pulegone, menthyl acetate and caryophyllene, by a comparison with authentic standard substances. Sabinene, menthofurane, neomenthone and cadinene were likely also present in the peppermint oil we analyzed here, but it was impossible to confirm whether these substances were indeed constituent elements of the oil because authentic standard substances for them were not available. It has been reported that natural peppermint oil contains α-pinene, β-pinene, limonene, cineol, isomenthone, neomenthone, menthofurane, menthyl acetate, caryophyllene, pulegone and menthol (Masada, 1975). The results we obtained here agree well with this previous result of GC–MS analysis of peppermint oil.

The individual effect of each identified constituent element on ambulatory activity in mice was then examined using the same method as for peppermint oil. α-pinene, β-pinene and (*R*)-(+)-limonene did not produce any apparent effect on ambulatory activity. In contrast, 1,8-cineol, menthone, isomenthone, menthol, (*R*)-(+)-pulegone, menthyl acetate and caryophyllene significantly increased ambulatory activity in a dose-dependent manner, as did peppermint oil. Thus, we concluded that these chemicals are the behaviorally active elements of peppermint oil. We could not examine the effects of sabinene, menthofurane, neomenthone and cadinene because these chemicals were not available. Therefore, it is probable that other behaviorally active chemicals exist within peppermint oil, and the effects of these chemicals should be examined in future research. Based upon the extent of the constituent elements, 400 mg/kg of peppermint oil which produces an apparent ambulation-promoting effect contains 30.32 mg/kg of 1,8-cineol, 80.04 mg/kg of menthone, 24.64 mg/kg of isomenthone, 147.92 mg/kg of menthol, 12.8 mg/kg of (*R*)-(+)-pulegone, 33.72 mg/kg of menthyl acetate and 14.68 mg/kg of caryophyllene. According to the result of Experiment 3, 147.92 mg/kg of menthol can produce the ambulation-promoting effect. In addition, because 100 mg/kg of menthone can produce the effect significantly, 80.04 mg/kg of menthone is supposed to be enough to produce the ambulation-promoting effect. On the other hand, the amounts of 1,8-cineol, isomenthone, (*R*)-(+)-pulegone, menthyl acetate and caryophyllene in 400 mg/kg of peppermint oil are not enough to produce their effect by itself. However, it is probable that these constituent elements may enhance the effects of menthol and menthone in all by their synergism.

Doses of the constituent elements, which produced the ambulation-promoting effect, are larger than those of other psychostimulants such as methamphetamine, cocaine and caffeine. Therefore, neurotoxicity of these constituent elements at such doses might affect the ambulatory activity of mice. However, we do not think that this is the case. It is known that $LD_{50}$ of menthol is 14200 mg/kg (ip) and 3100 mg/kg (po) in mice (National Toxicology Program), therefore, 400–800 mg/kg (ip) of menthol, which produces the ambulation-promoting effect, is much lower than $LD_{50}$, suggesting that the effect is not a result of toxic effect of menthol. In addition, all active constituent elements did not produce writhing, tremor, convulsion, stereotyped behaviors

and catalepsy, but did ataxia, which is recoverable. This is similar to the case of MK-801, an NMDA antagonist that produces an ambulation-promoting effect and ataxia in mice (Kuribara et al., 1992b). Therefore, it is highly probable that the changes in the ambulatory activity are relevant to the pharmacological action of them.

We initially reasoned that these chemicals likely produce their effects via peripheral stimulation. For example, menthol peripherally stimulates cold receptors (Nishino et al., 1997; Orani et al., 1991; Sekizawa et al., 1996). Thus, we examined the effects of intravenous administration of these chemicals on ambulatory activity in mice, and found that intravenous administration of menthone, 1,8-cineol, isomenthone, menthol, (*R*)-(+)-pulegone and menthyl acetate all significantly increased ambulatory activity in mice in a dose-dependent manner. All constituent elements examined produced an increasing effect on ambulatory activity almost immediately after injection. In addition, these chemicals caused their effect at a much lower dosage than was the cases for intraperitoneal administration. In general, psychoactive drugs intravenously administered produce their actions very quickly at much lower doses than is the case for intraperitoneal administration. Thus, it is highly probable that these constituent elements of peppermint oil produce their effect by acting on the central nervous system. The present results taken as a whole indicate that peripherally injected peppermint oil produces its behavioral action after absorption of its constituent elements into the blood stream, and then by passing through the blood–brain barrier, where they produce their effect on the neurons in the brain in the same manner as known psychoactive drugs. The neuronal mechanism underlies the effects of the constituent element remains unclear at the present time. This issue should be examined in future research. Recently, we observed that dopamine antagonists such as haloperidol, chlorpromazine and fluphenazine inhibited the ambulation-promoting effect of menthol (data not shown), suggesting that dopamine is involved in the effect of menthol. However, menthol may not be a direct dopamine agonist given that large dose of menthol produce ataxia but not stereotyped behaviors. Thus, it is likely that menthol acts on some other target(s) and dopamine mediates the effect.

The fact that we found in the present study supports our hypothesis in which EOs possess pharmacological effects on brain functions. In addition, we demonstrated that an effect of peppermint oil, an EO, comes from its constituent elements.

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