Sedative effects of the essential oil and headspace air of *Ocimum basilicum* by inhalation in mice

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Abstract

The sedative effects of the essential oil released by living *Ocimum basilicum* plants were investigated using a mouse activity monitoring system. *O. basilicum* plants were nursed in a hydroponic chamber, and either the headspace air from the hydroponic chamber or the essential oil from the grown plants were administered by inhalation to mice in an open field test. The most effective dose of *O. basilicum* essential oil for reducing the locomotor activity of mice was found to be 4.0×10^{-3} mg/cage. The headspace air was administered to mice in a glass cage via a Teflon tube connected to a hydroponic chamber containing *O. basilicum* plants. A significant decrease in locomotor activity was observed when the hydroponic chamber contained nine plants. The results of this study showed that the headspace air of living basil plants could effectively reduce the locomotor activity of a mice.

Keywords

sedative, inhalation, *Ocimum basilicum* L., eugenol, (*R*)-(-)-linalool

Introduction

The aromas from herbs are said to exert stimulant and sedative effects on humans, but to date there have been no confirmatory studies based on scientific data reported in the literature. Effects such as sedation[1] or appetite enhancement[2] have been proven in studies using the essential oil fractions from herbs, but no studies involving living plants have been reported so far. It is often said that greenery and smells from plants in the living environment can help stress reduction, but this has not yet been proved scientifically. In this study, the sedative effects of the essential oil and the headspace air from *Ocimum basilicum* plants cultivated in a hydroponic chamber were investigated via inhalation administration in mice.

Results and Discussion

GC and GC-MS analyses

The results of the GC and GC-MS analyses of *O. basilicum* essential oil (OBEO) extracted by hydrodistillation are presented in Table 1. A total of 14 compounds were identified, with the main components being: eugenol (44.5%), linalool (21.2%), methyl eugenol (10.0%), eucalyptol (6.7%), and α-bergamotene (3.9%). *O. basilicum* is known to contain several types of oil[3]; the type of basil used in this study contained predominantly eugenol. Stereochemical characterization of the linalool in the oil was carried out by GC using a Chirasil-Dex column; (*R*)-(-)-linalool was found to be the dominant form (the abundance ratio of *R*-(−) : *S*-(+) linalool was 2.25 : 1). (*R*)-(-)-Linalool and α-humulene have been previously investigated for their sedative effects with a single administration by inhalation; (*R*)-(-)-linalool was found to have a sedative effect[4] while α-humulene had no sedative effect[5] in mice.

Inhalation administration of essential oil from O. basilicum

Figure 1 shows the AUC (area under the curve) for mouse activity after inhalation administration of basil essential oil. A significant decrease in locomotor activity was observed with doses of 4.0×10^{-5}, 4.0×10^{-4}, 4.0×10^{-3}, and 4.0×10^{-2} mg/cage. However, the mice displayed abnormal activity, such as jumping and rearing, at a dose of 4.0×10^{-2} mg/cage, so the reduced activity at this dose was not considered to be a true sedative effect. Figure 2 shows the locomotor activity results after the administration of eugenol. Locomotor activity was reduced at a dose of 4.0×10^{-2} mg/cage, and this was also considered not to be a true sedative effect.

Fujiwara *et al.*[6] studied the sedative effect of clove oil, which contains 75% eugenol, and found no sedative effect at doses from 4.0×10^{-4} to 4.0×10^{-2} mg/cage, which is in accordance with the results obtained in this work. On the other hand, (*R*)-(-)-linalool has been previously revealed to be a potent sedative[4]. The sedative effect of basil oil may be explained by the sedative activity of its component (*R*)-(-)-linalool.

Inhalation administration of basil vapor emitted from living plants

Figure 3 shows the AUC for mouse activity following the administration of basil vapor by direct transfer of the air from a hydroponic chamber containing live basil plants. The lowest AUC was observed with nine plants in the chamber; with three plants in the chamber, a significant sedative effect was also observed.

The results of the SPME (solid-phase microextraction)-GC-MS analysis of the air in the hydroponic chamber are presented in Figure 4. Eugenol and linalool were the main compounds to be detected, and it was assumed that they had been emitted by the basil in the hydroponic chamber.

The results here indicate that direct inhalation of fragrance emitted from basil plants

caused a sedative effect in mice. According to a previous report, inhalation administration of linalool (4.0×10^{-5} mg/cage) resulted in a strong sedative effect, decreasing the locomotor activity of mice by 65%[4]. Direct inhalation of the headspace air from the hydronic chamber containing nine plants resulted in a 45.4% decrease in mouse activity versus the 47.1% decrease observed with administration of basil essential oil at a dose of 4.0×10^{-3} mg/cage. Comparison of the results obtained in this work with the earlier study using just linalool showed that mixtures of compounds including linalool and eugenol provided weaker sedative activities than that of pure linalool. This may be explained by the synergistic effects of the compounds, as described by Fujiwara *et al.*[6]. Eugenol was supposed to be a potent reducer of the sedative activity of linalool, which is why the sedative effect of basil oil was much weaker than that expected from calculation of the linalool content in the oil.

Conclusion

In this study, a glass cage and a hydroponic chamber were connected with a Teflon tube so that the sedative effects of the headspace air of *O. basilicum* cultivated in the hydroponic chamber could be investigated via inhalation administration in mice. Direct inhalation of the headspace air from nine plants growing in the hydroponic chamber resulted in a 45.4% decrease in mice locomotor activity, so the sedative effects were proven using not only essential oil but also the headspace air from living plants.

This study makes a valuable contribution to the literature by providing a scientific basis for the sedative effects of live basil plants. Living plants are adopted in real life easily because they are familiar to our lives. In conclusion, using living plants promotes self-medication because people can obtain a sedative effect routinely.

Experimental Methods

Materials

O. basilicum seeds for hydroculture, coated with cali clay and produced in India, were used in this study. They were germinated and grown in a hydroponic chamber (W 54.4 × L 26.2 × H 30.5 cm). Begetable Life A water-soluble fertilizers for hydroponic growing were obtained from OAT Agrio Co., Ltd., Tokyo, Japan. Benzylacetone and eugenol were purchased from Tokyo Kasei University, Japan. Triethyl citrate (Merck KGaA, Germany), an odorless solvent, was used to dissolve the fragrance components. All other chemicals and reagents used in this study were of the highest grade available.

Animals

Animal experiments were designed following the recommendations of the animal research committee of Kyoto University, Kyoto, Japan (approval number 14-14). Male 4-week-old ddY (20–30 g) mice were purchased from Japan SLC (Shizuoka, Japan). The mice were housed in colony cages at an ambient temperature of 25 ± 2 ℃ and a relative humidity of 50 ± 10% with a 12-h light–dark cycle before being used for the experiments. They were fed standard pellet chow and water *ad libitum*. All behavioral observations were conducted between 10:00 and 17:00 at the same temperature and humidity.

Extraction of essential oil

The essential oil of *O. basilicum* was extracted by hydrodistillation of the dried aerial parts of the plants for 2 h using a Clevenger apparatus according to the crude drug test procedure (9.1. Essential oil content) from the Japanese Pharmacopeia (JP ⅩⅦ). The oil was collected in hexane, dried with anhydrous sodium sulfate, and then concentrated. The obtained essential

oil was stored in sealed vials at 4 ℃ until analysis.

GC and GC-MS analyses

GC analyses were performed on a G-5000 (Hitachi) equipped with a flame ionization detector with the following conditions: a fused silica capillary column (Inert Cap-Wax, 60 m × 0.25 mm, film thickness 0.25 μm, GL Sciences) and a chiral column (CP-Chirasil-Dex CB, 25 m × 0.25 mm, film thickness 0.25 μm); column temperature program: 60 ℃ for 2 min, increasing to 220 ℃ at a rate of 4 ℃/min, then held at 220℃ for 15 min; the injector was set at 60 ℃ and the detector was set at 230 ℃[7]; carrier gas: helium (1 ml/min); injection volume: 1 μl; split ratio: 99 : 1.

GC-MS and SPME were performed on an Agilent 6850 series gas chromatograph connected to an Agilent MSD 5975 mass spectrometer under the following operating conditions: fused silica capillary column (DB-WAX, 60 m × 0.25 mm, film thickness 0.25 μm, Agilent Technology); SPME fiber: 100 μm polydimethylsiloxane (Supelco); the column temperature program was the same as that used for the GC analysis; ionization energy: 70 eV; carrier gas: helium (1 mL/min); injection volume: 1 μl; split ratio: 99:1. The identities of most of the separated compounds were confirmed by comparison of the retention indices (RI) and mass spectra patterns (MS library: NIST 2 and flavors). RI was accordant with past data.

Open field test

The sedative activities of the fragrant components were evaluated by monitoring their effects on the spontaneous motor activity of mice using an open field test, as described in a previous report[8]. The distilled oils were dissolved in triethyl citrate (400 μl total) at concentrations

ranging from 4.0×10^{-7} to 4.0×10^{-2} mg/cage. The dissolved oil sample was then dropped onto four filter paper disks (100 µl per disk), which were placed on the walls of the glass cage using adhesive tape. The solution vapor was allowed to fill the cage by natural diffusion for 60 min. A mouse was placed in the center of the cage and was monitored by video camera for 60 min. The frequency at which the mouse crossed the lines drawn on the bottom of the cage at 10-cm intervals was counted every 5 min for 60 min. The AUC which was calculated from a graph with time (min) on the *x*-axis and the *y*-axis was measured by the trapezoidal rule.

Open field test with hydroponic chamber connected to the cage

In Figure 5, a schema of the experimental equipment is presented. The vents of the hydroponic chamber (W 54.4 × L 26.2 × H 30.5 cm, air supply from vents 3.06×10^{-1} m^3/h and dark state) were covered with a funnel (9 cm diameter) that was connected to a Teflon tube (10 mm inside diameter, 34 cm length) using adhesive tape. The other end of the Teflon tube was placed into the glass cage (W 60 × L 30 × H 34 cm) so that the exhaust air from the hydroponic chamber flowed into the glass cage.

After 12 minutes of aeration from the hydroponic chamber to the glass cage, a mouse was placed in the center of the cage and monitored by a video camera for 60 min. The frequency at which the mouse crossed the lines drawn on the bottom of the cage at 10-cm intervals was counted every 5 min for 60 min. The AUC, indicating the total locomotor activity over 60 min, was calculated by the trapezoidal rule. The number of plants kept in the hydroponics equipment was either three, nine, or 27(height of the plant was about 23 cm).

Statistical analyses

Data are expressed as the mean ± standard error of the mean. Statistical analyses were

performed using one-way analysis of variance (ANOVA) followed by Dunnett's test using GraphPad InStat (GraphPad Software, San Diego, CA, USA). A probability level of $P < 0.05$ was considered to be statistically significant.

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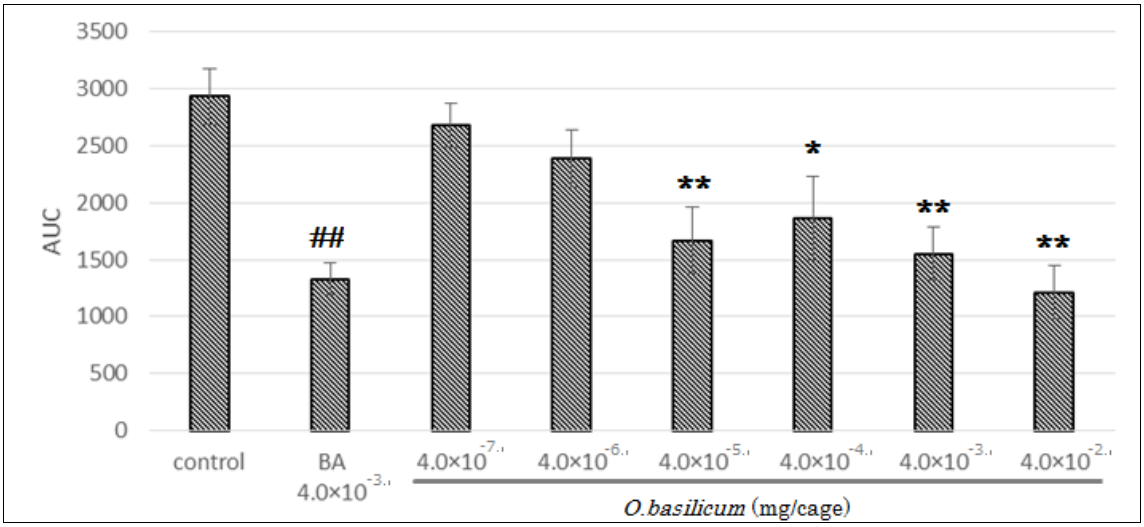
Table 1. Components of the essential oil from *Ocimum basilicum*

Compound	RI[a]	Peak area (%)
β-Pinene	1053	0.6
β-Phellandrene	1060	t[b]
β-Myrcene	1081	0.5
Eucalyptol	1208	6.7
β-Ocimene	1227	1.2
Linalool	1474	21.2
α-Bergamotene	1498	3.9
α-Humulene	1645	0.7
α-Terpineol	1654	1.1
Germacrene D	1665	2.6
γ-Cadinene	1689	1.9
Methyl eugenol	2010	10.0
Eugenol	2092	44.5
t-Cadinol	2095	t[b]
Total	**-**	**94.9**

a: retention index, b: t = trace (<0.01%)

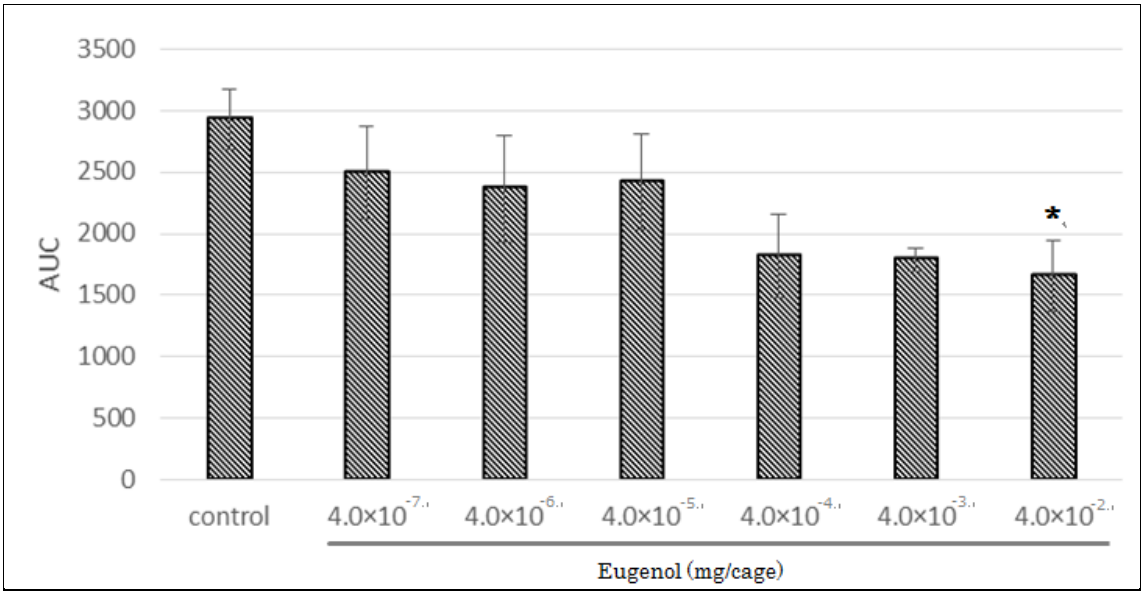
Peak area % was calculated from the GC charts. Compound identification was achieved by GC-MS.

Figure 1. Spontaneous motor activity of mice treated with *Ocimum basilicum* essential oil



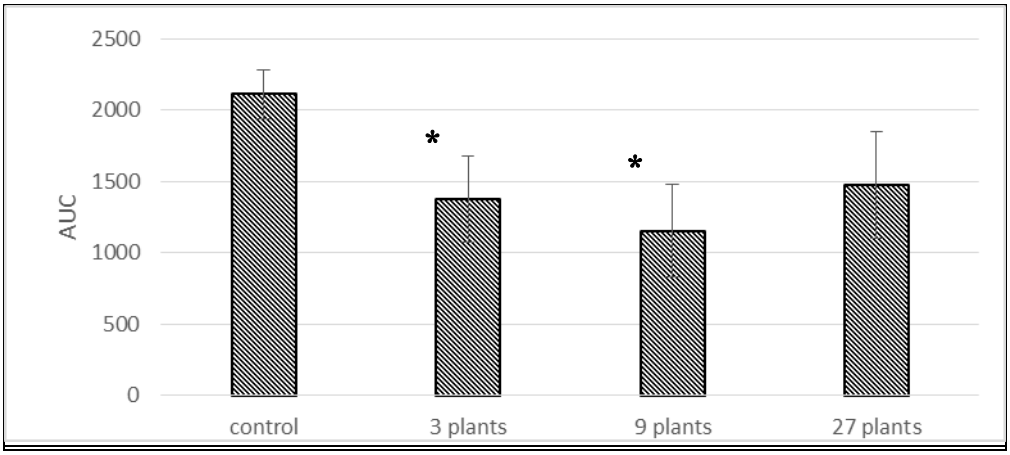
Data are shown as the mean values ± standard error of the mean for five mice. Statistical differences were calculated using one-way ANOVA followed by Dunnett's test. *$p < 0.05$, **$p < 0.01$ compared to the control group (triethyl citrate). Positive control: benzyl acetone (4.0×10^{-3} mg/cage).

Figure 2. Spontaneous motor activity of mice treated with eugenol



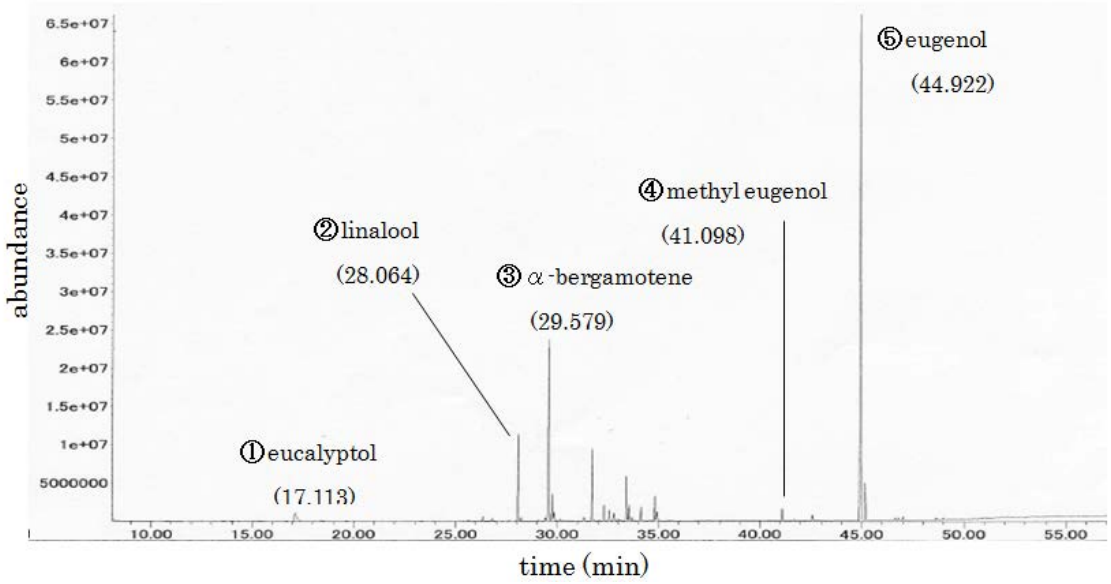
Data are shown as the mean values ± standard error of the mean of five mice. Statistical differences were calculated using one-way ANOVA followed by Dunnett's test. *$p < 0.05$ compared to the control group (triethyl citrate).

Figure 3. Spontaneous motor activity of mice treated with headspace air directly transferred from the hydroponic chamber



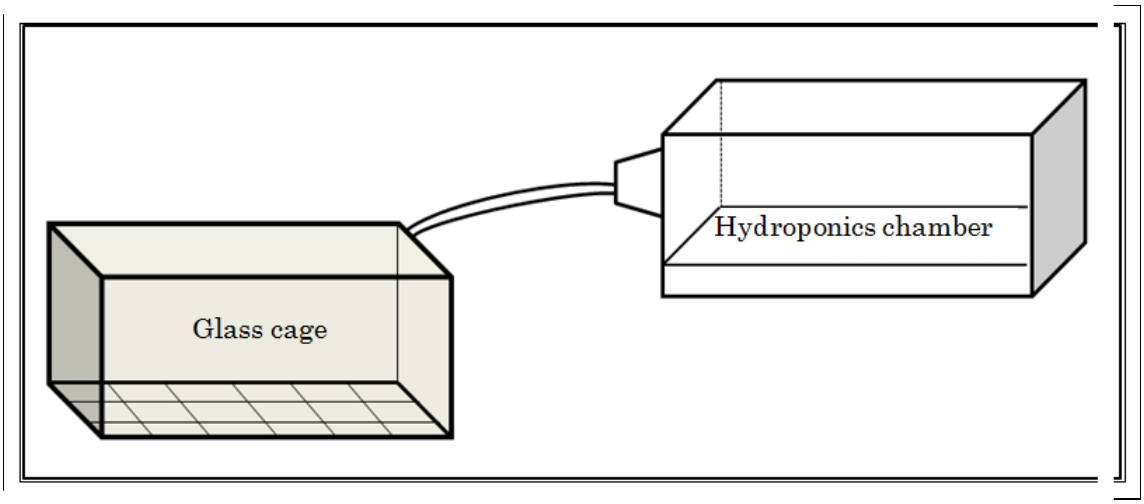
Data are shown as the mean values ± standard error of the mean for five mice. Statistical differences were calculated using one-way ANOVA followed by Dunnett's test. *p < 0.05 compared to the control group.

Figure 4. SPME-GC-MS chromatogram for air in the hydroponic chamber



The numbers in parentheses indicate the peak retention times.

Figure 5. Schema of experimental equipment for open field test with hydroponic chamber connected to glass cage



On the left is a glass cage (W 60 × L 30 × H 34 cm) and on the right is a hydroponic chamber (W 54.4 × L 26.2 × H 30.5 cm). They are connected with a Teflon tube (10 mm inside diameter, 34 cm length) so that the exhaust air from the hydroponic chamber flows into the glass cage.