Research Square

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Sugi (Cryptomeria japonica) fragrance improved stress-induced memory impairment via decreasing stress hormone

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Abstract

The purpose of this study is to clarify the biological activity of the aromatic components of Sugi () essential oil on stress hormones. The effect of Sugi wood aroma on memory disorders was investigated in chronic corticosterone-treated (CORT) mice that were exposed to the aroma of Sugi wood essential oil (SEO), δ-cadinene, α-muurolene, and β-cadinene complex fraction (CDS) separated from SEO, and δ-cadinene for 28 days. The effect of SEO and the above fractions on improving memory impairment was assessed using a behavioral test in mice. To elucidate this mechanism, changes in CORT levels in the hippocampus after SEO inhalation were measured. SOE inhalation improved memory disorder in the novel object recognition and Barnes maze tests. Observation of Immunohistochemistry staining of brain tissues and mRNA quantification and production of brain-derived neurotrophic factor (BDNF) in the hippocampus revealed that the inhalation of SEO and CDS was effective in neuroprotection. Additionally, SEO inhalation promoted the discharge of injected hippocampal corticosterone, indicating suppression of neuronal cell damage in the hippocampus to improve stress-related memory disorder. To the best of our knowledge, this is the first report to demonstrate a decrease in stress hormone levels and identify the active components of SEO. The study also elucidated the mechanism of memory improvement effect of inhalation of SEO and suggested its potential as medical aromatherapy for the treatment of diseases of the brain and nervous system such as depression and dementia.

Introduction

Approximately 70% of Japan's national land area (37.79 million hectares) is covered by forests (25.05 million hectares), of which planted forests cover 10.2 million hectares. Japanese cedar (Sugi: *Cryptomeria japonica*) accounts for approximately 44% of Japan’s planted forest area (Agency forestry in Japan, 2021, 2017). Sugi wood is known for durability, workability, high humidity control, and good odor; therefore, Japanese people use it as a building material for the interior of their houses.

However, the demand and self-sufficiency rate of Sugi wood has been declining owing to the aggressive introduction of inexpensive imported wood and decrease in the number of forestry workers since the 1960s. On the other hand, rooms in which wood has been used are expected to have a relaxing effect with a good odor and soft touch. Unfortunately, the physiological effects of Sugi wood fragrance have not yet been investigated or scientifically researched. Therefore, we believe that studying the physiological effects of Sugi wood essential oil (SEO) will increase the demand and add value to its properties and applications.

Plant essential oils are often used in aromatherapy, a practice within naturopathy, utilized for beauty, mental health, and body health[3]. In the EU region, medical aromatherapy using essential oils of plants is applied for the functional improvement of health and body in the medical and welfare fields [4]. After inhalation through the nose, volatile compounds of essential oils dissolve in the olfactory mucosa of the nasal cavity and bind to olfactory receptors located on the cilia at the tips of olfactory cells, which initiates transmission of the stimulus combined with the volatile component-olfactory receptor to the

olfactory cells as an electrical signal. This signal is then transmitted to the limbic system[5]. The limbic system includes the hippocampus and hypothalamus, which are involved in memory and emotion, the autonomic nervous system, and the endocrine system; thus, essential oil volatile components have the potential to affect the limbic system function. Therefore, essential oils are expected to prevent psychiatric disorders such as depression, dementia, and Alzheimer's disease (AD). Scientific studies on these disorders have focused on oils obtained from the leaves, fruits, and flowers[3, 6–8]. This report focuses on essential oil obtained from wood, which has rarely been reported, and the components that contribute to their effects.

The human body responds to several types of stressors. The stress response system is divided into two types: the hypothalamic-pituitary-adrenal (HPA) axis and sympathetic-adrenal-medullary (SAM) axis. The SAM system induces the adrenal medulla to release adrenaline/noradrenaline, which initiates physiological (sympathetic) arousal and produces fight, flight, and freeze responses[9]. The SAM system responds to perceived danger or acute stress and is also involved in metabolic and immune responses; sympathetic nerves play more significant role than parasympathetic nerves in responses such as pupil dilation, tracheal dilation, and enhanced alpha-amylase secretion. These nerves work relatively to the organism to maintain homeostasis and respond to stress. The HPA system involves the adrenal cortex, which produces corticosteroids such as cortisol, which not only helps the body to recover after stress but also has negative effects. A negative feedback mechanism is thought to play an important role in suppressing overexposure to stress hormones in neuronal cells[10]. However, chronic stress destroys this mechanism and excretes excessive stress hormones, causing dysfunction of the brain. One reason for this is the decrease in BDNF levels. BDNF is important for memory and neurogenesis; therefore, a decrease in BDNF levels induces memory disorders such as senility, AD, and depression[11–13]. In addition, chronic stress causes functional and structural changes, such as a decrease in neurotransmitters, glucocorticoid receptors, number of neurons, and synapses. Chronic administration of CORT in mice is frequently used to mimic physiological conditions of chronic stress[14–16]. Chronic CORT-treated mice receive daily administration of corticosterone, a rodent stress hormone, for approximately one month. After treatment, the HPA system and hippocampal function and structure of the mice are disrupted. Therefore, mice are widely used as models of depression-like and memory disabilities.

We investigated the effect of SEO inhalation on memory learning disabilities caused by chronic exposure to stress hormones using chronic corticosterone-injected mice. Additionally, we identified active components in SEO that contribute to the prevention of memory disorder. Studies on identification of compounds contributing to the fragrance functionality are limited. We believe that the study findings are particularly important for promoting the use of fragrances in medical aromatherapy.

Results and Discussion

Quantification of components of SEO

SEO contains sesquiterpene hydrocarbons 71% (area ratio) and sesquiterpene alcohols 18% (area ratio from GC-MS; Fig. 1A, Table S1). Sesquiterpene hydrocarbons main compounds were δ-cadinene 29.6% (area ratio), α-muurolene 22.26% (area ratio), and *cis*-calamenene 2.61% (area ratio).

Main compounds of sesquiterpene alcohols were cubenol 7.33% (area ratio) and epi-cubenol 7.22% (area ratio). Absolute calibration curve using δ-cadinene and *cis*-calamenene (Fig. 1B, C) revealed that SEO contained 0.2397 ± 0.02 mg/μL of δ-cadinene and 0.06272 ± 0.0002 mg/μL of *cis*-calamenene. Cadinene fraction was composed of cadinene skeletal formula 99% (area ratio), and the breakdown was δ-cadinene 52.9% (area ratio), α-muurolene 29.9% (area ratio), and β-cadinene 13.33% (area ratio). δ-cadinene concentration of 0.4332 ± 0.021 mg/μL was measured using absolute calibration method of δ-cadinene (Fig. 1B).

cis-Calemenene was isolated from SEO, and a few compounds were found to contaminate it. However, its purity was too low to detect ^{1}HNMR, ^{13}CNMR spectrum derived from *cis*-calamenene. The NMR results revealed the identification of these components as cis-calmenene compared with previous NMR spectra[17,18].

To confirm the concentrations of δ-cadinene and *cis*-calamenene in SEO (diluted 100-fold with ethanol) in each sprayed sample, the cadinene complex component (diluted at 181-fold), δ-cadinene (diluted at 417-fold), and *cis*-calamenene isolated from SEO (diluted at 1594-fold) were used.

SEO inhalation improved body weight reduction by CORT

Fig. 2 (A), (B), and (C) show the total changes in body, adrenal, and liver weights. Body weight gain was suppressed in the CORT group compared with the vehicle group after two days (Fig. 2(B)). On the other hand, SEO, δ-cadinene, and cis-calmenene groups showed suppression of body weight gain. In the adrenal glands, the CORT group (0.001058 ± 0.00022) significantly decreased adrenal gland weight than compared with the vehicle group (0.002096 ± 0.00020) ($F(5, 35) = 3.49$, $p < 0.05$). No difference was observed in the liver weight between the groups. This suggests that the administration of chronic stress hormones continued to surpass the secretion of stress hormones from the adrenal cortex, resulting in the dysfunction and atrophy of the adrenal glands. Therefore, the above result also indicates that SEO inhalation excretes injected stress hormones from the body and alleviates adrenal gland dysfunction.

These results represent the mean ± SEM of values for each group. # $p<0.05$, ## $p<0.01$; one-way ANOVA followed by Tukey's test.

Vehicle: control solvent injected and inhaled of ethanol, CORT:corticosterone solvent (40 mg/kg) injected and inhaled of ethanol, SEO: corticosterone solvent (40 mg/kg) injected and inhaled of SEO (1 mg /mL), CDS: corticosterone solvent injected and inhaled of cadinenes fraction (0.55 mg/mL), δ-cadinene: corticosterone solvent injected and inhaled of δ-cadinene (0.24 mg/mL), *cis*-calamenene: corticosterone solvent injected and inhaled of *cis*-calamenene (0.064 mg/mL *cis*-calamenene).

SEO inhalation improved memory disorder

Novel recognition test results are presented in Fig. 3 (A). The cognitive index of the vehicle (0.366 ± 0.113), SEO (0.136 ± 0.075), CDS (0.385 ± 0.107), and δ-cadinene groups (0.103 ± 0.071) specifically increased compared with the CORT group (-0.385 ± 0.157) ($F(5, 35) = 6.39$, $p < 0.05$ vs. SEO and δ-cadinene, $p < 0.01$ vs. CDS and vehicle, Dunnett's test). Additionally, it was specifically decreased in the *cis*-calamenene group (-0.151 ± 0.103) than compared with the vehicle and CDS groups. This result suggests that SEO inhalation improves short memory impairment caused by excessive stress hormone, and δ-cadinene, α-muurolene, and β-cadinene present in SEO contribute to the effect. The novel recognition test evaluates short-term memory by assessing preferences for novel objects 24 h after treatment[19,20]. Normal mice exhibited an increased interest in novel objects on the second day, indicating memory retention on the first day. The cognitive index, representing interest in novel objects, was significantly lower in the CORT group, CDS, SEO, and δ-cadinene groups than compared with the vehicle group (Fig. 3 (B)). These results suggest that inhalation of Sugi wood essential oil, cadinene complex, and δ-cadinene alleviates short-term memory impairment induced by chronic CORT administration.

Barnes maze test results from the acquisition phase are shown in Fig. 3 (D), and the probe test results are shown in Fig. 3 (E), (F), and (G). During the acquisition phase, the CORT group's escape latency was higher than that of the vehicle group at days 3 and 4 ($F (5, 35) = 2.49$ s, 2.40 s $p < 0.05$ vs. CORT, Dunnett's test). In contrast, the escape latencies of the sample groups were similar to that of the vehicle group.

In the probe test, the CORT group's escape latency (69.3 s ± 23.7) was specifically higher than compared with the vehicle (11.3 s ± 4.0), SEO (13.9 s ± 4.3), and CDS (7.38 s ± 1.42) groups ($F (5, 35) = 3.34$, $p < 0.05$, Dunnett's test). Additionally, the vehicle, SEO, and CDS groups tended to have a lower number of errors and hole deviation scores than those of the CORT group. The number of errors and hole deviation scores could determine whether the escape latency value was due to chance; therefore, the observed decrease in escape latency of the SEO and CDS groups was not considered a coincidence. Therefore, inhalation of SEO was considered to be effective in improving CORT-induced spatial memory impairment, and the presence of δ-cadinene, α-muurolene, and β-cadinene in SEO contributes to the effect.

The Barnes maze test assesses spatial memory learning ability. Normal mice learned and remembered the dark box (escape box) using visual cues, with decreasing escape latency over successive trials for five days. Mice that successfully learned the location of the dark box during acclimatization could still find it after its removal. However, the CORT group showed a significantly increased escape latency than compared with the vehicle group on days 3 and 4 during acclimatization (Fig. 3 (D)). In the probe test, the escape latencies of vehicle, CDS, and SEO groups were significantly lower than that of the CORT group, indicating that the spatial memory learning deficits was improved by inhalation of Sugi wood essential oil and cadinene complex, with δ-cadinene also showing a tendency to ameliorate deficits (Fig. 3 (E)).

Vehicle: control solvent injected and inhaled of ethanol, CORT: corticosterone solvent (40 mg/kg) injected and inhaled of ethanol, SEO: corticosterone solvent (40 mg/kg) injected and inhaled of SEO (1

mg /mL), CDS: corticosterone solvent injected and inhaled of cadinenes fraction (0.55 mg/mL), δ-cadinene: corticosterone solvent injected and inhaled of δ-cadinene (0.24 mg/mL), *cis*-calamenene: corticosterone solvent injected and inhaled of *cis*-calamenene (0.064 mg/mL *cis*-calamenene).

SEO inhalation improved BDNF expression in the hippocampus

BDNF expression results are shown in Fig. 4 (A). BDNF expression in the CORT (0.46 ± 0.04), δ-cadinene (0.44 ± 0.02), and cis-calamenene (0.53 ± 0.03) groups was specifically decreased than compared with the vehicle group (1 ± 0.14), ($F(5, 17) = 4.75$, $p < 0.05$, Dunnett's test). In contrast, the expression in the SEO (0.86 ± 0.18) and CDS (0.76 ± 0.07) groups was considerably higher than that of the CORT group. BDNF is an important protein involved in memory, neurogenesis, and neuronal cell protection. Therefore, this result indicates that inhalation of Sugi wood essential oil can increase the expression of BDNF and protect the hippocampus from excess stress hormones, and the presence of δ-cadinene, α-muurolene, and β-cadinene contributes to the effect.

BDNF mRNA expression results are shown in Fig. 4 (B). BDNF expression in the CORT group (0.46 ± 0.14) was specifically decreased compared with the vehicle (1 ± 0.3), CDS (1.33 ± 0.13), and δ-cadinene (0.88 ± 0.09) groups ($F(5, 35) = 7.59$, $p < 0.01$, Tukey's test). BDNF mRNA expression ratio among the groups was similar to that of BDNF protein expression. Therefore, these results indicate that SEO inhalation can increase the mRNA and protein expression of BDNF, and the presence of δ-cadinene, α-muurolene, and β-cadinene contributes to the effect.

Immunohistochemical staining of the hippocampal tissue revealed BDNF, indicated by brown staining (Fig. 4 (C), arrow). Moreover, the CORT group had a lower staining intensity (brown pigment intensity) than that of the vehicle group (Fig. 4 (C)). The BDNF-stained brown area was lower in the CORT group than in the vehicle group. The essential oil group showed higher BDNF expression than that of the CORT group (Fig. 4 (D)). Trends among the groups were similar to those of the western blotting and real-time PCR results. Accordingly, this result corroborates the western blot results that inhalation of Sugi essential oil increases BDNF protein levels.

Hippocampal BDNF production is important for memory, learning, and neuroprotection. BDNF is crucial for memory consolidation and learning and is decreased in patients with depression, dementia, and memory impairment[12]. Therefore, ameliorative effect of SEO inhalation on memory deficits was likely due to increased BDNF levels. The combined effects of δ-cadinene, α-muurolene, and β-cadinene contribute to this effect, with a lesser contribution from cis-calamenene. The CDS group showed higher effect than that of the δ-cadinene group, suggesting δ-cadinene's effects on memory deficits and BDNF increase may be enhanced by α-muurolene and β-cadinene. The complex components of δ-cadinene, α-muurolene, and β-cadinene improve memory learning impairment. This was demonstrated using a 100-fold dilution with ethanol. The physiological effects of essential oil aroma components vary with their concentrations, necessitating further investigation.

SEO inhalation reduced CORT levels in the hippocampus

The results of CORT level in the hippocampus (pg/μg protein) are shown in Fig. 5 (A). The protein concentration was measured using bicinchoninic acid assay kit. CORT level of the CORT group (794 pg/ μg protein ± 88) was specifically increased compared with the vehicle (75 pg/μg protein ± 4.5), SEO (269 pg/10 μg protein ± 56), CDS (275 pg/ μg protein ± 85), and cis-calamenen (290 pg/10 μg protein ± 67) groups (F (5, 17) = 8.524, CORT vs. vehicle = $p < 0.01$, CORT vs. SEO and CDS = $p < 0.05$, Tukey's test).

The results of temporal CORT levels in the hippocampus (pg/10 μg protein) are shown Fig. 5 (B). The result show that the inhalation of Sugi wood essential oil has the potential to excrete the injected CORT from the hippocampus. Hence, we investigated the time-related changes in CORT hippocampus levels after CORT injection.

We measured temporal changes (60 and 90 min after CORT injection) in CORT levels during SEO inhalation. The hippocampal CORT level of the SEO group (60 min: 118 pg ± 4.5, 90 min: 40.4 pg/10 μg protein ± 2.3) significantly decreased compared with that of the CORT group (60 min: 145 pg/10 μg protein ± 7.3, 90 min: 72.2 pg/10 μg protein ± 5.6) (60 min: F(3,11) = 23.406, $p < 0.01$, Tukey's test, 90 min: (F (3,11) = 101.568, $p < 0.01$, Tukey's test). It is known that the hippocampal CORT concentration is increased by the administration of CORT, reaching its peak at approximately 45 min after administration, and then returning to the basal level over 90 min, as observed in the CORT group(Droste et al., 2008). In addition, given the almost complete absence of corticosterone-metabolizing enzymes[22], the decrease in hippocampal CORT concentration observed in the SEO group indicated that CORT dynamics were affected by the inhalation of SEO.

This is the first report, to the best of our knowledge, to demonstrate that inhaled essential oils containing no monoterpenes improve memory and learning disabilities. In addition, this is the first study to identify the contributing components on a component-by-component basis, with the cadinene complex as a significant contributor. Furthermore, we demonstrated that inhalation of cedarwood essential oil promotes a reduction in CORT levels within the hippocampus, indicating this mechanism improves memory impairment. Although studies on the physiological effects of essential oil aroma components have primarily focused on monoterpenes, sesquiterpenes merit increased attention in future studies.

Conclusion

We demonstrated that Sugi wood essential oil ameliorated stress-induced memory disorder. The combined components of δ-cadinene, α-muurolene, and β-cadinene contribute to this effect, with the effect of δ-cadinene being enhanced by α-muurolene and β-cadinene. This mechanism may cause a decrease in CORT levels in the hippocampus, which is affected by inhalation of Sugi wood essential oil. Further experiments are required to elucidate the mechanism of action. This report demonstrates, for the first time, the potential of volatile compounds in Sugi wood to improve stress-induced disorders, and identifies their contributing components for the first time. We believe that the scientific elucidation of the physiological effects of Sugi wood fragrance will contribute to the forest health, stress reduction in humans, and enhancement of the added value of Sugi (*Cryptomeria japonica*).

Materials and methods

Chronic Corticosterone Treatment

Specific pathogen-free adult male mice were purchased from SLC Japan. After one week pre-breeding, they were distributed into six groups (n=6): vehicle group (ethanol), CORT group, CORT + Sugi essential oil (1 mg/mL Sugi essential oil) group, CORT + cadinene fraction (0.55 mg/mL cadinene fraction) group, CORT + δ-cadinene (0.24 mg/mL δ-cadinene) group, CORT + δ-cadinene (0.064 mg/mL *cis*-calamenene) group. Each sample concentration was detected based on δ-cadinene and *cis*-calamenene contented concentration of Sugi essential oil (δ-cadinene: 0.2397 mg/μL *cis*-calamenene: 0.064 mg/mL).

The vehicle group was treated with control solution (Saline + 0.1% DMSO, 0.1% Tween-80), while the CORT groups were treated with CORT solution (40 mg/mL) dissolved in the control solution by subcutaneous administration once a day for 3 weeks. After injecting each solution, mice inhaled 4 mL of each flavor solution atomized by an aroma diffuser (At Aroma, Inc., SQUARE) for 1 h every day. Behavioral tests (Barnes Maze and Novel Object Recognition Test) on memory were conducted from day 22 to 31, and dissection was performed after those tests. Mice were maintained under a 12-h light/dark cycle (7:00-19:00,22 ± 1) with free access to food (Oriental Yeast Co., Ltd., MF powder) and water. Body weight, food intake, and water intake were measured every 2 days. All animal experiments were conducted following the Guide for the Care and Use of Laboratory Animals at Gifu University, Japan (approval number. 2022-111).

Behavioral test

The Novel Object Recognition test

This examination takes advantage of the characteristics of preferencing novelty objects and is able to evaluate short memory (24 h)[19,20]. 24h after the last injection, the novel object recognition test (NORT) was performed. On the first day, the mice were placed in the box (50×35×50 cm) and explored the room for 5 min to acclimate to the area. On the Second day, a watermelon toy object (5 cm × 5 cm in height and diameter) was introduced on both sides of the box, and mice memorized them for two and a half minutes. On the third day one of them was changed to a dice toy and the time of interest behavior to the dice and the watermelon was measured. Assuming watermelons to be old objects and dice new objects, the following formula was used to divide the cognitive index. The interest behavior is defined when the animals' mouths and noses are <1 cm away from the object or directly touching the object.

Barnes maze

This examination procedure was developed by Carol Barnes to study spatial memory in mice[23–25]. This Barnes Maze consisted of a plastic circular platform with 20 holes placed 2.5 cm from the edge, and all holes were equally distributed around the surface. Its surface platform was 92 cm in diameter and 92 cm above the ground. The Barnes Maze takes advantage of the animal’s natural reluctance to open up lit places. Therefore, subjects are motivated by a bright light to locate an escape hole that leads

to a dark box. The Barnes Maze test consisted of three phases: an adaptation period, an acquisition period, and a probe trial. Before starting the test on the first day, a preliminary test (adaptation period) was performed. In this study, the acquisition trial consisted of 5 days and then a probe trial was performed twenty-four hours after the final acquisition trial. The behavior of the experimental subject was captured by a video camera and was recorded on the computer. Four parameters were observed in this assay: the number of errors (number of wrong holes explored during acquisition trail), latency (the time taken to escape from target hole during acquisition trial days), and the percentage of time spent during the probe trial

Western blot PCR, and Immunohistochemistry

Western blot, PCR and immunohistochemistry were conducted according to the method described in supplemental section.

Corticosterone in hippocampus measured method

Hippocampus was extracted for 2 hours after the final treatment of corticosterone. The hippocampus was lysed with radioimmunoprecipitation assay (RIPA) buffer at 3500 rpm for 20 sec. After centrifugation at 10000 rpm for 10 min, solution determined protein concentration using BCA kit. The hippocampus corticosterone concentration was measured by enzyme-linked immunosorbent assay kit (Corticosterone ELISA kit, ENZ, ADI-900-097). The results were normalized to the protein concentration.

Sample Appreciation

Sugi essential oil (9.2 mL) was collected by steam distillation from Sugi wood chips (10 kg). (+)-δ-cadinene was purchased from TCI (Tokyo Chemical Industry Co., Ltd. C3734). The extraction and separation of Sugi essential oil and GC-MS analysis were described in supplemental section.

Longitudinal changes in hippocampal CORT levels induced by inhalation of Sugi wood essential oil

Specific pathogen-free adult male mice were purchased from SLC Japan. After one week pre-breeding, they were distributed into six groups (n=15): vehicle group (ethanol), CORT group, CORT+ Sugi essential oil (1 mg/mL Sugi essential oil) group. The Vehicle group was treated with control solution (Saline + 0.1% DMSO, 0.1% Tween-80), while the CORT groups were treated with CORT solution (40 mg/mL) dissolved in the control solution. Following the injected solution, mice were immediately placed on a shelf under the essential oil atmosphere atomized by aroma diffusers. 15, 30, 60, 90, 120 minutes after injected, mice were killed by cervical dislocation and hippocampus was promptly removed. The hippocampus was extracted 2 hours after the final treatment of corticosterone. This hippocampus was lysed with radioimmunoprecipitation assay (RIPA) buffer at 3500 rpm for 20 sec. After centrifugation at 10000 rpm for 10min, solution determined protein concentration using BCA kit. The corticosterone concentration in hippocampus was measured by enzyme-linked immunosorbent assay kit (Corticosterone ELISA kit, ENZ, ADI-900-097). The results were normalized to the protein concentration.

Statistical analysis

Comparisons between multiple groups were made by Tukey and Dunnet's multiple comparison test after ANOVA (analysis of variance1), assuming a normal distribution of the data. Statistical significance levels of $p< 0.05$ and $p< 0.01$ were considered as significant differences. The results of the analysis of variance were presented as F-values with degrees of freedom.

Declarations

Declaration of competing interest

All authors declare that they have no competing interests.

Author Contribution

A.F. and H.T. conducted experiments. K.Y. and T.M. supervised the study. H.T. wrote the manuscript and K.Y. and T.M. reviewed and edited it.

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References

1. Agency forestry in japan. Forestry and mountain villages. Annual Report on Forest and Forestry in Japan 2021;1:91–112.
2. Agency forestry in japan. Forest resource statistics. Annual Report on Forest and Forestry in Japan 2017;2–3.
3. Ali, B. et al. Essential oils used in aromatherapy: A systemic review. Asian Pac J Trop Biomed 2015;5:601–611.
4. Farrar, A. J. & Farrar, F. C. Clinical Aromatherapy. Nursing Clinics of North America 2020;55:489–504.
5. Donoshita, Y., Choi, U.-S., Ban, H. & Kida, I. Assessment of olfactory information in the human brain using 7-Tesla functional magnetic resonance imaging. Neuroimage 2021;236:118212.
6. Rashed, A. A., Rahman, A. Z. A. & Rathi, D. N. G. Essential oils as a potential neuroprotective remedy for age-related neurodegenerative diseases: a review. Molecules 2021;26.
7. Sánchez-Vidaña, D. I. et al. Lavender essential oil ameliorates depression-like behavior and increases neurogenesis and dendritic complexity in rats. Neurosci Lett 2019;701:180–192.

8. Igarashi, M., Ikei, H., Song, C. & Miyazaki, Y. Effects of olfactory stimulation with rose and orange oil on prefrontal cortex activity. Complement Ther Med 2014;22:1027–1031.
9. Godoy, L. D., Rossignoli, M. T., Delfino-Pereira, P., Garcia-Cairasco, N. & Umeoka, E. H. de L. A comprehensive overview on stress neurobiology: Basic concepts and clinical implications. Front Behav Neurosci 2018;12:Preprint at https://doi.org/10.3389/fnbeh.2018.00127
10. Leistner, C. & Menke, A. Hypothalamic–pituitary–adrenal axis and stress. Handb Clin Neurol 2020;175:55–64.
11. Schaaf, M. J. M., De Jong, J., De Kloet, E. R. & Vreugdenhil, E. Downregulation of BDNF mRNA and protein in the rat hippocampus by corticosterone. Brain Res 1998;813:112–120.
12. Amidfar, M., de Oliveira, J., Kucharska, E., Budni, J. & Kim, Y. K. The role of CREB and BDNF in neurobiology and treatment of Alzheimer's disease. Life Sci 2020;257.
13. Castrén, E. & Monteggia, L. M. Review Brain-Derived Neurotrophic Factor Signaling in Depression and Antidepressant Action. doi:10.1016/j.biopsych.2021.05.008
14. Xie, X. et al. Chronic corticosterone-induced depression mediates premature aging in rats. 2018. doi:10.1016/j.jad.2017.12.073
15. Dieterich, A. et al. Chronic corticosterone administration induces negative valence and impairs positive valence behaviors in mice. doi:10.1038/s41398-019-0674-4
16. Xu, Z. et al. Chronic corticosterone administration from adolescence through early adulthood attenuates depression-like behaviors in mice. 2010. doi:10.1016/j.jad.2010.11.005
17. Bunko, J. D., Ghisalberti, E. L. & Jefleries, P. R. (lR,4S)-Calamenene. Chemical Correlation with the Diterpene Analogues of Eremophila*. Aust. J. Chem 1981;34.
18. Nakashima, K. et al. molecules Total Synthesis of (-)-(7S,10R)-Calamenene and (-)-(7S,10R)-2-Hydroxycalamenene by Use of a Ring-Closing Metathesis Reaction. A Comparison of the cis-and trans-Isomers. Molecules 2002;7.
19. Antunes, M. & Biala, G. The novel object recognition memory: Neurobiology, test procedure, and its modifications. Cogn Process 2012;13:93–110.
20. Ennaceur, A. One-trial object recognition in rats and mice: Methodological and theoretical issues. Behavioural Brain Research 2010;215:244–254.
21. Droste, S. K. et al. Corticosterone Levels in the Brain Show a Distinct Ultradian Rhythm but a Delayed Response to Forced Swim Stress. Endocrinology 2008;149: 3244–3253.
22. R W Brown, R. D. A. C. R. Y. V. K. J. J. M. M. H. K. J. R. S. The ontogeny of 11 beta-hydroxysteroid dehydrogenase type 2 and mineralocorticoid receptor gene expression reveal intricate control of glucocorticoid action in development. Endocrinology 1996;137:794–7.
23. Pitts, M. W. Barnes Maze Procedure for Spatial Learning and Memory in Mice. doi:10.21769/BioProtoc.2744
24. Harrison, F. E., Hosseini, A. H. & McDonald, M. P. Endogenous anxiety and stress responses in water maze and Barnes maze spatial memory tasks. Behavioural Brain Research 2009;198:247–251.

25. Barnes, C. A. Memory deficits associated with senescence: a neurophysiological and behavioral study in the rat. J Comp Physiol Psychol 1979;93:74–104.

Figures

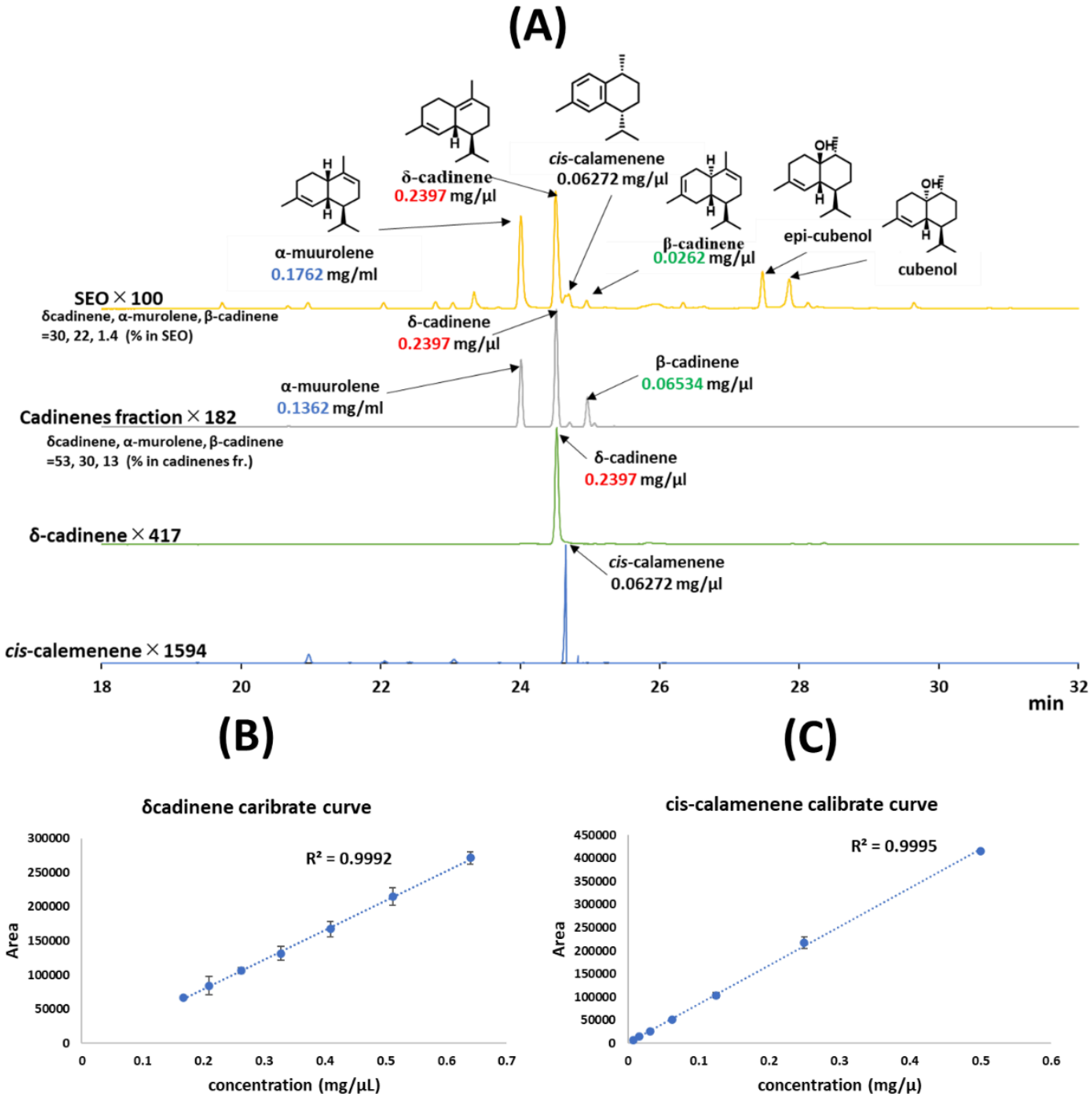


Figure 1

(A) TIC of each sample on GC-MS analysis and typical compound structure.

(B) Absolute calibration curve (prepared using pure δ-cadinene) to quantify δ-cadinene in the essential oil samples. (C) Absolute calibration curve (prepared using pure cis-calamenene) to quantify cis-calamenene in the essential oil samples.

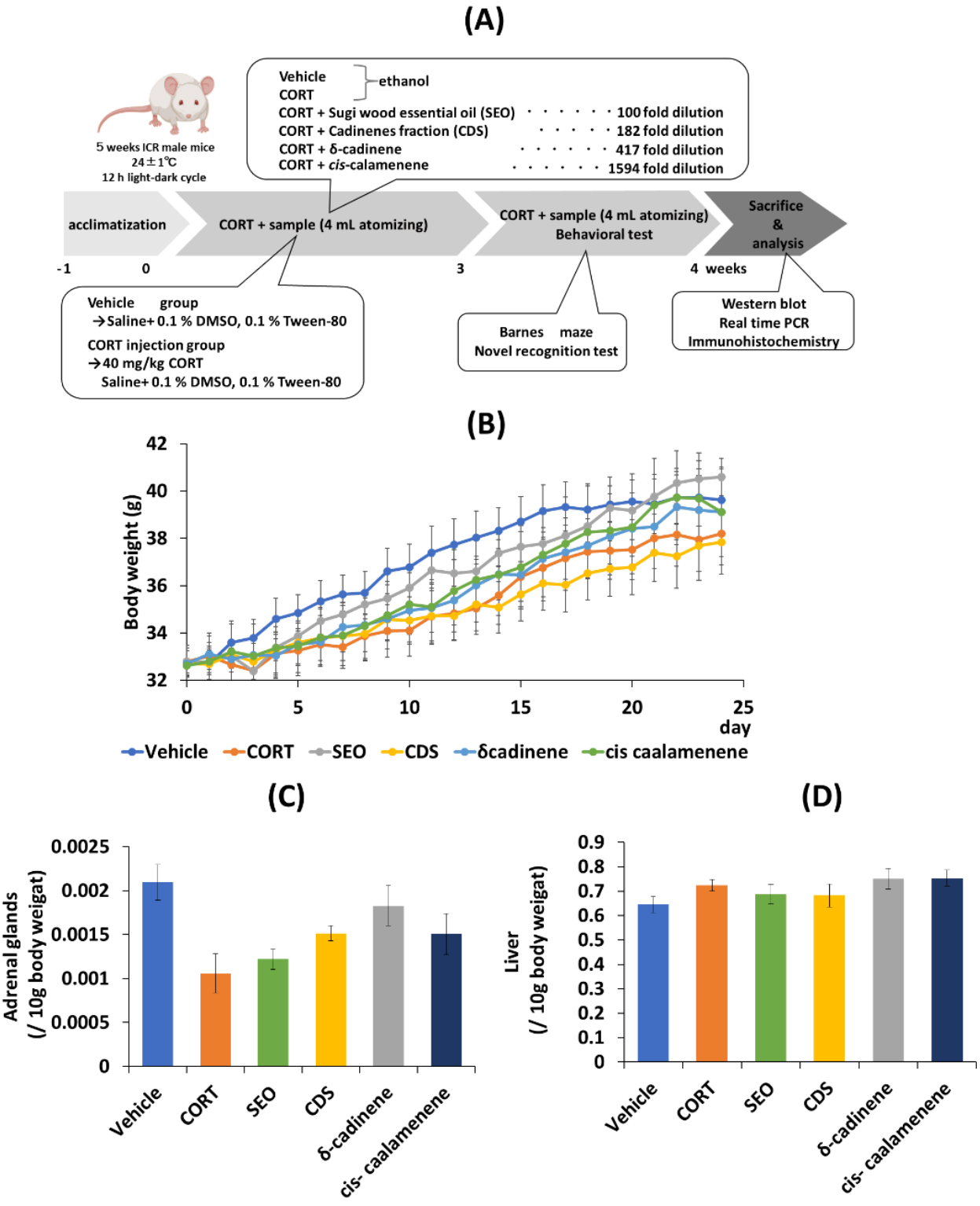


Figure 2

(A) Breeding protocol. (B) Body weight transition of all groups (n=6). (C)Adrenal glands weight of each group(g/ 10 g body weight) (n=6). (D)Liver weight of each group. (n=6).

These results represent the mean ± SEM of values for each group. # p<0.05, ##p<0.01; one-way ANOVA followed by Tukey's test.

Vehicle: control solvent injected and inhaled of ethanol, CORT:corticosterone solvent (40 mg/kg) injected and inhaled of ethanol, SEO: corticosterone solvent (40 mg/kg) injected and inhaled of SEO (1 mg /mL), CDS: corticosterone solvent injected and inhaled of cadinenes fraction (0.55 mg/mL), δ-cadinene: corticosterone solvent injected and inhaled of δ-cadinene (0.24 mg/mL), *cis*-calamenene: corticosterone solvent injected and inhaled of *cis*-calamenene (0.064 mg/mL *cis*-calamenene).

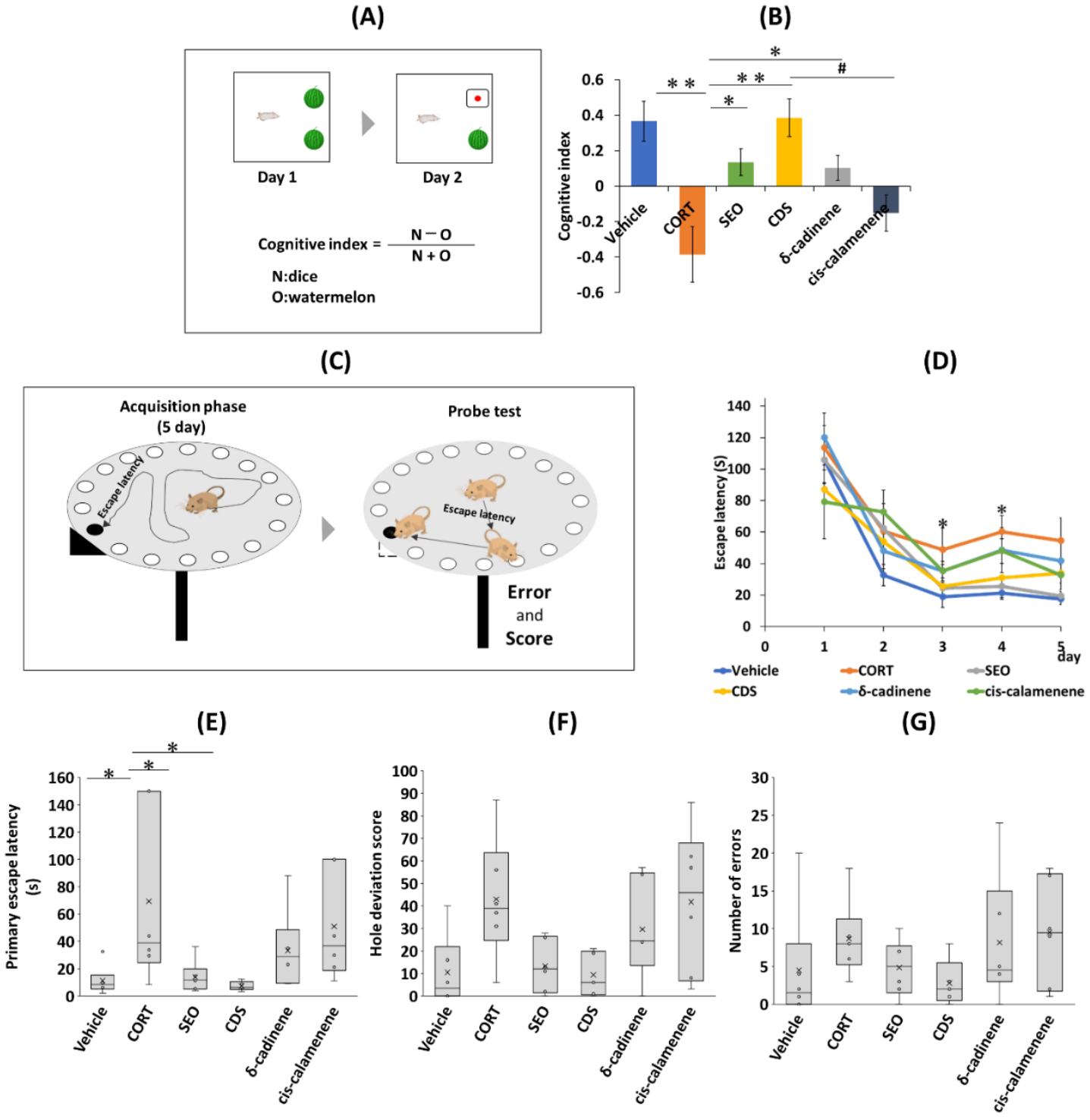


Figure 3

(A) Outline of novel recognition test. (B) Result of Nobel recognition test in all groups. Results represent the mean ± SEM of values for each group (n = 6). (C) Outline of Barnse maze. (D) Primary escape latency of each group in acquisition phase (1 day -5 day). (E) Primary escape latency in the probe test. (F) Number of errors in the probe test. (G) Hole deviation score in the probe test. Results represent the mean ± SEM of values for each group (n = 6).

# $p<0.05$; One-way ANOVA followed by Tukey's test. *$p<0.05$, **$p<0.01$; One-way ANOVA followed by Dunnet's test vs CORT

Vehicle: control solvent injected and inhaled of ethanol, CORT: corticosterone solvent (40 mg/kg) injected and inhaled of ethanol, SEO: corticosterone solvent (40 mg/kg) injected and inhaled of SEO (1mg /mL), CDS: corticosterone solvent injected and inhaled of cadinenes fraction (0.55 mg/mL), δ-cadinene: corticosterone solvent injected and inhaled of δ-cadinene (0.24 mg/mL), *cis*-calamenene: corticosterone solvent injected and inhaled of *cis*-calamenene (0.064 mg/mL *cis*-calamenene).

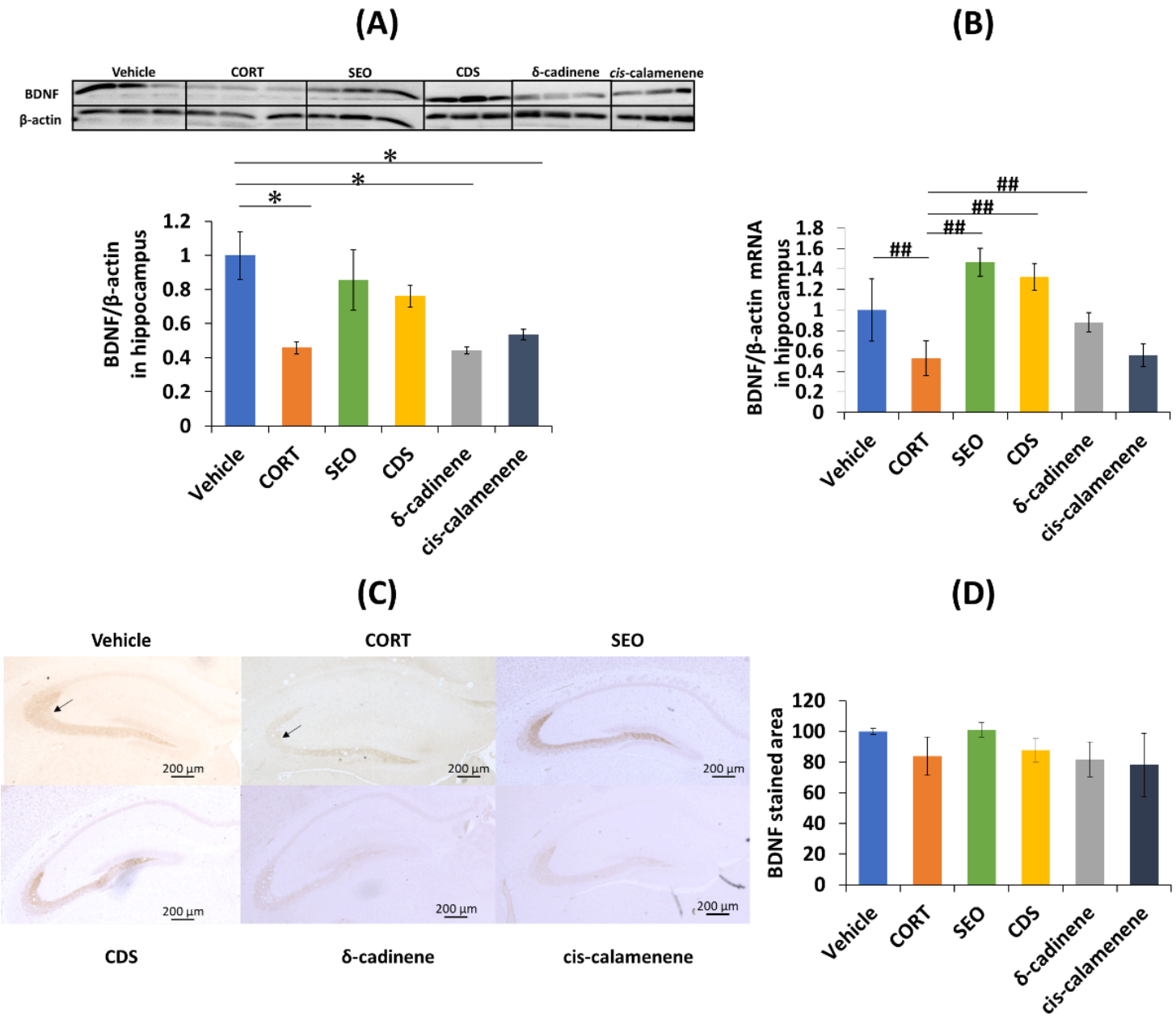


Figure 4

(A) BDNF protein expression in hippocampus. This result represents the mean±SEM of values for each group. * p<0.05, ** p<0.01; one way ANOVA followed by Dunnet's test, n=3. (B) BDNF mRNA expression in hippocampus. This result represents t the mean ± SEM of values for each group. # p<0.05, ## p<0.01; one way ANOVA followed by Tukey's test, n=3.

(C) Immunohistochemistry of BDNF in hippocampus. Arrows show stained BDNF (D) Measured stained area by image j. Value shows each data vs vehicle group. This result representsthe mean ± SEM of values for each group. n=3.

Vehicle: control solvent injected and inhaled of ethanol, CORT: corticosterone solvent (40 mg/kg) injected and inhaled of ethanol, SEO: corticosterone solvent (40 mg/kg) injected and inhaled of SEO

(1mg /mL), CDS: corticosterone solvent injected and inhaled of cadinenes fraction (0.55 mg/mL), δ-cadinene: corticosterone solvent injected and inhaled of δ-cadinene (0.24 mg/mL), *cis*-calamenene: corticosterone solvent injected and inhaled of *cis*-calamenene (0.064 mg/mL *cis*-calamenene).

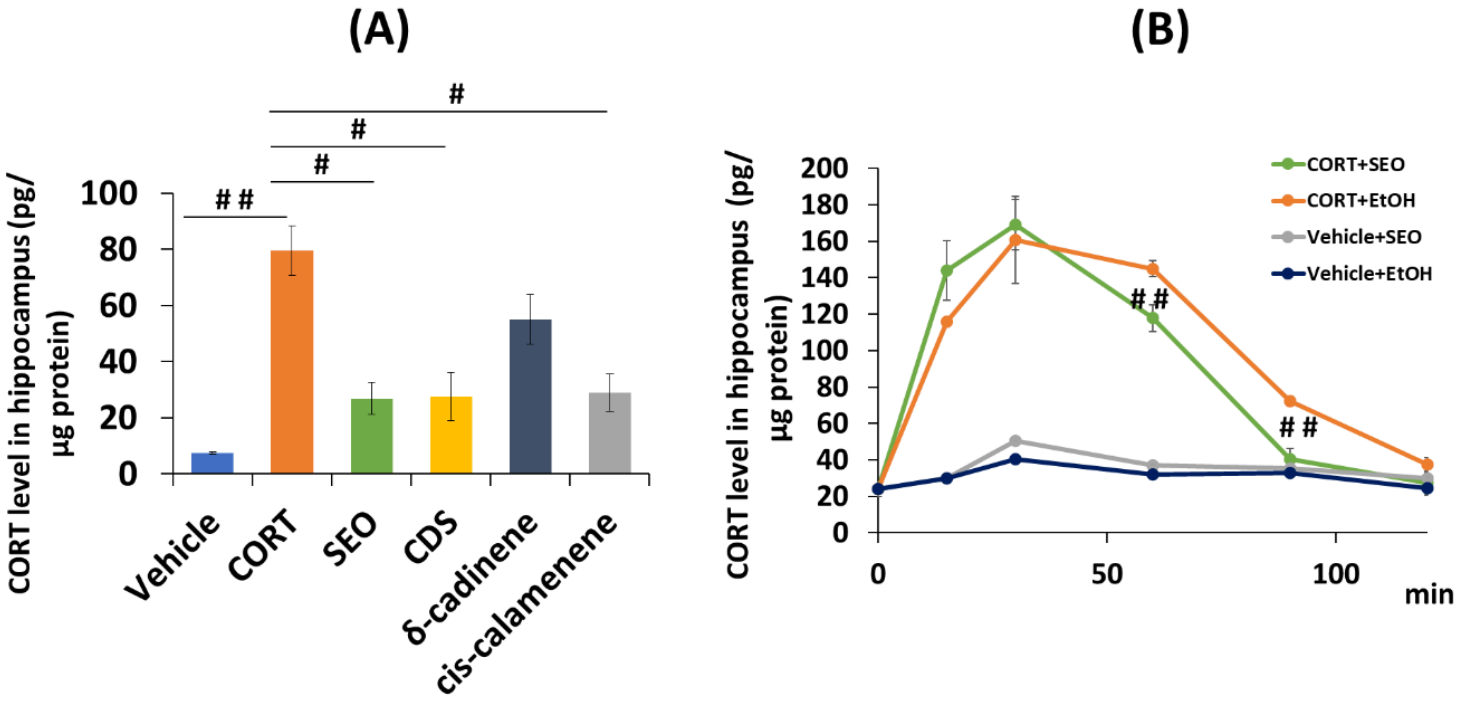


Figure 5

(A)CORT level in hippocampus (pg /µg protein) These results represent the mean ± SEM of values for each group. # p<0.05, ##p<0.01; one way ANOVA followed by Tukey's test, n=3. Protein concentration measured by BCA assay.

(B) Temporally change of CORT level in hippocampus (pg /µg protein) at the inhalation of Sugi wood essential oil. These results represent the mean ± SEM of values for each group. # p<0.05, ##p<0.01; one way ANOVA followed by Tukey's test, each plot n=3. Protein concentration measured by BCA assay.

Vehicle: control solvent injected and inhaled of ethanol, CORT: corticosterone solvent (40 mg/kg) injected and inhaled of ethanol, SEO: corticosterone solvent (40 mg/kg) injected and inhaled of SEO (1 mg /mL), CDS: corticosterone solvent injected and inhaled of cadinenes fraction (0.55 mg/mL), δ-cadinene: corticosterone solvent injected and inhaled of δ-cadinene (0.24 mg/mL), *cis*-calamenene: corticosterone solvent injected and inhaled of *cis*-calamenene (0.064 mg/mL *cis*-calamenene).

Supplementary Files

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- Supplementarymaterial.docx
- floatimage1.png