Investigation of Anxiolytic- and Antidepressant-like Effects of Essential Oils from Six Traditional Korean Herbal Prescriptions

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Essential oils and aromatherapy have traditionally been used for the treatment of anxiety and depression with few side effects. This study aimed to investigate the effects of essential oils from six herbal prescriptions known to be effective in treating anxiety and depression in Korean medicine. The neuroprotective and anti-neuroinflammatory effects of six essential oils, including Gamisachil-tang (GMSCT), Guibi-tang (GBT), Sihogayonggolmoryeo-tang (SYM), Danchisoyosan (DCSYS), Sihosogansan (SHSGS), and Soyosan (SYS), were examined in PC12 and BV2 cells. In corticosterone (CORT)-stimulated PC12 cells, all six essential oils ameliorated the CORT-induced decrease in cell viability at a concentration of 10 μg/ml. GMSCT, GBT, and SHSGS recovered CORT-induced cytotoxicity at concentrations of 1 μg/ml and 10 μg/ml. In lipopolysaccharide (LPS)-stimulated BV2 cells, GBT (10 μg/ml) decreased interleukin (IL)-1β production, whereas SHSGS (1 μg/ml) inhibited tumor necrosis factor (TNF)-α production. In the MK-801-induced anxiety in zebrafish, electroencephalogram (EEG) assessment indicated that GMSCT and SHSGS induced recovery in the delta and beta power densities and reduced theta/beta and delta/beta ratios. DCSYS and SYS decreased theta power density and theta/beta ratio, whereas GBT and SYM showed no effects on EEG signals. In the tail suspension test (TST) in mice, GBT, DCSYS, SHSGS, and SYS exhibited antidepressant-like effects by decreasing immobility time. These results suggest that the essential oils from the six herbal prescriptions, except SYM, may have beneficial effects on anxiety and/or depression. Further studies should be conducted to investigate the molecular signaling pathways that mediate the effects of these essential oils on anxiety and depression.

keywords : Herbal prescription, Essential oil, Depression, Anxiety, Tail suspension test, Electroencephalogram

Introduction

Anxiety and depression are the most common psychological disorders and the leading causes of disability and a reduction in the quality of life of patients with these disorders[1]. Anxiety is characterized by excessive worry, fear, and difficulty in controlling nervousness[2]. The core symptoms of depression include a depressed mood, loss of interest and pleasure in almost all activities, and suicidal ideation in severe cases[3]. During the COVID-19 pandemic, quarantine, social distancing, and lockdown policies implemented in many countries for a long duration resulted in negative psychological consequences with a remarkable increase in the number of psychiatric patients. Based on the results of a meta-analysis, the prevalence of anxiety and depression during the COVID-19 pandemic ranged from 18.3% to 27.6% and 13.5% to 24.3%, respectively[4]. In addition, anxiety and depression commonly co-occur. A previous study indicated that more than 80% of patients with depression showed symptoms of anxiety and vice versa[5].

The hypothalamic-pituitary-adrenal (HPA) axis is a major neuroendocrine system involved in the pathogenesis of stress-related psychiatric disorders, including depression and anxiety disorders[6,7]. Hyperactivation of the HPA axis in response to stress results in excessive secretion of glucocorticoids (cortisol in humans and corticosterone in rodents) from the adrenal gland[8]. Chronic stress with elevated levels of glucocorticoids causes neuronal damage in the hippocampus and prefrontal cortex (PFC), which are important brain regions that control mood and behavior[9]. Neuroinflammation has been suggested to play a crucial role in the progression of many psychiatric disorders including anxiety and depression[10]. Microglia are major

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immune cells that modulate the neuroinflammatory response in the central nervous system (CNS). In disease conditions, activated microglial cells produce a variety of pro-inflammatory molecules, such as interleukin (IL)-1β, IL-6, tumor necrosis factor α (TNF-α), and nitric oxide (NO), which can induce neuronal damage and are associated with anxiety and depression behaviors[11]. Therefore, inhibiting neuroinflammation and neuronal damage may be effective in the treatment of depression and anxiety disorders.

The first-line treatments for anxiety and depression are selective serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs)[12,13]. However, the long-term administration of these drugs is associated with numerous side effects, including seizures, bleeding, and cardiovascular and gastrointestinal side effects[14]. Previous studies have demonstrated that several essential oils from herbal medicines have therapeutic effects on mood disorders, including anxiety and depression, with few side effects[15,16]. These data suggest that aromatherapy or the use of essential oils from herbal medicines may be a promising alternative treatment for anxiety and depression.

In this study, we selected six herbal prescriptions that were traditionally used for anxiety and depression treatment, according to the Clinical Practice Guideline of Korean Medicine for Anxiety Disorder (National Institute for Korean Medicine Development, NIKOM) and the Korean Medicine Clinical Practice Guideline for Depression (Korea Institute of Oriental Medicine, KIOM). The six prescriptions include Gamisachil-tang (GMSCT), Guibi-tang (GBT), Sihogayonggolmoryeo-tang (SYM), Danchisoyosan (DCSYS), Sihosogansan (SHSGS), and Soyosan (SYS) (Table 1). The effects of essential oils from these herbal prescriptions on neurotoxicity and neuroinflammation were examined using corticosterone (CORT)-induced PC12 cells (neuronal cell model) and lipopolysaccharide (LPS)-induced BV2 microglial cells. Moreover, animal models (zebrafish and mice) were used to investigate the anxiolytic and antidepressant-like effects of these essential oils.

Materials and Methods

1. Preparation of essential oils from six herbal prescriptions

The extraction of essential oils from six herbal prescriptions (Table 1) was conducted using the simultaneous distillation extraction (SDE) method with n-hexane as the solvent. All herbs in the six prescriptions were purchased from Omniherb (Daegu, Korea) and authenticated by Professor In-Jun Yang (Dongguk University, Gyeongju, South Korea). Each sample was immersed in distilled water (1:10, w/v) in a 1000 mL flask, and n-hexane was placed in a 100 mL flask. The SDE procedure was performed for > 4 h. The oil captured in n-hexane was dried over anhydrous Na_2SO_4. n-Hexane was subsequently evaporated using a rotary evaporator (EYELA, Tokyo, Japan). Subsequently, the essential oils were weighed and stored at −20 ℃. The yield (%) of the essential oils is shown in Table 1.

Table 1. Composition and essential oil yield of six herbal prescriptions

Herbal prescription	Composition	Essential oil yield (%)
Gamisachil-tang (GMSCT)	Pinelliae Rhizoma 4 g, Citri Pericarpium 4 g, Poria 4 g, Massa Medicata Fermentata 3 g, Aurantii Immaturus Fructus 3 g, Arisaematis Rhizoma 3 g, Citrii Unshiu Immaturi Pericarpium 2 g, Magnoliae Cortex 2 g, Perilla Herba 2 g, Arecae Semen 2 g, Amomi Fructus 2 g, Amomi Rotundus Fructus 1.5 g, Alpiniae Fructus 1.5 g, Zingiberis Rhizoma Recens 10 g	0.54
Guibi-tang (GBT)	Angelicae Gigantis Radix 3 g, Longanae Arillus 3 g, Zizyphi Spinosae Semen 3 g, Polygalae Radix 3 g, Ginseng Radix 3 g, Astragali Radix 3 g, Atractylodis Rhizoma Alba 3 g, Hoelen cum Pini Radix 3 g, Aucklandiae Radix 1.5 g, Glycyrrhizae Radix 0.9 g	0.59
Sihogayonggolmoryeo-tang (SYM)	Bupleuri Radix 6 g, Pinelliae Rhizoma 4.5 g, Rhei Rhizoma 3 g, Scutellariae Radix 2.5 g, Fossilia Ossis Mastodi 2.5 g, Ostreae Concha 2.5 g, Cinnamomi Ramulus 2.5 g, Poria 2.5 g, Zingiberis Rhizoma Recens 2.5 g, Ginseng Radix 2.5 g, Zizyphi Fructus 2.5 g	0.61
Danchisoyosan (DCSYS)	Angelicae Gigantis Radix 6 g, Bupleuri Radix 5 g, Paeoniae Radix Alba 5 g, Atractylodis Rhizoma Alba 5 g, Poria 5 g, Moutan Cortex 3 g, Gardeniae Fructus 3 g, Glycyrrhizae Radix 2 g, Menthae Herba 2 g, Zingiberis Rhizoma Recens 2 g	0.43
Sihosogansan (SHSGS)	Bupleuri Radix 6 g, Citri Pericarpium 6 g, Cnidii Rhizoma 4 g, Cyperi Rhizoma 4 g, Aurantii Fructus 4 g, Paeoniae Radix Alba 4 g, Glycyrrhizae Radix 2 g	0.62
Soyosan (SYS)	Glycyrrhizae Radix 3 g, Angelicae Gigantis Radix 6 g, Poria 6 g, Atractylodis Rhizoma Alba 6 g, Bupleuri Radix 6 g, Paeoniae Radix Alba 6 g	0.25

2. Cell culture

PC12 cells were obtained from the Korea Cell Line Bank (Seoul, Korea) and were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin (Welgene, North Gyeongsang, Korea) at 37℃ in a 5% CO_2 humidified incubator.

BV2 cells were provided by the Korea Institute of Oriental Medicine (KIOM, Daegu, Korea) and were maintained in Dulbecco's Modified Eagle Medium (DMEM) high glucose medium supplemented with 10% fetal bovine

serum and 1% penicillin/streptomycin (Welgene, North Gyeongsang, Korea) at 37℃ in a 5% CO_2 humidified incubator.

3. Cell viability

The effects of the essential oils on the viability of PC12 and BV2 cells were evaluated using the MTT assay. Briefly, cells were seeded in 96-well plates and treated with essential oils (1, 10, and 100 μg/ml) for 24 h. To evaluate the neuroprotective effect of essential oils, PC12 cells were pre-incubated with essential oils (1 and 10 μg/ml) for 1 h before stimulation with corticosterone (CORT, 200 μM) (Sigma-Aldrich, St. Louis, MO, USA) for 24 h. After treatment, MTT reagent (5 mg/ml, 10 μl/well) (Sigma-Aldrich, St. Louis, MO, USA) was added and incubated for 4 h. Absorbance was measured at 570 nm using an iMark microplate absorbance reader (Bio-Rad Laboratories, Hercules, CA, USA).

4. Enzyme-linked immunosorbent assay (ELISA)

To evaluate the anti-neuroinflammatory effects of essential oils, BV2 cells were pre-incubated with essential oils (1 and 10 μg/ml) for 1 h before stimulation with lipopolysaccharide (LPS, 1 μg/ml) (Sigma-Aldrich, St. Louis, MO, USA) for 24 h. The levels of IL-1β, IL-6, and TNF-α in the cell culture media were evaluated using commercial ELISA kits (LABISKOMA, Seoul, Korea), according to the manufacturer's protocols. Absorbance was measured at 450 nm using an iMark microplate absorbance reader (Bio-Rad Laboratories, Hercules, CA, USA).

5. Electroencephalogram (EEG) recording in zebrafish

Adult zebrafish (*Danio rerio*, wild-type, AB strain) were maintained at Zefit Co., Ltd. (Daegu, Korea) under a 14 h:10 h light: dark cycle. The water used for animal maintenance was purified using the reverse osmosis method and kept at pH 6.5-7.5. The housing tank was maintained at 27±1 ℃ and 30-70% humidity. Adult zebrafish were placed in a 300 mL water bath containing 16 mg/l (v/v) eugenol to induce stage 3 anesthesia. MK-801 (Sigma-Aldrich, St. Louis, MO, USA) was used to induce an anxiety-like model in zebrafish. After 30 min of pretreatment with MK-801, essential oils (10 mg/l) or haloperidol (a positive control, 9 μM) were administered for another 30 min, and EEG was measured for 20 min using non-invasive electrodes. An MP36 device (Biopac Systems Inc., CA, USA) was used to measure and process EEG signals. The fast Fourier transform method was used to analyze the frequency of the signals. The relative power spectral densities (slow oscillation, pure delta, delta, theta, alpha, beta1, beta2, beta, and slow gamma) were recorded. The delta/beta and theta/beta ratios are considered anxiety-related markers.

6. Tail suspension test (TST) in mice

Male ICR mice (5 weeks old, 25-29 g) were purchased from Koatech (Gyeonggi, South Korea) and allowed to acclimatize for seven days before the experiments. All animal experimental procedures were approved by the Institutional Animal Care and Use Committee of Dongguk University (Approval No. IACUC-2021-15). The mice were divided into seven groups (n = 4 per group): Control (CON) (vehicle (3% Tween-80 in saline)-treated group) and six essential oil-treated groups (20 mg/kg, dissolved in the vehicle), including GMSCT, GBT, SYM, DCSYS, SHSGS, and SYS. The dose of essential oils was selected based on previous studies that intranasal administration of essential oils at doses from 12.5 to 50 mg/kg exhibited beneficial effects in the murine models of neurological and psychiatric diseases[17,18]. The mice were treated intranasally with essential oils or vehicle (10 μL/mouse) 30 min before TST to investigate the antidepressant effect of the essential oils. TST is a standard behavioral test for depression. In the TST, the mice were exposed to short-term stress by attaching their tail to a bar (50 cm above the floor) using adhesive tape (length, 15 cm). In response to this inescapable condition, the mice will exhibit an immobility behavior. The total immobility time (s) of each mouse in a six-minute session of TST was recorded using the SMART v3.0 video tracking system (Panlab, Barcelona, Spain) and used as a marker of depression-like behavior.

7. Statistical analysis

All experiments were performed in at least three independent experiments. GraphPad Prism 9.0 (GraphPad Software, CA, USA) was used for the statistical analysis. The results are presented as the mean ± standard deviation (SD), followed by statistical significance (two-tailed unpaired Student's t-test) with a p-value < 0.05.

Results

1. Effects of six essential oils on CORT-induced cytotoxicity in PC12 cells

CORT-induced PC12 cells were used to investigate the neuroprotective effects of essential oils from the six prescriptions. The results from the MTT assay showed that none of the six essential oils at a dose of 1-10 μg/ml had

cytotoxic effects on PC12 cells (Fig. 1A). Hence, these concentrations were used in subsequent studies. Fig. 1B indicates that CORT (200 μM) significantly reduced the viability of PC12 cells ($p < 0.05$). In contrast, pretreatment with GMSCT, GBT, and SHSGS reversed the CORT-induced decrease in cell viability at both 1 and 10 μg/ml, whereas SYM, DCSYS, and SYS showed beneficial effects at a higher dose (10 μg/ml) ($p < 0.05$) (Fig. 1B).

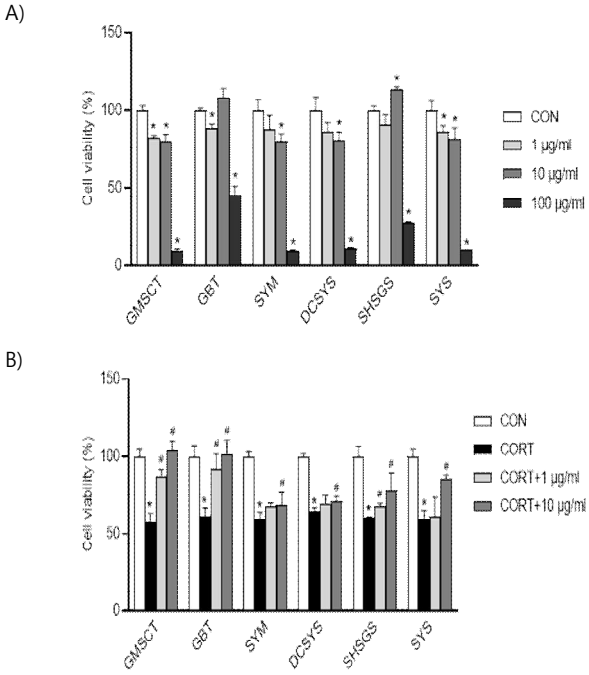


Fig. 1. Effects of six essential oils on CORT-induced cytotoxicity in PC12 cells. (A) PC12 cells were treated with essential oils (1, 10, 100 μg/ml) for 24 h. Effects of essential oils on cell viability were evaluated using MTT assays. (B) PC12 cells were pretreated with essential oils (1, 10 μg/ml) for 1 h, then stimulated with CORT (200 μM) for 24 h. Effects of essential oils on CORT-induced cytotoxicity in PC12 cells were evaluated using MTT assays. Data are presented as mean ± SD (n = 3 per experiment). ∗$p < 0.05$ vs. normal control (CON), #$p < 0.05$ vs. CORT-treated cells (two-tailed unpaired Student's t-test).

2. Effects of six essential oils on LPS-induced inflammation in BV2 cells

We used LPS-stimulated BV2 cells as an in vitro model of neuroinflammation to examine the effects of the six essential oils. The MTT-assay results showed that none of the six essential oils at a dose of 1-10 μg/ml had cytotoxic effects on BV2 cells (Fig. 2A). Hence, these concentrations were used in subsequent studies. Fig. 2B-D indicate that LPS (1 μg/ml) significantly increased the production of inflammatory cytokines, IL-1β, IL-6, and TNF-α in BV2 cells ($p < 0.05$). In contrast, pretreatment with GBT (10 μg/ml) reversed the LPS-induced production of IL-1β ($p < 0.05$) (Fig. 2B), whereas SHSGS (1 μg/ml) inhibited TNF-α production in LPS-treated BV2 cells ($p < 0.05$) (Fig. 2D). However, none of the six essential oils reduced IL-6 secretion in LPS-stimulated BV2 cells (Fig. 2C).

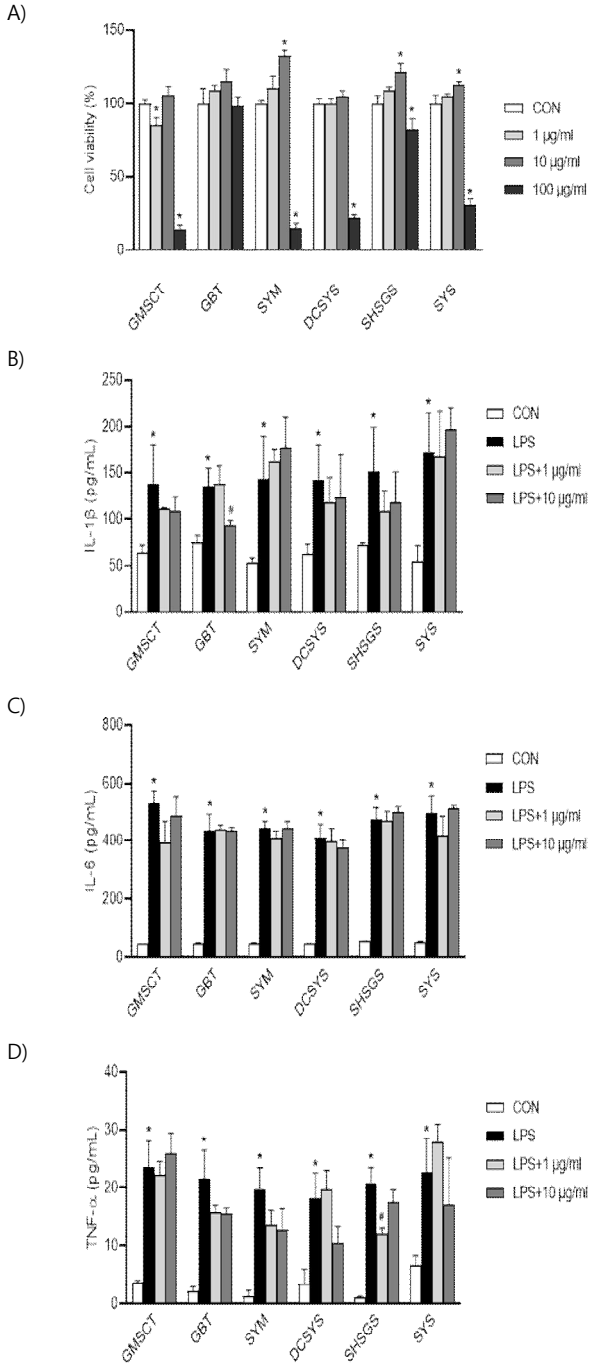
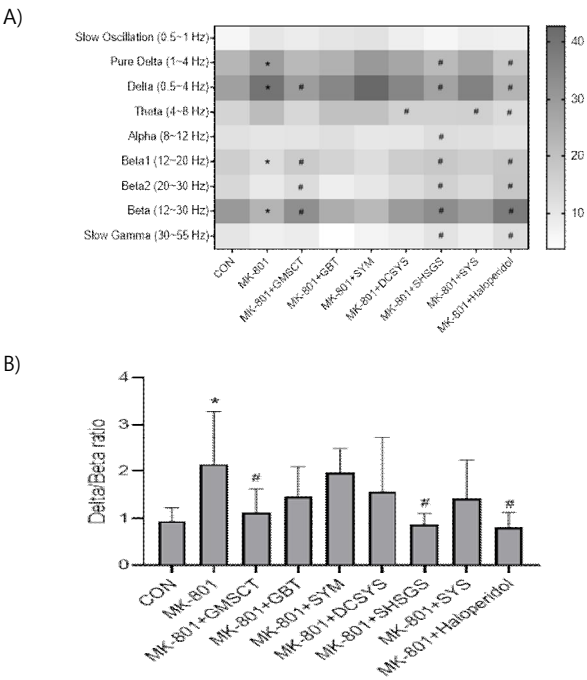


Fig. 2. Effects of six essential oils on LPS-induced inflammation in BV2 cells. (A) BV2 cells were treated with essential oils (1, 10, 100 μg/ml) for 24 h. Effects of essential oils on cell viability were evaluated using MTT assays. (B-D) BV2 cells were pretreated with essential oils (1, 10 μg/ml) for 1 h, then stimulated with LPS (1 μg/ml) for 24 h. Effects of essential oils on LPS-induced production of IL-1β, IL-6, and TNF-α in BV2 cells were evaluated using commercial ELISA kits. Data are presented as mean ± SD (n = 3 per experiment). ∗$p < 0.05$ vs. normal control (CON), #$p < 0.05$ vs. LPS-treated cells (two-tailed unpaired Student's t-test).

3. Effects of six essential oils on MK-801-induced anxiety in zebrafish

We examined the anxiolytic effects of essential oils in MK-801-induced zebrafish. Essential oils at the concentration of 10 μg/ml did not show any cytotoxic effects in vitro (Fig. 1-2), hence, 10 mg/L of each essential oil was treated to zebrafish to evaluate anxiolytic-like effects. Compared to the CON group, the MK-801 group showed significant increases in the relative power spectral densities of pure delta and delta ($p < 0.05$) and a significant decrease in the relative power spectral densities of beta1 and beta ($p < 0.05$) (Fig. 3A). These changes were reversed by SHSGS treatment ($p < 0.05$). In addition, SHSGS also increased the relative power spectral densities of alpha, beta2, and slow gamma compared to the MK-801 group ($p < 0.05$). GMSCT significantly reduced the relative power spectral density of delta ($p < 0.05$) and increased the relative power spectral densities of beta1, beta2, and beta ($p < 0.05$) compared with the MK-801 group (Fig. 3A). Treatment with DCSYS and SYS significantly decreased the relative power spectral density of theta waves ($p < 0.05$) in MK-801-treated zebrafish. Fig. 3B and 3C indicate that MK-801 stimulation significantly increased the delta/beta and theta/beta ratios in zebrafish ($p < 0.05$), which was reversed by treatment with GMSCT and SHSGS ($p < 0.05$). The DCSYS and SYS treatments only decreased the theta/beta ratio ($p < 0.05$). GBT and SYM did not affect the relative power spectral densities or the delta/beta and theta/beta ratios in MK-801-induced zebrafish.



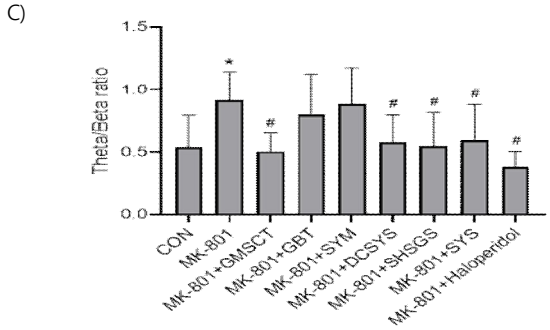


Fig. 3. Effects of six essential oils on EEG signals in an MK-801-induced anxiety-like model in zebrafish. (A) Heat map representing EEG relative power spectrum. (B-C) Delta/beta and theta/beta ratios. Data are presented as mean ± SD (n = 8 per group). ＊$p < 0.05$ vs. normal control (CON) group, #$p < 0.05$ vs. MK-801 group (two-tailed unpaired Student's t-test).

4. Effects of six essential oils on TST in mice

To investigate whether the six essential oils have antidepressant-like effects on TST, mice were intranasally treated with 20 mg/kg of essential oils 30 min before the test. As shown in Fig. 4, GBT, DCSYS, SHSGS, and SYS significantly decreased the immobility time in TST ($p < 0.05$). However, GMSCT and SYM did not significantly decrease the immobility time(Fig. 4).

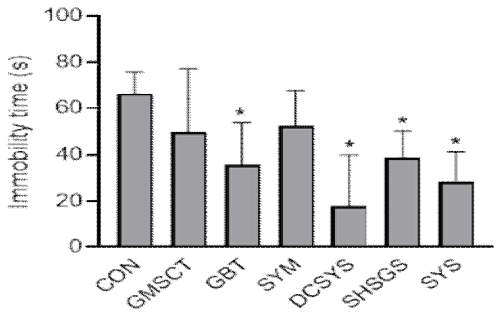


Fig. 4. Effects of six essential oils on the immobility time in the TST in mice. Data are presented as mean ± SD (n = 4 per group). ＊$p < 0.05$ vs. normal control (CON) group (two-tailed unpaired Student's t-test).

Discussion

Essential oils have been considered common alternative medicines for the treatment of psychiatric disorders, including anxiety and depression, with high effectiveness and few side effects[19,20]. In Korean medicine, several herbal prescriptions have been commonly used to treat anxiety and depressive syndromes, such as GMSCT, GBT, SYM, DCSYS, SHSGS, and SYS, and their efficacy has been demonstrated previously in clinical studies[21,22]. This study investigated the potential therapeutic effects of essential oils from these herbal prescriptions on anxiety and depression

using in vitro and in vivo models.

Clinical studies have indicated that hyperactivity of the HPA axis and elevated glucocorticoid levels are commonly observed in patients with anxiety and depression disorders[23]. In addition, chronic administration of CORT leads to a reduction in hippocampal volume and cell proliferation, associated with anxiety and depression behaviors in mice[24]. Clinical studies have demonstrated that cortisol levels are negatively correlated with hippocampal volume[25] and patients with mood disorders exhibit decreased hippocampal volume[26]. The pheochromocytoma PC12 cell line is commonly used in neuroscience research because of its typical features in neuronal cells[27]. PC12 cells show high expression of glucocorticoid receptors, and when stimulated with high concentrations of CORT may be used as an in vitro model of neuronal damage caused by glucocorticoids[28]. Hence, agents that show protective effects against CORT-induced toxicity in PC12 cells may have the potential for the treatment of anxiety and depression. In this study, all six essential oils from herbal prescriptions ameliorated CORT-induced neurotoxicity, indicating their potential therapeutic effects on psychiatric disorders by alleviating neuronal damage.

Accumulating evidence suggests that neuroinflammation is associated with the development of various psychiatric diseases such as anxiety and depression[29]. Microglia are the main immune cells in the central nervous system that modulate neuroinflammatory responses by secreting a variety of proinflammatory mediators, including IL-1β, IL-6, and TNF-α[30]. Elevated production of these inflammatory cytokines may cause neuronal damage and induce anxiety and depression symptoms[10]. Previous studies have demonstrated that lipopolysaccharides (LPS), the main component of the outer membrane of Gram-negative bacteria, can activate microglial cells to induce neuroinflammation and anxiety- and depression-like behaviors in mice[31]. LPS-treated BV2 microglia are commonly used to investigate the anti-neuroinflammatory effects of drugs in vitro[32]. In this study, LPS induced the secretion of TNF-α, IL-1β, and IL-6 in BV2 cells. In contrast, GBT reduced IL-1β production and SHSGS decreased TNF-α production, suggesting that these essential oils may partially suppress microglial activation and neuroinflammation to modulate anxiety and depression symptoms.

Recently, a zebrafish model has been developed and used in studies of psychiatric diseases, including anxiety[33]. Zebrafish are considered a good model for drug screening and development because they are cost-effective to maintain, and treatments can be easily performed by administering drugs to the housing tank[34]. A previous study showed that exposure to MK-801, an antagonist of the N-methyl-D-aspartate receptor, induced anxiety-like behavior in a zebrafish model[35]. EEG has been considered an effective method to study brain functions and psychiatric disorders, including anxiety[36]. EEG frequency bands consist of delta, theta, alpha, beta, and gamma. Among them, the lower frequency bands, such as delta and theta are involved in the regulation of motivation and emotion, while the higher frequency bands like beta are responsible for attentional control and cognition[37,38]. Increased theta/beta and delta/beta ratios were reported to be associated with changes in brain activities and showed a positive correlation with the severity of social anxiety disorder, hence they might be used as markers of anxiety[39-41]. In this study, MK-801 administration induced changes in the delta and beta EEG power densities and associated with increases in theta/beta and delta/beta ratios. In contrast, treatment with GMSCT and SHSGS essential oils reversed the changes in the EEG signals and decreased the theta/beta and delta/beta ratios. The results suggest that these essential oils exert anxiolytic-like effects by regulating the EEG power spectrum.

The antidepressant-like effects of essential oils from the six herbal prescriptions were investigated using TST in mice. A previous study suggested that intranasal administration of clove essential oils at doses lower than the intraperitoneal route exerted the same effects on behavioral impairment[42]. In addition, intranasal administration of drugs can induce rapid-acting effects with high bioavailability by avoiding first-pass metabolism[43]. In this study, mice were intranasally administered essential oils 30 min before behavioral testing. TST is commonly used to examine the antidepressant effects of drugs by evaluating the immobility time of the mice when being suspended by their tail. Agents with antidepressant potential show a decrease in immobility time and an increase in escape behavior[44]. We showed that treatment with GBT, DCSYS, SHSGS, and SYS significantly reduced the immobility time in the TST, indicating the antidepressant potential of these essential oils. However, our study still has some limitations. A positive control-treat group, such as fluoxetine was not included and only one dose of essential oils was used in the TST. In this study, we aimed to screen the antidepressant effects of the six essential oils in mice. Hence, dose-dependent experiments using effective essential

oils with a positive control group should be conducted in the future for further investigation.

Among the six essential oils evaluated, SHSGS may be the most effective candidate, with both anxiolytic- and antidepressant-like effects, as well as neuroprotective and anti-neuroinflammatory effects. Our results are consistent with a previous study that showed that oral administration of a water extract of SHSGS at a dose of 1 g/kg ameliorated stress-induced anxiety and depression in mice[45]. In our study, small doses of SHSGS (20 mg/kg in mice or 10 mg/l in zebrafish) were sufficient to exhibit anxiolytic- and antidepressant-like effects. This demonstrates the advantages of intranasal administration of essential oils in the development of drugs for anxiety and depression. However, further studies are required to investigate the molecular mechanisms of action of SHSGS in the treatment of these psychiatric disorders.

Conclusion

This study examined the effects of six essential oils from herbal prescriptions on anxiety and depression, using in vitro and in vivo models. All six essential oils showed neuroprotective effects by ameliorating CORT-induced cytotoxicity in PC12 cells, whereas GBT and SHSGS inhibited LPS-induced production of IL-1β and TNF-α in BV2 cells. In a zebrafish model, GMSCT, SHSGS, DCSYS, and SYS improved MK-801-induced anxiety-related changes in the EEG power spectrum. Additionally, GBT, DCSYS, SHSGS, and SYS decreased the immobility time in the TST in mice, indicating that these essential oils may have antidepressant-like effects. Among the six essential oils, SHSGS may be the most effective candidate, with both anxiolytic- and antidepressant-like effects, as well as neuroprotective and anti-neuroinflammatory effects. Further research should be performed to investigate the mechanism of action of these essential oils in psychiatric disorders.

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