Inhibitory Effects of the Essential Oil from SuHeXiang Wan on the Central Nervous System after Inhalation

Byung-Soo Koo,a Seung-II Lee,a Jeoung-Hee Ha,a and Dong-Ung Lee*,d

a Department of Oriental Neuropsychiatry, College of Oriental Medicine, Dongguk University; Seoul 135–010, Korea; b School of Life and Health, Sorabol College; Gyeongju 780–711, Korea; c Department of Pharmacology, College of Medicine, Yeungnam University; Daegu, 705–717 Korea; and d Department of Biotechnology, College of Natural Science, Dongguk University; Gyeongju 780–714, Korea. Received October 23, 2003; accepted December 8, 2003

The present study was performed to evaluate the central nervous system inhibitory effects of the essential oil from SuHeXiang Wan (Storax pill), a prescription usually used for treating epilepsy in traditional Chinese medicine, on fragrance inhalation (aroma therapy). Preinhalation of the fragrance oil markedly delayed the appearance of pentetrazole-induced convulsion, but showed weak activities on picrotoxin- and strychnine-induced convulsions, which implies this drug may inhibit the convulsion by GABAergic neuromodulation. This essential oil inhibited the binding of [3H]Ro15-1788, a selective antagonist for the benzodiazepine receptor and also the binding of [3H]flunitrazepam, a selective agonist for the receptor, in the presence of γ-aminobutyric acid (GABA) and NaCl, showing a positive GABA shift, which suggested the strong possibility of the agonistic activity of the essential oil to the GABA/benzodiazepine receptor complex in rat cerebral cortices. Furthermore, inhalation inhibited the activity of GABA transaminase as the inhalation period was lengthened. The GABA level was significantly increased and glutamate content was significantly decreased in mouse brain by preinhalation of the essential oil. The above results suggest that the anticonvulsive effect of this essential oil can also originate from the enhancement of GABA level in the mouse brain, because convulsion depends partially on the GABAergic neuromodulation. This essential oil to the GABA/benzodiazepine receptor complex in rat cerebral cortices. Furthermore, inhalation increased the pentetrazole-induced sleeping time as inhalation time was lengthened and inhibited brain lipid peroxidation, to which the anticonvulsive action is attributed; this also supported the above results, confirming the inhibitory effects of the essential oil of SuHeXiang Wan on the CNS via the GABAergic system.

Key words SuHeXiang Wan; essential oil; anticonvulsive effect; GABAergic neuromodulation; sedative effect; aroma therapy

SuHeXiang Wan (SHXW), a Chinese traditional medicinal prescription (Storax pill), consists of 15 crude herbs, among them, Suhexiang (Liquidambar orientalis), Muxiang (Saussurea lappa), Chenxiang (Aquilaria agallocha), Tanxiang (Santalum album), Ruxiang (Bowellia Carterii), Dingxiang (Euuga Caryophyllata), Anxiang (Styrax benzoin), Longnaoxiang (Dryobalanops aromatica), and Xiangfuzi (Cyperus rotundus) have the term “Xiang” (fragrance) in their Chinese plant names, which may hint that the essential oils in this prescription play an important role in their physiological effects.

This prescription has been used orally for the treatment of seizures, infantile convulsion, Qi (spirit) obstruction, sudden loss of consciousness, stroke and so forth.1 Because this composite drug has a strong fragrance, its essential oil components may be closely related with any effect on CNS after direct inhalation of its fragrance (aroma therapy). Six kinds of major volatile constituents of SHXW have been reported.2

Pure fragrance compounds and essential oils with sedative properties influenced the motility of mice in inhalation studies under standardized conditions.3 To date, only a few papers have reported on sedative or activating properties of some essential oils on animals after fragrance inhalation under standardized experimental procedures.4–7 We recently introduced the inhibitory effects of the fragrance inhalation of essential oil from Acorus gramineus on CNS: preinhalation of the fragrance oil delayed the appearance of pentetrazole-induced convulsion, inhibited γ-aminobutyric acid (GABA) transaminase activity, increased brain GABA level, prolonged the pentetrazole-induced sleeping time and inhibited brain lipid peroxidation.8

We now report the effects of an essential oil mixture of the fragrance containing herbs in the Chinese prescription SHXW on the sedative effect, anticonvulsant property and antioxidative activity after fragrance inhalation. The possible anticonvulsant action mechanism will also be discussed.

MATERIALS AND METHODS

Materials All medicinal plants were purchased from a traditional herb market located in Yeongchon, Korea and were identified by Prof. Byung-Soo Kang, College of Oriental Medicine, Dongguk University, Gyeongju, Korea. GABA, glutamate, vigabatrin, α-ketoglutaric acid, β-NADP, pentetrazole, α-phthaldialdehyde, 2-aminoethylisothio-uro-nium bromide, pyridoxal-5-phosphate, chlorpromazine hydrochloride, and sodium pentobarbital were obtained from Sigma Co. (St. Louis, MO, U.S.A.). Ro15-1788 was provided by Hoffmann-Roche Co. (Basel, Switzerland). [3H]Flunitrazepam and [3H]Ro15-1788 were bought from NEN Life Science Products (Boston, MA, U.S.A.). Scintillation cocktail (Aquasol-2) was bought from Packard Instruments B.V. Chemical Corporation (Groningen, Netherlands). The bichinchoninic acid protein assay kit was purchased from Pierce Chemicals (Rockford, IL, U.S.A.). All other chemicals and reagents were of the highest grade available.

Animal Studies Male 8-week-old outbred ICR mice with a mean weight of 28.5 g were housed in groups of seven under standardized conditions (room temperature: 21 ± 2 °C, relative humidity: 50—60%, light–dark rhythm: 12 h cycle). Male Sprague-Dawley rats weighing 250—350 g were used for the receptor preparation. A special cage (Three-Shine...
Co., Seoul, Korea) was used for inhalation of the fragrance: 2 g of fragrance oils on a petri dish (8.5 cm diameter) was put in each cage (W 26×L 22×H 20 cm) and allowed to evaporate. The cage cap was equipped with a special filter which passed minimum breathing air. Concentration of the fragrance in the cage was not determined. Essential oil was inhaled two times per day (for 3 h every morning and afternoon) for 7—14 d, respectively.

Preparation of Essential Oil A total of 200.02 g of the mixture of Liquidambar orientalis (22.86 g), Saussurea lappa (22.86 g), Aquilaria gallocha (17.14 g), Santalum album (22.86 g), Boswellia carterii (22.86 g), Eugenia caryophyllata (22.86 g), Cyperus rotundus (22.86 g), Styrax benzoin (22.86 g), and Dryobalanops aromatica (22.86 g) was pulverized and extracted once with 1 l of 25% (v/v) hexane at room temperature for 48 h, then filtered. The filtrate was evaporated under 80 °C to remove hexane, which was further eliminated in vacuo for 5 min at room temperature to give 29.7 g of clear pale brown essential oil.

Anticonvulsant Activity Assay Each animal inhaled for 7 d, then pentylentetrazole (70 mg/kg), picrotoxin (5.0 mg/kg) and strychnine (2.5 mg/kg) were respectively injected subcutaneously 1 h after the last inhalation. Onset time of convulsion and lethality were recorded.

Receptor Binding Assay Assay was performed as in our previous report9 using 8.0 mg/ml of the essential oil in Tris–citrate buffer. In brief, the receptor was prepared using the cerebral cortex of rats, then the binding of [3H]Ro15-1788 (specific activity = 87.0 Ci/mmol) and [3H]flunitrazepam (specific activity = 82.0 Ci/mmol) to the plasma membranes was assayed using a filtration technique and the radioactivity retained by the filters was measured in a liquid scintillation spectrometer (Wallac 1410, Turku, Finland). Protein was determined by bichinchonic acid method. In addition, ‘GABA shift’ assays were employed to determine the pharmacological characteristics of the essential oil interacting with benzodiazepine receptor. If an additional increase in the ability of the samples to inhibit [3H]flunitrazepam binding was observed in the presence of GABA (final concentration of 20 μM) and NaCl (final concentration of 120 mM), this was interpreted as a ‘positive GABA shift’, suggesting that the receptor ligand in the sample had agonistic properties.

GABA Transaminase Assay Animals were anesthetized with diethyl ether and perfused with normal saline to exclude any disturbance from intravascular substances. The whole brain was isolated, and then homogenized with a glass Teflon homogenizer in 4 volumes of 0.1 M potassium phosphate buffer (pH 7.4). Homogenates were centrifuged in 600×g for 10 min at 4°C, supernatant was collected and recentrifuged in 10000×g for 20 min at 4°C. Postmitochondrial fractions were ultracentrifuged (Kontron T-2080, Switzerland) in 105000×g for 1 h, and the supernatant was used as an enzymatic source in the GABA transaminase assay. GABA, α-ketoglutaric acid, 0.15 M potassium phosphate buffer (pH 8.0) and tissue homogenates were incubated in 37°C for 30 min, followed by the addition of NADP+. The amount of NADPH generated in the brain tissue for 20 min was measured by spectrophotometer (Ultrspec 2000, Pharmacia, U.S.A.) at 340 nm as an activity of GABA transaminase.

Determination of GABA/Glutamate Levels in Brain Concentrations of GABA and glutamate in the brains of PTZ-treated mice used for the anticonvulsant activity assay were measured using a modified method of Allen and Griffiths.10 Tissues were homogenized in 0.3 M triethanolamine buffer (pH 6.8) containing 1 mM of aminomethylisothiourea boronide and 2 mM pyridoxal-5’-phosphate, then centrifuged at 15000×g for 20 min. Postmitochondrial fraction from each extract was resuspended in 200 mM potassium phosphate buffer, deproteinized, and then centrifuged. Supernatants were filtered by membrane filter (0.2 μm; 13 mm), o-phthaldialdehyde-derivated GABA and glutamate were used to detect fluorescence in the HPLC measurement (LC-10AD, Shimadzu, Japan). An Inertsil ODS-3 (150×4.6 mm I.D., 5 μm) column was used. HPLC was performed using gradients of methanol (20% to 0% in 40 min) in 10 mM potassium acetate buffer (pH 6.5), at a flow rate of 0.6 ml/min.

Pentobarbital Induced-Sleeping Time Assay Mice were administered sodium pentobarbital (50 mg/kg in saline) intraperitoneally, 1, 2, 5 and 10 h after fragrance inhalation. Sleeping time was recorded from the disappearance of the righting reflex until its recovery and compared with those of the control group and the chlorpromazine-treated group (positive control).

Lipid Peroxidation Assay According to the method of Ohkawa et al.,11 tissue homogenates, 8.1% sodium dodecyl sulfate, 20% acetate buffer (pH 3.5) and 0.8% 2-thiobarbituric acid were incubated for 1 h at 95°C, and then cooled to room temperature. The thiobarbituric acid reactive substance (pink color) in the reactant was transferred to a mixture of n-butanol : pyridine (15 : 1) and its absorbance was measured at 532 nm as the degree of lipid peroxidation. The level of lipid peroxides was expressed in terms of MDA (malondialdehyde) equivalents (nmoles MDA/g of tissue).

GC/MS Analysis For qualitative and quantitative analysis of the components of an essential oil, gas chromatography–mass spectrometry (GC–MS) was performed on a Hewlett Packard 5890 series II instrument connected to an Automass 50 (JEOL). The operating conditions were as follows: column fused silica capillary column, TC-wax (Hewlett Packard), 60 m×0.25 mm, film thickness = 0.25 μm; column temperature: 40—300°C increasing at 5°C/min to 150°C, then 15°C/min to 300°C, ending at 300°C for 5 min; injector: 180°C; carrier gas: nitrogen at a flow rate of 30 cm/s; column head pressure: 180 kPa; injection volume: 0.5 μl; ionization energy: 70 eV; ion source temperature: 200°C. Chemical components were identified by comparing their retention times and mass spectra with those of authentic samples or by comparing their mass spectra with those in the MS data library (NBS library). The relative amount of each component was determined by calculating the peak area of the TIC chromatogram.

Statistical Analysis Data are expressed as the mean± S.E. of the number (n) of experiments. Statistical analysis of difference was determined by Student’s t-test or analysis of variance (ANOVA), followed by Neuman–Keuls multiple comparision analysis package (Systat Inc., Evanston, IL, U.S.A.).

RESULTS

Anticonvulsant Activity The effects of the preinhalation
of an essential oil on pentylentetrazole (PTZ)-, picrotoxin (PCX)-, and strychnine (STN)-induced convulsions in mice were observed. All inhaling groups showed a delayed appearance of convulsion compared with control groups, (Table 1) however, their effects were differed. In the PTZ-treated group the onset time was significantly (p<0.05) lengthened to 112.5% compared with control, but the PCX- (26.6%) and STN-treated group (13.9%) were weak. Lethality was strongly decreased from 42.8% for control to no death in the PTZ-treated group, but was not reduced in the PCX- or STN-treated group.

**Agonistic Effect on GABA/Benzodiazepine Receptor Complex** The essential oil inhibited the specific binding of [3H]Ro15-1788, a selective benzodiazepine receptor antagonist, to rat cerebrocortical membranes in a dose-dependent manner (Fig. 1) and enhanced [3H]flunitrazepam, a selective agonist for the receptor, binding to the receptor. However, in the presence of GABA, the essential oil inhibited [3H]flunitrazepam binding: in the presence of 20 μM GABA and 120 mM NaCl, percent inhibition of [3H]flunitrazepam binding (63.7±1.1) was significantly (p<0.05) higher than in its absence (−7.0±2.6) (Fig. 2).

**GABA Transaminase Inhibition** The inhalation of an essential oil led to inhibition of the activity of GABA transaminase. In all groups that inhaled the fragrance, production of NADPH (nmol/mg protein/h) by GABA transaminase was significantly (p<0.05) lower than that of the PTZ-treated group (Fig. 3). The levels of NADPH at 7 and 14 d inhalation were respectively decreased to 17.3% and 36.2% (p<0.05) of the PTZ-treated group (Fig. 3). Vigabatrin, a known anticonvulsant, exhibited 66.8% reduction against PTZ-treated group.

**GABA and Glutamate Levels** Preinhalation of an essential oil increased the brain GABA level and decreased glutamate level compared to the PTZ-treated group as shown in Table 2. Brain GABA contents (μM) were significantly (p<0.05) decreased in the PTZ-treated mice compared with the control group. However, GABA levels were significantly (p<0.05) increased by preinhalation, almost to the control level by 14 d inhalation of the fragrance. In the same experiment, brain glutamate content was strongly increased in the PTZ-treated group and decreased almost to the control level (p<0.05) after fragrance inhalation for 14 d.

**Sleeping Time Prolongation** Preinhalation of fragrance time-dependently prolonged the pentobarbital-induced sleep (Fig. 4). At the longest period (10 h inhalation), the sleeping time was significantly (p<0.05) increased by preinhalation, almost to the control level by 14 d inhalation of the fragrance. In the same experiment, brain glutamate content was strongly increased in the PTZ-treated group and decreased almost to the control level (p<0.05) after fragrance inhalation for 14 d.

### Table 1. Effect of the Preinhalation of Fragrance Oil on Pentylenetetrazole (PTZ)-, Picrotoxin (PCX)-, and Strychnine (STN)-Induced Convulsions in Mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Onset time (min)</th>
<th>Lethality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.4±0.22</td>
<td>42.8</td>
</tr>
<tr>
<td>Fragrance</td>
<td>5.1±0.95*</td>
<td>0</td>
</tr>
<tr>
<td>Vigabatrin</td>
<td>15.3±2.88*</td>
<td>0</td>
</tr>
</tbody>
</table>

Mice inhaled the essential oil two times per day (3 h every morning and afternoon) for 7 d. Vigabatrin was orally administered at a dose of 10 mg/kg. Inhalation for 14 d led to longer onset time (7.2±1.25*) in PTZ-treated mice. Values represent the mean±S.E. (n=7). * p<0.05, significantly different from the control group.

### Table 2. Effect of the Preinhalation of Fragrance on the Brain GABA and Glutamate Levels in PTZ-Treated Mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>GABA (μM)</th>
<th>Glutamate (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.54±0.56</td>
<td>3.37±0.32</td>
</tr>
<tr>
<td>PTZ</td>
<td>0.81±0.08</td>
<td>4.15±0.45</td>
</tr>
<tr>
<td>7 Days</td>
<td>1.84±0.12</td>
<td>4.06±0.38</td>
</tr>
<tr>
<td>14 Days</td>
<td>2.31±0.21</td>
<td>3.57±0.32</td>
</tr>
</tbody>
</table>

Mice inhaled an essential oil two times per day (3 h every morning and afternoon) for 7 and 14 d. Values represent the mean±S.E. (n=7). a p<0.05, significantly different from control; b p<0.05, significantly different from PTZ-treated group.
time was increased by 88.1% of the control. Chlorpromazine hydrochloride (10 mg/kg, p.o.), a positive control, showed 119.9% prolongation as compared with the control group.

**Anti-Lipid Peroxidation** Preinhalation of fragrance for 7 d had an inhibitory effect on lipid peroxidation as shown in Fig. 5. Brain lipid peroxidation (MDA mmoles/g of tissues) was significantly (p<0.05) increased in the PTZ-treated mice compared with control (34.9% increase). However, lipid peroxides were significantly (p<0.05) diminished by 17.1% compared with the level of the PTZ-treated group by fragrance inhalation.

**GC/MS Analysis** The results of GC-MS analysis of the essential oil are shown in Table 3. The content of borneol and iso-borneol was over 50%. The reported components2) (endo-Caryophyllene, endo-Borneol) were partially corrected and some others were additionally detected under our analytical conditions.

### DISCUSSION

Preinhalation of the essential oil of SuHeXiang Wan (SHXW) markedly delayed the appearance of pentyleneetetrazole (PTZ)-induced convulsion and lethality (Table 1). PTZ is known to block the action of GABA in the CNS, inducing convulsion.13) Therefore, this result indicated that the inhalation of the SHXW essential oil inhibited the convulsion by agonistic action to the GABA/benzodiazepine receptor, activating the chloride channel. Picrotoxin (PCX)- and strychnine (STN)-induced convulsions were not significantly inhibited by fragrance inhalation.

Although we did not measure the blood level of the essential oil components in animals, the fragrance inhalation apparently affected the nervous system more than control, suggesting that some active components affected GABA- and glutamate-neurons in the CNS. In connection with this, we examined the effect of essential oil on the radioligands to the GABAA-benzodiazepine receptor complexes of rat cerebral cortices. In an *in vitro* test, the essential oil inhibited the binding of[^1^H]Ro15-1788, a selective antagonist for the benzodiazepine receptor, in a dose-dependent manner (Fig. 1), of which the effect corresponded to the diazepam equivalent (mg/g of tissue weight) of 4.93±0.22. Furthermore, this essential oil significantly (p<0.05) inhibited the binding of[^1^H]fluunitrazepam, a selective agonist for the receptor, in the presence of 20 μM GABA and 120 mM NaCl compared to the control (absence of GABA) (Fig. 2), and this ‘positive GABA shift’ supported the strong possibility of the agonistic activity of the essential oil to the receptor.

Positive modulation on the GABAergic neurotransmission by endogenous benzodiazepine receptor agonists found in mammalian brain has been reported to be increased in the presence of GABA.13—15) These results suggest that the SHXW essential oil can allosterically modulate the GABAergic neurotransmission via enhancement of the binding of endogenous receptor agonist in the presence of GABA, a major inhibitory neurotransmitter in the mammalian brain.

The inhalation of the SHXW essential oil inhibited GABA transaminase, a degrading enzyme for GABA as the inhalation period was lengthened from 7 to 14 d (Fig. 3). This result of enzyme inhibition supported the above anticonvulsant effect of fragrance inhalation on PTZ-induced convulsion, because the proper level of GABA in brain plays an important role in anticonvulsion. Next, we determined the level of GABA as well as the content of glutamate, an excitatory neurotransmitter, in mouse brain. The GABA level was significantly (p<0.05) increased and glutamate content was significantly (p<0.05) decreased by preinhalation of the essential oil compared with each control group (Table 2). The above results suggest that the anticonvulsive effect of the essential oil of SHXW is partially supported by the enhancement of GABA level in the mouse brain, because convulsion depends

---

**Table 3. Composition of the Fragrance Oil Analyzed by GC-MS**

<table>
<thead>
<tr>
<th>Retention time (min)</th>
<th>Compound[^a^]</th>
<th>Content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.86</td>
<td>Fenchol</td>
<td>0.50</td>
</tr>
<tr>
<td>12.76</td>
<td>Camphor</td>
<td>1.11</td>
</tr>
<tr>
<td>13.10</td>
<td>Borneol</td>
<td>21.30</td>
</tr>
<tr>
<td>13.38</td>
<td>endo-Borneol</td>
<td>33.30</td>
</tr>
<tr>
<td>14.71</td>
<td>Octyl acetate</td>
<td>0.54</td>
</tr>
<tr>
<td>18.60</td>
<td>Eugenol</td>
<td>5.93</td>
</tr>
<tr>
<td>20.19</td>
<td>β-Caryophyllene</td>
<td>0.72</td>
</tr>
<tr>
<td>22.83</td>
<td>Eugenyl acetate</td>
<td>2.19</td>
</tr>
<tr>
<td>26.33</td>
<td>Benzyl benzoate</td>
<td>5.38</td>
</tr>
<tr>
<td>28.98</td>
<td>Benzyl cinnamate</td>
<td>1.95</td>
</tr>
</tbody>
</table>

[^a^] Compounds having over 0.5% content are listed.
partially on GABA concentration which can be properly preserved by inhibiting GABA transaminase.

To investigate whether the preinhalation of this fragrance also affects sedation in the CNS, the prolongation of sleep was estimated. Fragrance inhalation progressively prolonged the pentobarbital-induced sleeping time as inhalation time was lengthened (Fig. 4). The effect of the longest inhalation time (10 h), however, was much weaker than that of chlorpromazine, a positive control.

Finally, we examined the antioxidative activity of the SHXW essential oil after fragrance inhalation in mice. It has been stated that the excitatory amino acid (e.g., glutamate) is released by oxidative stress conditions to afford neuronal excitation\(^\text{16}^\) and the anticonvulsive effect of the plant extract may be attributable to the antioxidant activity of its active components.\(^\text{17}^\) The content of brain peroxy-lipids (MDA moles/g of tissue) was significantly \((p<0.05)\) increased in PTZ-treated mice compared with control. However, this lipid peroxidation was significantly \((p<0.05)\) diminished almost to the level of control by preinhalation of the essential oil fragrance (Fig. 5). This antioxidant activity may be related to the anticonvulsant effect of the essential oil.

SHXW essential oil was analyzed by a GC-MS system to exhibit ten compounds (over 0.5% content) including \textit{endo}-borneol (33.3%) and borneol (21.3%) as main components (Table 3). Instead of the presence of iso-borneol and the absence of borneol in the previous study,\(^\text{2}^\) our analytical conditions detected \textit{endo}-borneol and borneol as monoterpenoids with high quality (over 92%).

In conclusion, the essential oil of the Chinese medicinal prescription, SuHeXiang Wan (Storax Pill) possesses anticonvulsive and sedative actions by direct inhalation of the essential oil fragrance, which may be useful for aroma therapy.

**Acknowledgement** This work was supported by grant No. R05-2002-000-00682-0 from the Basic Research Program of the Korea Science & Engineering Foundation.

**REFERENCES**