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# Effects of the Essential Oil from Modified SuHeXiang Wan (Storax Pill) in Mice after Inhalation, Oral Administration, and Inunction

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## 加減蘇合香元 精油香氣의 吸入, 經口投與 및 皮膚塗擦에 따른 鎭痛效果 比較

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요 약

목적 : 소합향원 (蘇合香元) 加減方 정유향기의 진통효과가 투여방법에 따라 어떤 차이가 있는지를 비교, 검토함으로서 본 약재의 새로운 적용방법을 탐색해 보고자 한다.

방법 : 가감소합향원에서 추출한 정유향기를 실험동물을 대상으로 7일간 경구 (50mg/kg, 100mg/kg), 흡입 (매일 3시간씩 1일 2회), 또는 피부도찰 (10mg) 방법으로 투여하여 페닐퀴논-유도 writhing test, 아세트산-유도 writhing test 및 hot-plate test를 실시하였다.

결과 : 가감소합향원 정유향기는 페닐퀴논-유도 writhing test에서는 피부도찰이 가장 효과적이었으며 그 효과는 양성 대조약물인 아세트아미노펜 보다 약간 더 우수하였다 ($p<0.05$). 아세트산-유도 writhing test에서도 피부도찰법이 가장 좋은 효과를 나타내었으며 양성대조 약물인 아미노피린보다는 좀 더 좋은 효과를 보여주었다 ($p<0.05$). 그러나 hot-plate test에서는 경구투여가 가장 좋은 진통효과를 나타내었는데 고용량 (100mg/kg)에서는 양성 대조약물인 아세트아미노펜보다 유의적으로 더 효과적이었으나 저용량 (50mg/kg)에서는 이보다 다소 약하였다. 이상의 연구결과, 가감소합향원의 정유향기는 피부도찰시에는 비특이적 화학자극제인 페닐퀴논이나 아세트산에 의한 통증의 완화에 보다 효과적이며, 경구투여시에는 중추신경계에 영향을 미치는 hot plate에 의한 열자극성 통증에 더 유효함을 의미한다.

결론 : 가감소합향원의 정유향기액은 향기흡입보다는 피부흡수나 경구투여방법으로 진통의 목적으로 응용될 수 있을 것으로 사료된다.

주제어 : 가감소합향원, 정유향기, 진통효과, writhing test, hot-plate test

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# I. Introduction

SuHeXiang Wan (SHXW), a Korean and Chinese traditional medicinal prescription (Storax Pill), consists of 15 crude herbs including Suhexiang ( *Liquidambarorientalis* ), Muxiang ( *Saussurealappa* ), Chenxiang ( *Aquilariaagalloch a* ), Tanxiang ( *Santalumalbum* ), Ruxiang ( *Boswelliacarterii* ), Dingxiang ( *Eugeniacaryophyllata* ), Anxixiang ( *Styraxbenzoin* ), Longnaoxiang ( *Dryobalanopsaromatica* ), and Xiangfuzi ( *Cyperusrotundus* ). All these herbs have the term "Xiang" (fragrance) in their Chinese plant names, providing a hint that the essential oils in this prescription play an important role in their physiological effects.

The Storax Pill has been used orally for the treatment of conditions that include seizures, infantile convulsion, Qi (spirit) obstruction, sudden loss of consciousness, and stroke[1]. Given the strong fragrance of this composite drug, it is conceivable that the essential oil components may be closely related with an effect(s) on the central nervous system after direct inhalation (aroma therapy). We have previously reported that preinhalation of the fragrance oil markedly delays the appearance of pentylenetetrazole-induced convulsion, inhibits the activity of ɣ-aminobutyric acid (GABA) transaminase, increases the GABA level in the mouse brain, and prolonges the pentobarbital-induced sleeping time, and that the essential oil itself exhibits agonistic activity to the GABA/benzodiazepine receptor complex in the rat cerebral cortex[2]. However, the analgesic effect of SHXW essential oil has not yet been investigated.

Pure fragrance compounds and essential oils with sedative properties have been shown to influence the motility of mice in inhalation studies under standardized conditions[3]. Todate, only a few papers have reported on the sedative or activating properties of some essential oils on animals after fragrance inhalation under standardized experimental procedures[4-7]. As a part of our continuing search for aroma therapy-delivered sedatives[2,8], we presently investigated the analgesic effects of the essential oil fragrance of SHXW after inhalation and compared its effect with the per os and inunction routes of administration.

# II. Materials and Methods

## 1. Materials

All medicinal plants were purchased from a traditional herb market located in Yeongchon, Korea. Phenylbenzoquinone, Tween 80, acetaminophen, 4 - dimethylaminoantipyrine ( aminopyrine ), chlorpromazine hydrochloride were obtained from Sigma - Aldrich ( St.Louis, MO, USA). Sodium pentobarbital was provided as an injectable solution ( Hallim Pharmaceutical, Seoul, Korea ). Ointment base for inunction was obtained from Sang-A Pharmaceutical ( Seoul, Korea ). All other chemicals and reagents were of the highest grade available.

## 2. 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 ℃, relative humidity of 50% - 60%, 12 h alternating cycle of light and dark). A special cage (W 26 cm × L 22 cm × H 20 cm) purchased from Three-Shine (Seoul, Korea) was used for inhalation of the fragrance. In the procedure, 2 g of fragrance oils on a 8.5 cm diameter Petri dish was put in each cage and allowed to evaporate. The cage cap was equipped with a special filter to minimize the passage of breathing air. Concentration of the fragrance in the cage was not determined. Essential oil was inhaled two times per day (3 h every morning and afternoon) for 7 consecutive days. In the skin absorption test, 10 mg of fragrance oil and acetaminophen was mixed with 10 mg of ointment base, and the mixture was applied to the tail of each tested mouse.

## 3. Preparation of essential oil

A total of 200.02 g of the prescription (Table 1) was pulverized and extracted once with 1 l of *n*-hexane at room temperature for 48 h, and then filtered. The filtrate was evaporated under 80℃ 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 oils.

Table 1. Prescription of the Modified SuHeXiang Wan (Storax Pill)

| Drug | Content (g) |
|---|---|
| Liquid ambarorientalis | 22.86 |
| Saussurea lappa | 22.86 |
| Aquilaria agallocha | 17.14 |
| Santalum album | 22.86 |
| Boswellia carterii | 22.86 |
| Eugenia caryophyllata | 22.86 |
| Cyperus rotundus | 22.86 |
| Styrax benzoin | 22.86 |
| Dryobalanops aromatica | 22.86 |
| Total amount | 200.02 |

## 4. Phenylquinone-induced writhing test

The test was carried out as previously described[9]. The essential oil was either inhaled, given orally (50 mg/kg and 100 mg/kg in 20% Tween 80+0.5% citric acid+4% benzylalcohol solution), or applied (10 mg) to the tail of each tested mouse. Each test was conducted daily for 7 consecutive days. Mice were injected intraperitoneally with 4.5 ml/kg body weight of 0.03% phenylquinone ( 0.1 ml) in 5% ethanol-saline 1 h after each application of essential oil. The number of writhing syndromes (a response consisting of abdominal wall contraction and pelvic rotation followed by hind limb extension) and writhing latency time were recorded for 20 min. Acetaminophen (0.1 ml, 80 mg/kg in EtOH-Tween80-saline 10 :10:80v/v%) was intraperitoneally administered as a positive control.

## 5. Acetic acid-induced writhing test

The Whittle's technique was utilized[10].

The essential oil fragrance was inhaled and given orally (50 mg/kg and 100 mg/kg) to the mice for 7 days. Mice were injected intraperitoneally with 10 ml/kg body weight of 0.7% acetic acid solution in saline 1 h after each administration of essential oil. The number of writhing syndromes and writhing latency time were recorded for 20 min. Aminopyrine (0.1 ml, 100 mg/kg in saline) was orally administered as a positive control.

### 6. Hot plate test

As described previously[11], mice were placed on a hot plate maintained at 50 - 55℃ for testing. The essential oil fragrance was inhaled and given orally (50 mg/kg and 100 mg/kg) to the mice for 7 days. The time between placement and responses, which included shaking or licking of the hind paws, or jumping, was recorded as the hot plate latency. A manual 40s cut-off time was used to prevent tissue damage. Acetaminophene (0.1 ml, 80 mg/kg in EtOH-Tween 80-saline 10:10:80v/v%) was intraperitoneally administered as a positive control.

### 7. Statistical analyses

Data were expressed as the mean ± S.E. of the number ($n$) of experiments. Statistical analysis of difference was determined by Student's $t$-test or ANOVA, followed by Neuman-Keuls multiple comparisons analysis package (Systat, Evanston, IL, USA).

## III. Results and Discussion

The analgesic effect of the essential oil of SHXW (Storax Pill) was investigated with inhalation, per os, and inunction routes of administration in mice. The effects were evaluated by chemically induced tissue damage using phenylbenzoquinone and acetic acid ( writhing test ) and thermal stimulus ( hot plate test ). As presented in Table 1, essential oil of SHXW inhibited the phenylbenzoquinone - induced writhing response, but differences were evident depending on the administration route.

Table 1. Analgesic Effect of Differently Administered Essential Oil on Phenylbenzoquinone-induced Writhing in Mice.

| | Route | Dose | Writhing frequency | Writhing latency (s) | Inhibition(%) |
|---|---|---|---|---|---|
| Control | | | 26.7±4.4 | 452.7±136.7 | - |
| Essential oil | inhalation | 6 h | 17.4±5.2 | 334.8±81.1 | 34.8 |
| | per os | 50 mg/kg | 18.4±1.7* | 357.4±43.1 | 31.1 |
| | | 100 mg/kg | 12.2±5.0* | 445.4±44.7 | 54.3 |
| | inunction | 10 mg | 4.4±1.5* | 559.2±86.5 | 83.5 |
| Acetaminophen | per os | 80 mg/kg | 8.0±4.0* | 582.8±178.0 | 70.0 |
| | inunction | 10 mg | 5.6±3.3* | 766.8±191.8 | 79.0 |

Each values represents the mean ± S.E. (n = 7). * $p<0.05$, significantly different from control.

External application of essential oil on mice skin inhibited the phenylbenzoquinone -induced writhing response most potently (83.5%) and significantly ($p<0.05$); the inhibition was superior to that of the positive control acetaminophen[12], as well as using the inunction (79.0%) and oral (70.0%) routes of administration. Inhalation of the essential oil fragrance showed an unexpectedly weak effect (34.8% inhibition), which was similar to that of oral administration at a dose of 50 mg/kg (31.1%) and much weaker than that observed at a dose of 100 mg/kg (54.3 %). We have previously demonstrated that inhalation of essential oil fragrance of SHXW results in the CNS inhibition as manifest by strong anti-convulsion induced by pentylenetetrazole, increase of the brain GABA level, and decrease of the brain glutamate level[2]. Presently, the change of writhing latency was similar to the writhing frequency. However, acetaminophen delayed the writhing response more than essential oil when applied by inunction. Several tissue stimulants are used in the writhing test for analgesic evaluation assay. These include bradykinin[13], hypertonicsaline[14], 5 - hydroxytryptamine[15], histamine[16], aceticacid[10], phenylbenzoquinone[17], and acetylcholine[18]. Among them, phenylbenzoquinone and acetic acid increase the peritoneal fluids of prostaglandins, serotonin, and histamine, and so commonly are used as a model for screening of peripheral analgesics[19].

To compare and confirm the analgesic effect of essential oil of SHXW, we performed the same writhing test using acetic acid as a stimulant. The results summarized in Table 2 showed that inunction of the essential oil and the analgesic aminopyrine[20] markedly decreased the writhing frequency by 62.0% and 55.5% ($p<0.05$), respectively, while other treatments revealed only marginal if any effect.

Table 2. Analgesic Effect of Differently Administered Essential Oil on Acetic Acid-induced Writhing in Mice.

| | Route | Dose | Writhing frequency | Writhing latency (s) | Inhibition(%) |
|---|---|---|---|---|---|
| Control | | | 27.4±6.2 | 363.2±38.7 | - |
| Essential oil | inhalation | 6 h | 26.6±4.2 | 263.4±23.3 | 2.9 |
| | per os | 50 mg/kg | 27.8±2.4 | 301.0±19.1 | 0 |
| | | 100 mg/kg | 18.2±6.6 | 335.0±44.9 | 33.6 |
| | inunction | 10 mg | 10.4±2.2* | 335.0±44.9 | 62.0 |
| Acetaminophen | per os | 100 mg/kg | 17.0±7.2 | 370.0±113.9 | 38.0 |
| | inunction | 10 mg | 12.2±1.7* | 390.8±34.7 | 55.5 |

Each values represents the mean ± S.E. (n = 7). * $p<0.05$, significantly different from control.

The starting time of the writhing syndrome was also markedly delayed when essential oil and aminopyrine was applied by inunction, as compared to the other administration routes. These observations indicate that the analgesic activity of SHXW essential oil and positive drugs can be exerted most effectively by direct application on skin in the chemically induced writhing test. It is known that absorption of the classic analgesic indometacin through the skin is very useful self-medication for pain relief[21]. In the latter study, the pain-relieving threshold of 0.75% indometacin ointment increased rapidly 1 h after application, producing an effect equal to that of compound products containing 1% indometacin.

Presently, the analgesic effect of SHXW essential oil was evaluated by another animal pain model, the hot plate test. This test is commonly used to examine central analgesic effects, because of its sensitivity to strong analgesics and limited tissue damage[11]. As shown in Table 3, the analgesic effect apparent using the hot plate test was observed regardless of the route of administration.

Table 3. Effect of Differently Administered Essential Oil on Pain Sensitivity Measured by Hot Plate Test in Mice.

| | Route | Dose | Jump latency (s) | Inhibition (%) |
|---|---|---|---|---|
| Control | | | 20.5±2.8 | - |
| Essential oil | inhalation | 6 h | 30.2±5.1* | 47.3 |
| | per os | 50 mg/kg | 31.4±5.3* | 53.2 |
| | | 100 mg/kg | 39.8±4.6* | 94.1 |
| | inunction | 10 mg | 32.3±3.8 | 57.6 |
| Acetaminophen | per os | 80 mg/kg | 35.0±5.0* | 70.7 |
| | inunction | 10 mg | 34.8±6.2 | 69.7 |

Each values represents the mean ± S.E. (n = 7). * $p<0.05$, significantly different from control.

However, contrary to the results obtained using the writhing test, the effect of oral administration was somewhat better than that of inunction. These observations suggest that the analgesic activity of SHXW essential oil could be related either to a peripheral site of action or to a central nervous mechanism. Most of the available evidence seems to support the role of monoamine neurotransmitters such as serotonin and of opiate neurons in the mechanism of pain control on the one hand and the mutual interactions of these systems on the other hand[22].

## IV. Conclusion

The essential oil fragrance of the modified SuHeXiang Wan (Storax Pill), a traditional medicinal prescription in Korea and China, possesses analgesic effect by either the oral route or by direct absorption into skin, similar to that produced by application of an ointment. Thus, the Storax Pill may be clinically useful for pain relief.

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