Effects of *Crocus sativus* L. on the Ethanol-Induced Impairment of Passive Avoidance Performance in Mice

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The acute effects of an alcohol extract of *Crocus sativus* L. (CS-extract) were studied on learning and memory in step through (ST) and step down (SD) tests in normal as well as in learning- and memory-impaired mice. A single oral administration of CS-extract had no effects on memory registration, consolidation or retrieval in normal mice. CS-Extract reduced the ethanol-induced impairment of memory registration both in ST and SD tests and the ethanol-induced impairment of memory retrieval in SD test. CS-Extract decreased the motor activity (MA) and prolonged the sleeping time induced by hexobarbital. These results suggested that CS-extract ameliorates the impairment effects of ethanol on learning and memory processes, and possesses a sedative effect.

**Keywords** *Crocus sativus*; ethanol; passive avoidance performance; sedative effect

*Crocus sativus* L. grows wild in southeast China, India and south European and has been used as a drug in these areas. Its medical value has been recognized and recorded in Yi-Lin-Ji-Yao, a traditional Chinese medical book comprised during the Ming Dynasty (A.D. 1514), and notable effects were described as promoting blood circulation to remove blood stasis. It has therefore commonly been used in the treatment of menstrual disturbances, thrombus disease, and some other high blood viscosity related diseases. Recently, it has been reported that an alcohol extract of *Crocus sativus* L. (CS-extract) inhibited skin chemical carcinogenesis and that its oral administration (200 mg/kg) had an anti-tumor activity in mice. It was also recorded that *Crocus sativus* L. was used in allaying fear, curing trance and some other disorders of the central nervous system, such as convulsion caused by fever and depression *etc.*, and these findings suggested that *Crocus sativus* L. exerted pharmacological actions on the central nervous system and may influence the central processes of learning and memory. However, there have been few reports on the effects of *Crocus sativus* L. on learning and memory in modern pharmacological research to date.

In this paper, the acute effects of CS-extract on learning and memory were evaluated in passive avoidance performances in normal and in learning- and memory-impaired mice.

**MATERIALS AND METHODS**

**Animals** Male Std-1dY mice, 5 weeks old, were kept under temperature- and humidity-controlled (22 ± 1°C, 55 ± 2%) and specific pathogen free conditions with food and water *ad libitum*, and used in our experiments after breeding for one week.

**Drug Preparation and Administration** The *Crocus sativus* L. used was artificially cultivated in Takoda prefecture of Ohita, Japan. The pistils of the plant were extracted with 50% ethanol overnight at room temperature 3 times. Then, the extract was concentrated in vacuo to yield 33%. CS-Extract was dissolved with physiological saline and given to the mice in a single oral administration. The selected doses were 125, 250 and 500 mg/kg.

**Passive Avoidance Performances. 1. Step Through (ST) Test** ST test was performed according to the method employed in our laboratory. The apparatus (PA M1, O'Mara & Co., Ltd., Tokyo, Japan) consisted of two compartments separated by a black wall with a hole in the lower middle part. One of the two chambers was illuminated and the other was dark. The test was conducted on two consecutive days at the same time of day. The first day (learning trial), the mouse was put into the lighted chamber. If it entered the dark compartment, it suffered an electric shock on its feet (36 V, 0.2 mA AC) through the stainless steel grid floor. The time when the mouse entered the dark chamber was recorded automatically and described as latency. On the second day (testing trial), the same test procedure was followed: mice were exposed in the light compartment for 60 s in the learning trial and 300 s in the testing trial. The latency and the number of mice which did not enter the dark compartment were recorded in the testing trial.

**2. Step Down (SD) Test** In the SD test, the chamber was equipped with the stainless steel grid floor through which an electric shock (50 V, 0.2 mA AC) was given. A platform was provided on part of the floor on which mouse could stay to avoid the electric shock. The first day (learning trial), mice were exposed to a 10 min-learning course during they were put on the platform and were punished by a foot shock if they got off the platform (error). The number of mice which did not step down and the number of errors in the latter half (5 min) of the learning trial were recorded. On the second day (testing trial), the same test procedures were performed at the same time of day, and mice were exposed for 3 min. The number of errors and the number of mice which did not step down to the floor were also recorded in the testing trial.

**3. Procedures of Drug Administration** Effects of CS-Extract on Passive Avoidance Performances in Normal Mice: CS-Extract was given i) 30 min before the learning trial, ii) immediately after the learning trial, or iii) 30 min before the testing trial, to evaluate its effects on the
registration, consolidation and retrieval of memory, respectively.

Effects of CS-Extract on Passive Avoidance Performances in Memory-Impaired Mice: i) CS-Extract was given 30 min before learning trial. Ethanol (30%, 10 ml/kg, p.o.) or scopolamine hydrobromide (0.5 mg/kg, i.p.) was given 20 min before the learning trial to cause memory registration impairment.

ii) CS-Extract was given 30 min before the testing trial. Ethanol (40%, 10 ml/kg, p.o.) was given 20 min before the testing trial to impair memory retrieval.

Measurement of the Motor Activity (MA), Fecal and Urinary Excretions A mouse was put into the testing cage which was a round (18 cm in diameter and 18 cm in height) tilting-type apparatus (Ambulometer MA001, O’Hara & Co., Ltd., Tokyo, Japan), and the amount of MA and the number of feces and urinary drops were recorded over a 30 min period. These measurements were conducted twice with a 24-h interval. CS-extract was given just before the first or second measurement.

Hexobarbital-Induced Sleeping Test Thirty min after CS-extract was given orally, mice were intraperitoneally injected with hexobarbital sodium (Teikoku Chemical Co., Ltd., Osaka, Japan) at the dose of 70 mg/kg. Chlorpromazine hydrochloride (CPZ, Sigma Chem. Co., Ltd.) was given (2 mg/kg i.p.) 20 min before hexobarbital injection. The time during the disappearance of the righting reflex was then recorded as sleeping time.

Statistics The latency in ST test, the number of errors in SD test and the number of feces and urinary drops were analyzed by Mann-Whitney’s U-test; the number of mice which did not make errors in either ST or SD test was analyzed by x²-test; and MA and the sleeping time, with ANOVA followed by Dunnett’s test.

RESULTS

Effects of CS-Extract on Memory Registration Effect of CS-Extract in Normal Mice: There were no differences between control and CS-extract-treated groups in either ST or SD test, suggesting that CS-extract had no effect on memory registration in normal mice (data not shown).

Effect of CS-Extract in Scopolamine-Treated Mice: CS-extract had no effect on scopolamine-induced impairment of memory registration in either of these tests (data not shown).

Effect of CS-Extract in Ethanol-Treated Mice: CS-extract improved the shortening of latency induced by ethanol significantly (Fig. 1A), but had no effect in increasing the number of successful mice decreased by

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Fig. 1. Effect of CS-Extract on Memory Registration in 30% Ethanol-Treated Mice in ST and SD Tests

A, learning trial; B, testing trial. A: the latency which indicated the time mice entered the dark compartments in ST test. B: mice which did not enter the dark compartment within 300 s were termed successful mice. C: the number of errors was the number of times of stepping down to the floor in SD test. D: the number of mice which made no errors in SD test. a) p<0.05, b) p<0.01 vs. control (cont); c) p<0.05 vs. EtOH control in Mann-Whitney’s U test; mean ± S.E.M., n=12.
Fig. 2. Effect of CS-Extract on Memory Retrieval in 40% Ethanol-Treated Mice in ST and SD Tests
For explanation, see Fig. 1.

Fig. 3. Effect of CS-Extract on MA in Mice
MA was measured immediately after CS-extract administration. , first measurement; , second measurement. A: CS-extract was given just before the first measurement. B: drug was given just before the second measurement. a) p<0.05, b) p<0.01 vs. control (cont) in ANOVA followed by Dunnett’s test; mean ± S.E.M., n=10—12.

ethanol in ST test (Fig. 1B). It also ameliorated the increase of errors induced by ethanol significantly (Fig. 1C), but had no effect on the decrease of the learned mice induced by ethanol in SD test (Fig. 1D). CS-Extract ameliorated the ethanol-induced memory registration impairment in both ST and SD tests (Fig. 1). The ameliorating effects were dose-dependent.

Effect of CS-Extract on Memory Consolidation in Normal Mice CS-Extract showed no effect on memory consolidation in normal mice (data not shown).
Table 1. Effects of CS-Extract on Fecal and Urinary Excretions in Mice

<table>
<thead>
<tr>
<th>Group</th>
<th>Dose (mg/kg)</th>
<th>No. of feces</th>
<th>No. of urinary drops</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1st-M</td>
<td>2nd-M</td>
</tr>
<tr>
<td>A:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cont.</td>
<td>7.4 ± 0.9</td>
<td>6.7 ± 0.9</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>CS-1</td>
<td>6.6 ± 1.0</td>
<td>7.7 ± 0.8</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>CS-2</td>
<td>5.5 ± 0.8</td>
<td>7.3 ± 0.7</td>
<td>0.5 ± 0.2</td>
</tr>
<tr>
<td>CS-3</td>
<td>5.6 ± 0.7</td>
<td>8.3 ± 0.9</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>B:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cont.</td>
<td>7.2 ± 0.6</td>
<td>8.2 ± 0.8</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>CS-1</td>
<td>6.9 ± 0.7</td>
<td>5.3 ± 0.9</td>
<td>0.5 ± 0.2</td>
</tr>
<tr>
<td>CS-2</td>
<td>6.6 ± 0.6</td>
<td>6.2 ± 0.7</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>CS-3</td>
<td>7.5 ± 0.6</td>
<td>5.2 ± 1.2</td>
<td>1.0 ± 0.3</td>
</tr>
</tbody>
</table>

CS-extract was given before the first (A) or the second (B) measurement and the number of feces and urinary drops eliminated by each mouse during MA measurement were counted. 1st-M, first measurement; 2nd-M, second measurement. a) p < 0.05, vs. Cont. in Mann-Whitney’s U test; mean ± S.E.M., n = 12.

Fig. 4. Effect of CS-Extract on Hexobarbital-Induced Sleeping in Mice

a) p < 0.05, b) p < 0.01 vs. control (cont) in ANOVA followed by Dunnett’s test, mean ± S.E.M., n = 10.

Effects of CS-Extract on Memory Retrieval Effect of CS-Extract in Normal Mice: CS-Extract showed no effect on memory retrieval in normal mice (data not shown).

Effect of CS-Extract in Ethanol-Treated Mice: CS-Extract significantly ameliorated the ethanol-induced impairment of memory retrieval in SD test (Fig. 2C, D), but not in ST test (Fig. 2A, B). The increase of errors and the decrease in number of learned mice were significantly ameliorated by CS-extract in SD test (Fig. 2C, D). The improving effects were dose-dependent.

Effects of CS-Extract on MA and Fecal and Urinary Excretions As shown in Fig. 3, when CS-extract was given just before the first or second measurement, MA was significantly decreased when the dose was more than 125 mg/kg, but this effect disappeared 24 h later. As shown in Table I, after CS-extract administration on the second day, fecal excretion was decreased significantly, and when given on the first day, it also showed a tendency to decrease.

Effect of CS-Extract on Hexobarbital-Induced Sleeping As shown in Fig. 4, the sleeping time induced by hexobarbital was significantly prolonged by administration of CS-extract, but its potency was obviously weaker than that of CPZ.

DISCUSSION

A single oral administration of CS-extract ameliorated the ethanol-induced impairment of memory registration in both ST and SD tests, and the ethanol-induced impairment of memory retrieval in SD test. It had no effect, however, on registration, consolidation or retrieval of memory in normal mice, and did not improve the scopolamine-induced impairment of memory registration in mice in either test. These results suggest that CS-extract has a specific ameliorating effect on ethanol-induced memory deficiency or the toxic effects of alcohol. Four possible mechanisms governing the effects are: 1) detoxification of alcohol by decreasing its absorption from the gastrointestinal tract; 2) acceleration of alcohol elimination from the brain by promoting its metabolism in the liver; 3) acceleration of alcohol elimination from the brain by promoting blood circulation; and 4) antagonization of the pharmacological effects of ethanol in the central nervous system. Experiments on these possibilities are now being conducted in our laboratory.

It has been reported that ethanol can cause severe memory deficiency in human beings, although the mechanism is not yet clear. Recent studies in animals have demonstrated that ethanol rather selectively affects several functional processes related to learning and memory in the central nervous system, and that it affects not only the central cholinergic system but the adrenergic system. More recently, it was reported that ethanol inhibited the responses mediated by NMDA type of glutamate receptors which is thought to play an important role in learning and memory processes.

In our previous studies (unpublished), oral administration of 30% or 40% ethanol to mice impaired memory registration or memory retrieval, respectively, in passive avoidance performances. But 30% ethanol did not prolong hexobarbital-induced sleeping time, and showed no influence on the motor activity or 0.7% acetic acid-induced writhing syndrome 20—30 min after its administration. This is the reason mice of which passive avoidance performances were impaired by ethanol have been used as one of the animal models in behavioral pharmacological research. The present results suggested that CS-extract ameliorated the ethanol-induced impairment of passive avoidance performances.

These performances can also be influenced by some other nonspecific effects of the drugs, in addition to their effects on learning and memory abilities. For example, passive avoidance performances are sometimes impaired by central excitation, anti-anxiety, analgesia, muscle relaxation, antiperspiration or lack of motor coordination; or are ameliorated by central depression, sedation, diaphoresis or algesia. From our evaluating results, it was found that CS-extract decreased MA and potentiated hexobarbital-induced sleeping, suggesting that CS-extract had a mild sedative effect and a direct effect which might be important in antagonizing the toxic effects of alcohol in the central nervous system.
Although the mechanisms of the action of CS-extract remain speculative, the present results suggest that it directly affects the learning and memory processes in the central nervous system to ameliorate the impairment of the passive avoidance performances induced by ethanol, or that the sedative effect of CS-extract induced the antagonization of the ethanol-induced impairment of the passive avoidance performances. Numerous studies of CS-extract on the central nervous system also remain to be tackled.

The decreasing effect of CS-extract on fecal excretion, but not on urinary excretion, suggested that the substance had an inhibitory effect on gastrointestinal mortality. Although this result does not support that CS-extract possesses an inhibitory effect on the absorption of alcohol, experiments on the four possibilities mentioned are still to be conducted.

REFERENCES

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