The Therapeutic and Prophylactic Effects of *Crocus sativus* L. (Saffron) in Senile Dementia

Hiroshi Saito
Hiroo 3-4-10 ; Shibuya-ku,
Tokyo 150-0012
Japan

Abstract

We investigated the effects of saffron extract and its ingredients on learning behaviors and long-term potentiation (LTP). Oral administration of *Crocus sativus* L. extract (CSE) antagonized both ethanol-induced memory impairment and ethanol-induced suppression of LTP generation. Crocin was the main active component in the CSE, with regards to both learning performance and LTP generation, and its pharmacological action was triggered via modulation of the central NMDA receptors. The results suggested that crocin improved learning deficits by attenuating the inhibitory effect of ethanol on LTP generation, and highlighted the possibility that crocin is a candidate for an ethanol-induced central nervous system disorders' therapeutic.

Saffron, a stigma of *Crocus sativus* L., has been used in medicines, spice, golden coloring, perfumes and incense for various purposes, since 3,500 years ago in Egypt and Middle East. It was exported to China in 13th century of the Yuan dynasty as healthfood; in 16th century of the Min dynasty as a medicine; and to Japan in the beginning of 17th century as a medicine. Since then, the Japanese have used saffron as medicine.

The first Figure shows the history of saffron in Japan. The original document on saffron in Japan was Chinese Materia Medica, Pen Tsao Kang Mu, written by Li Shin Chen in 1578, which was introduced to Japan in 1603. In 1543, the Portuguese introduced matchlock muskets. Since then, Portuguese trade began, and saffron was imported directly from Europe. Though in 1639 the national seclusion policy was established to all Westerners except the Dutch, saffron was imported constantly until now. The use of imported saffron was documented frequently during the Edo dynasty.

In 1716, after the import permission for European medical books, European medicines were introduced to Japan. Japanese recognized the picture of *C. sativus* for the first time by European Materia Medica, R. Dodonaeus's botanical illustrated book and noticed the mistakes of document on saffron in Pen Tsao Kang Mu. They (Chinese) thought saffron as a petal of kind of safflower, so they called *C. sativus* as Tibetan, Arabic or Western safflower. At the same time, Japanese doctors began to use it to build into the context of the original Japanese medicines, and made new formulas in combination with Japanese traditional medicines.

In 1886, the first Japanese Pharmacopoeia was announced and saffron was accepted as medicine. In the newest version of Japanese Pharmacopoeia, 14th edition in 2002, saffron is still accepted as medicine among non-prescription drug, such as drugs for women's diseases and children's sedatives. I will show you the list of these drugs afterward.

Between 1910 and 1916 in Japan, Bunpei Kira in Takeda, Oh-ita prefecture, developed new cultivation method of *C. sativus*. This method was the rest from people's hard labor gathering saffron in the basket.

Recently Chinese farmers learned this technique and used it. So Takeda's farmers produce pistils only about 30 Kg a year.

Figure 2 shows recent medical use of saffron in Japan. We use saffron as medicine in non-prescription drug, but not frequently. All of these preparations (articles) were registered as non-prescription drugs since almost 50 years ago. Some of them, which were made as new formulas in combination with Japanese traditional medicine by Japanese doctors, were used as medicine since more than 100 years ago. We use saffron as an herb

Proc. I[st] IS on Saffron
Eds: J.-A. Fernández & F. Abdullaev
Acta Hort 650, ISHS 2004

for women whose health is adversely affected by cold, and improvement of women's circulation of the blood. There are 37 scientific articles documenting this use. We also use saffron as a constituent of formula for women's dizziness, congestion of the brain and hysterics, bad health before and after woman's confinement, and a menopausal disorder. 140 articles are accepted as drugs for women's diseases in which 45 articles include saffron in combination with Japanese traditional herbs such as Chinese Angelica (*Angelica sinensis*), Sichuan Lovage (*Ligusticum wallichii*), peony root (*Paeonia suffruticosa*), cinnamon bark (*Cinnamomum zeylanicum*), licorice (*Glycyrrhiza glabra*), Poria cocos (*Poriae cocos*), ginseng (*Panax ginseng*), and others.

Moreover, we use saffron as a constituent of formula for children's sedatives, that is, for children's nervousness, meningism, spasm, infirmity, milk vomiting, extensive weeping at night, poor digestion, indigestion and diarrhea. We have 45 peer-reviewed articles in which 17 articles include saffron in a combination with ginseng, buffalo gall stone, aloeswood et al. We also use saffron as a constituent of formula for a cardiacs. There are 228 articles documenting its use as a non-prescription drug. Among those, 119 articles include saffron in a combination with cake of Toad skin secretion, Buffalo gall stone, ginseng, Musk deer, etc.

Modern pharmacological studies have demonstrated pharmacological effect such as antitumor, heart suppressive, hypotensive, vasodilating, anti-arteriosclerosis, hepatopathy improving, anti-platelet aggregation, anti-hyperlipidemia, myometrium exciting, breath exciting, renal-vasoconstrictive, anti-alcohol and learning and memory improving effects of saffron extract (shortened to CSE) and its ingredient.

Among the constituents of CSE, crocetin derivatives are mainly responsible for these pharmacological activities. Saffron's medical effect recorded in Chinese Materia Medica was that notable effects were described as promoting blood circulation to remove blood stasis. It was also used in allying fear, curing trance and some other disorders of the CNS. These medical findings suggested that saffron exerts pharmacological actions on the CNS, and influences the central processes of learning and memory. Moreover I was very interesting in the sentence, that "saffron is effective on hangover and the fresh one is better" which was written in The Greek Herbal of Dioscorides and Naturalis Historia of Gaius Plinius Secundus, we researched and found anti-alcohol effect, and learning and memory improving effect of saffron. I will show you our work in this paper.

Thereafter, we studied the effect of CSE and its ingredients on the ethanol-induced impairment of learning behavior and ethanol-induced blockade of LTP. We used alcohol-aqua extract of saffron. CSE and its constituents were kindly offered from Prof. Masahiro Shoyama, University of Kyushu.

First, we studied the effect of CSE on learning and memory using one trial passive avoidance learning response, in both step-through and step-down tests.

Oral pre-administration of CSE had no influences on acquisition, retention and retrieval of learning and memory, and motor-incoordination in mice (Zhang et al., 1994). But CSE had showed weak sedative effect, as CSE depressed motor activity, prolonged pentobarbital-induced sleeping time and inhibited alcohol-induced increase of motor activity.

Moreover, CSE improved memory acquisition and retrieval impairments induced by alcohol, and the memory retention impairment induced by electro convulsive shock in both tests. Figure 3-1 shows the effects of CSE on ethanol-induced memory acquisition deficits in both step through and step down tests (Zhang et al., 1994). Figure 3-2 shows the effect of CSE, on ethanol-induced memory retrieval deficit in both tests (Sugiura et al., 1995a). These results indicated that CSE had beneficial effect on alcohol memory disorders.

How is memory formed in the brain? Hebb proposed that memory is formed by a plastic change in synaptic functions, depending on neuronal activity. The input of stronger information may increase the efficiency of neurotransmission at specific synapses. An electrophysiological experiment using an anaesthetized rabbit was performed, and it demonstrated that application of high-frequency stimulation to

presynaptic fibers in the hippocampus produced a long-lasting increase in post-synaptic potentials. This phenomenon was termed long-term potentiation, shortened to LTP. LTP is induced most easily and reproducibly in the hippocampus, a brain region that is crucially involved in learning and memory. Then, hippocampal LTP has been extensively studied as a candidate mechanism underlying memory formation using anaesthetized rats in vivo.

Oral administration of CSE (250 mg/kg) did not affect the basal synaptic transmission as well as the generation of LTP in anaesthetized rat hippocampal dentate gyrus (Sugiura et al., 1995a). We studied the preventive effect of orally administered CSE on LTP inhibited by ethanol. CSE (p.o) dose-dependently suppressed the inhibitory effects of ethanol (p.o., i.v. and i.c.v.), and acetaldehyde (i.v.) on LTP generation. Figures 4-1, -2 and -3 show preventive effect of orally administrated CSE on LTP inhibited by ethanol, administered orally, intravasculary and intracerebroventriculary. These results suggested that CSE antagonized the action of ethanol and acetaldehyde on LTP generation at the central nervous system level (Sugiura et al., 1995a).

Figure 5 shows ingredients in stigmas of saffron. We, therefore, focused on these three constituent substances in saffron, crocin (crocetin di-gentiobiose ester), crocetin gentiobiose glucose ester and crocetin di-glucose ester, and picrocrocin, the most abundant compound in saffron, whose % content of saffron extract and chemical structure are shown in Figure 5. Crocin (p.o.) dose-dependently suppressed the inhibitory effect of ethanol (i.v.) on LTP generation in anaesthetized rat hippocampal dentate gyrus (Sugiura et al., 1994).

Intracerebroventricural administration of ingredients in saffron and their related compounds did not alter the basal synaptic transmission and the LTP generation. Crocin (i.c.v.) dose-dependently suppressed the inhibitory effect of ethanol (i.v.) on LTP generation, and crocetin gentiobiose glucose ester suppressed the inhibitory effect of ethanol (i.v.) on LTP generation at twice larger dose of crocin (Sugiura et al., 1994). On the other hand, crocetin di-glucose ester, picrocrocin, the most abundant component in CSE, β-carotene, the main structure of unsaturated carbohydrate without gentiobiose residue, glucose, and bi-, tri-, tetra- and penta saccharides, β(1-3) combination, did not affect the ethanol (i.v.)-induced inhibition of LTP generation. Result of LTP experiment using these ingredients, suggested that crocin is the main active component in saffron in terms of ethanol-induced inhibition of LTP generation and also that gentiobiose residues in the terminals of β-carotene structure were necessary for manifestation of the activity.

Figure 6 shows preventive effect of crocin on LTP inhibited by ethanol. Crocin was given orally, and ethanol, intravascularly. Other ingredients administered orally had no effect (Sugiura et al., 1994).

Then, we studied preventive effects of intracerebroventriculary administered saffron ingredients, on ethanol-induced inhibition of LTP. Ethanol was given intravasculary. Intracerebroventricular administration of crocin prevented ethanol-induced inhibition of LTP generation. Figure 7 shows the result of the structure activity relationship of crocin. Crocetin gentiobiose glucose ester, which lacks one sugar molecule compared with crocin, prevented ethanol-induced inhibition of LTP in a similar manner to crocin, but was less potent than crocin. Crocin di-glucose ester, which lacks two sugar molecules compared with crocin, was ineffective even at high doses. Gentiobiose attached to the fatty acid chain has been considered to be responsible for the most of pharmacological activities of saffron extracts.

Other ingredient in saffron and compounds used in this experiment had no effect on LTP generation (Sugiura et al., 1994).

What is the molecular target of crocin concerning the antagonism against ethanol? The induction of LTP in CA1 and dentate gyrus regions of the hippocampus essentially requires the activation of the N-methyl-D-aspartate (NMDA) type of glutamate receptors. Furthermore ethanol has been considered to inhibit the induction of LTP by suppressing the activity of NMDA receptor channel complex.

Figure 8 shows preventive effect of crocin on LTP inhibited by ethanol in the

region of CA1 of rat hippocampal slices.

Crocin did not affect the baseline of synaptic response, but significantly prevented the LTP-suppressing action of ethanol within the hippocampus (Sugiura et al., 1995b).

Ethanol inhibited the responses mediated by NMDA type of glutamate receptor, which is thought to play an important role in learning and memory process. Then, we studied the effect of crocin on the ethanol-induced suppression of NMDA receptor response in the dentate gyrus of rat hippocampal slice. Figure 9 shows antagonizing effect of crocin on ethanol-induced inhibition of NMDA-receptor mediated synaptic response. Superfusion of crocin (10μM) inhibition the ethanol-induced suppression of NMA receptor response (Sugiura et al., 1995b). These results indicated that one of the sites of crocin action was a NMDA receptor.

Then, we conducted learning experiment using ingredients of saffron. Except crocin, ingredients used in this experiment had no effect on the ethanol-induced memory deficit in both step through and step down tests. Figure 10-1 shows the preventive effect of crocin (p.o.) on ethanol-induced memory acquisition deficit in both step-through and step-down tests (Sugiura et al., 1995c). Figure 10-2 shows the effect of crocin on ethanol-induced memory retrieval deficit in both step-through and step-down tests. The preventive effect of crocin on ethanol-induced memory retrieval deficit in step-down test is very clear (Sugiura et al., 1995c). Picrocrocin and other related compounds of crocin did not ameliorate the deficits of memory acquisition and memory retrieval. These results suggest that the main active component in CSE in terms of learning performances is crocin.

It is possible that crocin alone affects learning and memory or that crocin interacts with substances other than ethanol. Indeed, we have found recently that intracerebroventricular administration of a higher dose of crocin alone promoted the maintenance of LTP (Figure11). These results also highlighted the possibility that crocin might be considered as a candidate therapeutics and prophylactives for the treatment of senile amnesia and dementia.

Table 1 shows pharmacological activities of saffron, *Ginkgo bilova*, and their ingredients, some of which can be effective for the treatment of senile dementia.

In the modern medicine, there are many prophylactics and therapeutics for the treatment of senile dementia, which each have different and their own pharmacological activities. Those prophylactics and therapeutics are given to the patient for long time at the same time in modern medicinal treatment.

In the left side column, several modern prophylactics and therapeutics for the treatment of senile dementia are listed. Right side column sub-classified the treatment into saffron and *G. bilova*.

G. bilova has been extensively used clinically in modern European medicine as the drug for the treatment of memory disorders. However, in spite of its positive indications, the effect of ginkgo remained pharmacologically obscure.

It is speculated that among the constituents of ginkgo, flavons act as free radical scavengers; terpens act as fibronolytics, and ginkgolide B acts as PAF inhibitors. These activities are not strong when constituents are applied separately, but all constituents cooperate and act as good cerebral circulation enhancers.

Saffron extract has pharmacological activities that are necessary for the treatment of senile dementia to a larger extend than ginkgo does. The results also highlighted the possibility that saffron might be considered a drug for the treatment of senile dementia, similarly to *G. bilova*.

Literature Cited

Suiura, M., Shoyama, Y., Saito, H., Abe, K. 1994. Crocin (crocetin di-gentiobiose ester) prevents the inhibitory effect of ethanol on long-term potentiation in the dentate gyrus in vivo. J Pharmacol. Exp. Ther. 271: 703-707.

Sugiura, M., Saito, H., Abe, K. 1995a. Ethanol extract of *Crocus sativus* L. Antagonizes the inhibitory action of ethanol on hippocampal long-term potentiation in vivo.

Phytother. Res. 9: 100-104.
Sugiura, M., Shoyama, Y., Saito, H., Abe, K. 1995b. The effects of ethanol and crocin on the induction of long-term potentiation in the ca1 region of rat hippocampal slices. Jap. J. Pharmacol. 67: 395-397.
Sugiura, M., Shoyama, Y., Saito, H., Nishiyama, N. 1995c. Crocin improves the ethanol induced impairment of learning behaviors of mice in passive avoidance tasks. Proc. Japan. Acad., 71, Ser. B.
Zhang, Y. X.; Shoyama, Y., Sugiura, M., Saito, H. 1994. Effects of *Crocus sativus* L. on the ethanol-induced impairment of passive avoidance performances in mice. Biol. Pharm. Bull. 17: 217-221.

Figures

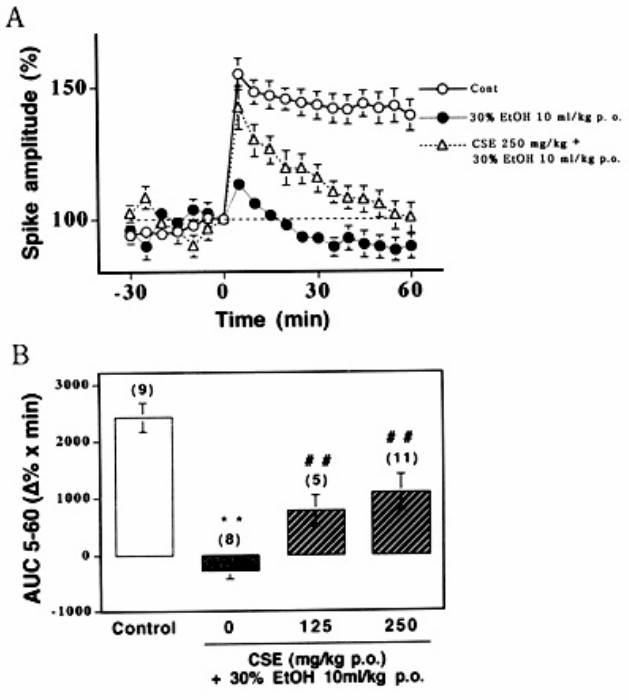

Fig. 1. Preventive effect of CSE (p.o.) on LTP inhibited by Ethanol (p.o.)

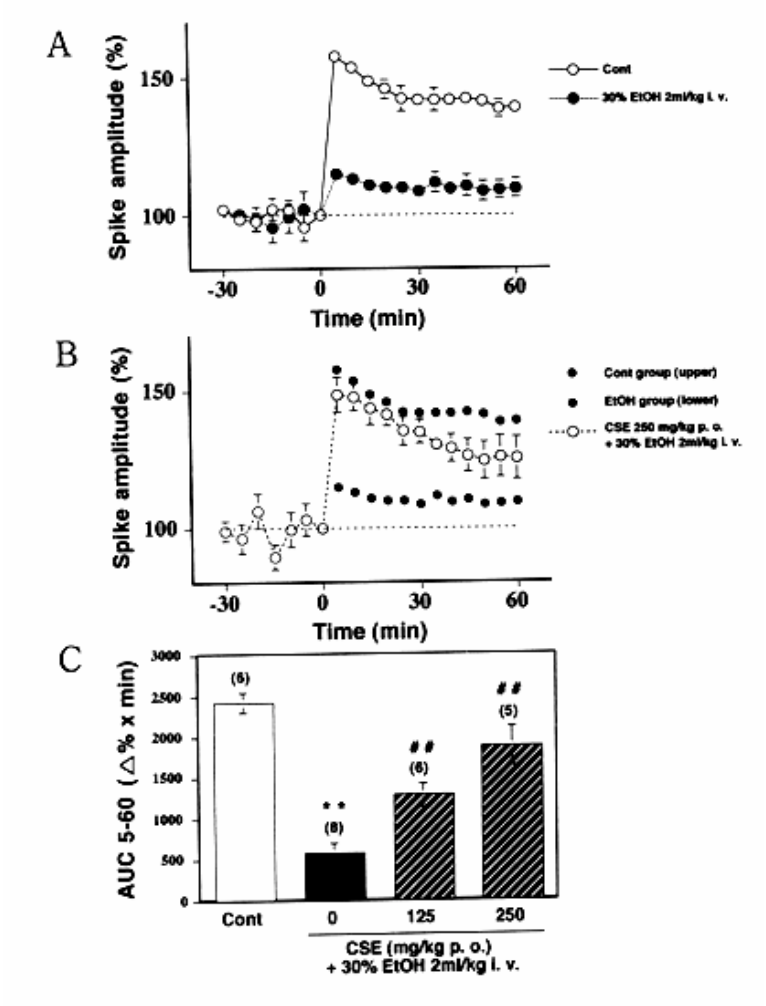

Fig. 2. Preventive effect of CSE (p.o.) on LTP inhibited by Ethanol (i.v.)

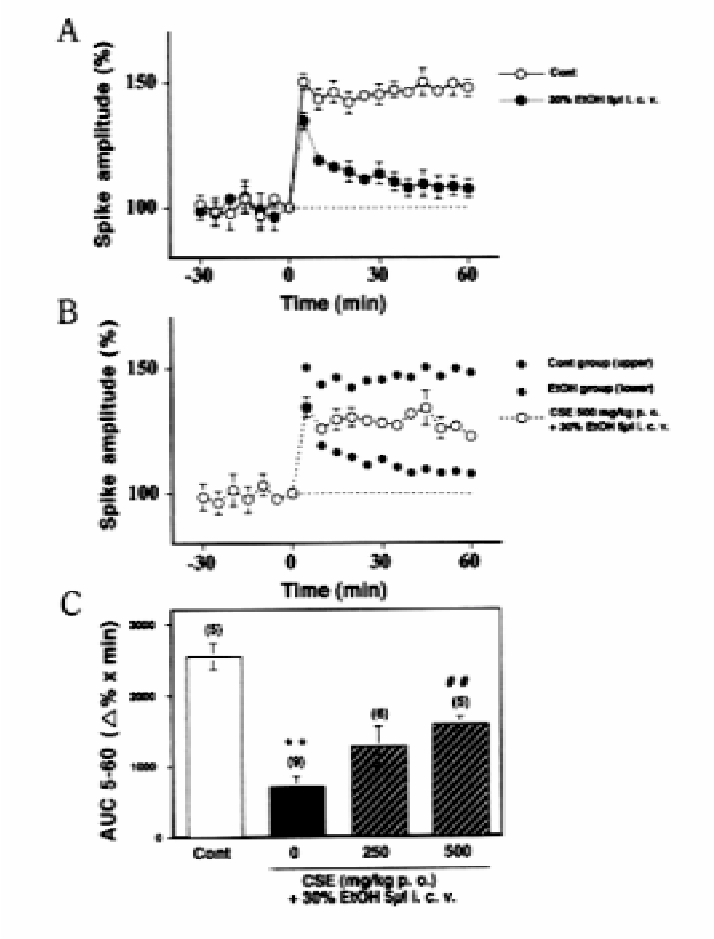

Fig. 3. Preventive effect of CSE (p.o.) on LTP inhibited by Ethanol (i.c.v.)

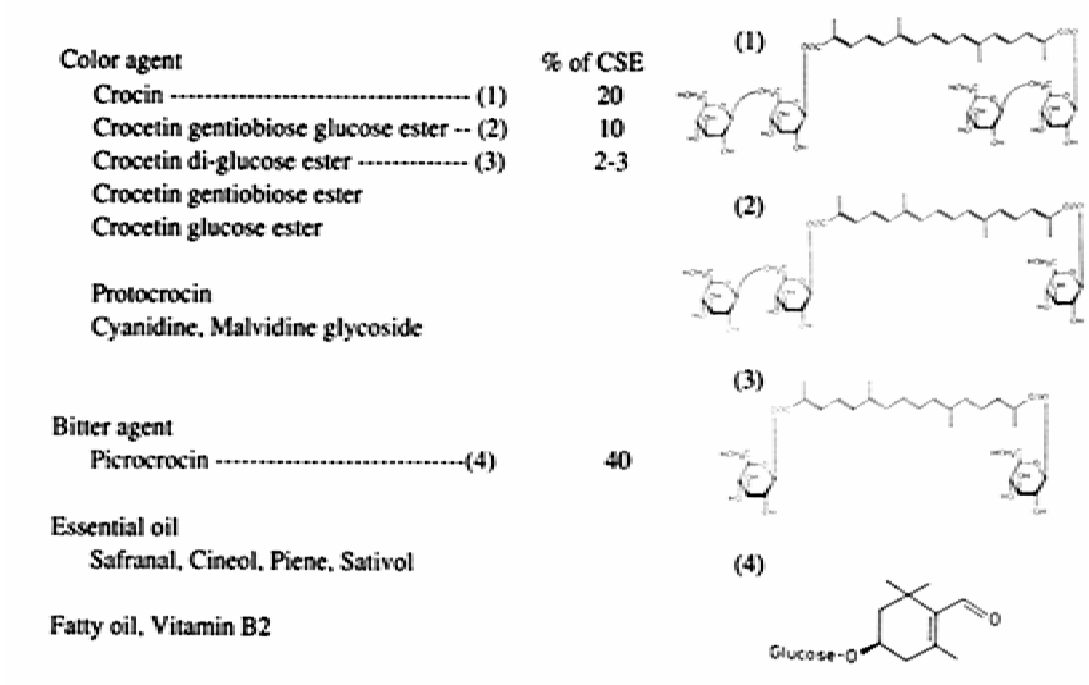

Fig. 4. Ingredients in pistils of *Crocus sativus* L.

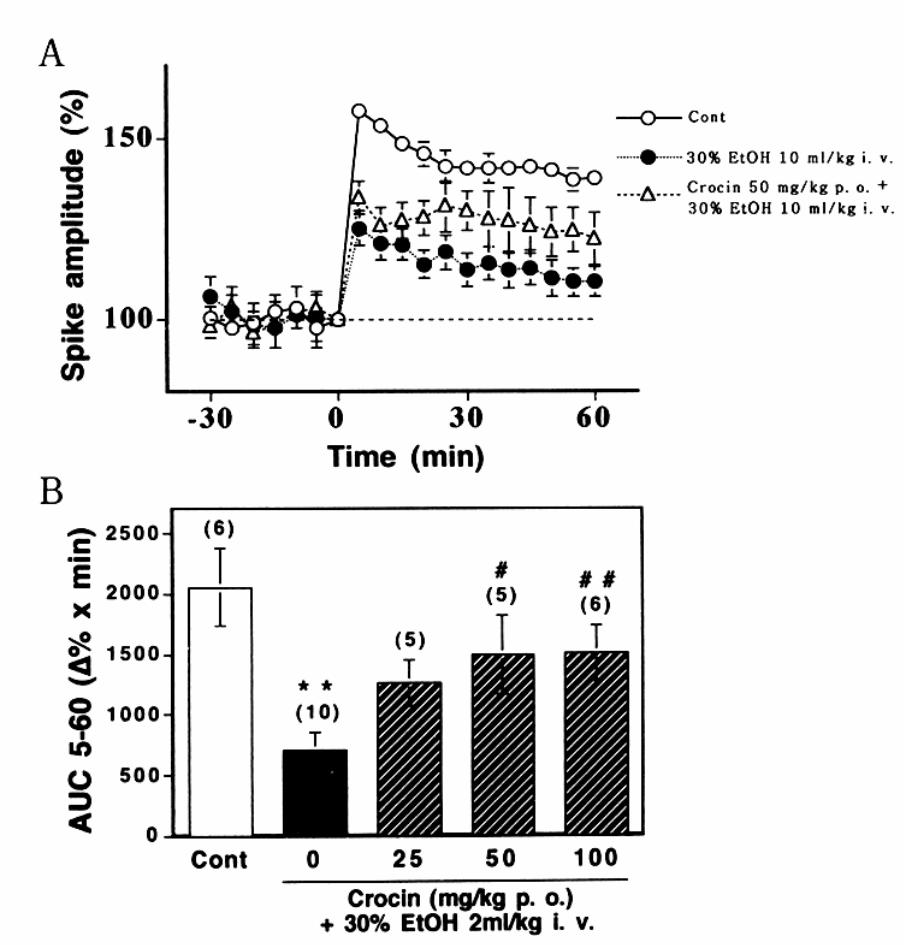

Fig. 5. Preventive Effect of Crocin (p.o.) on LTP inhibited by Ethanol (i.v)

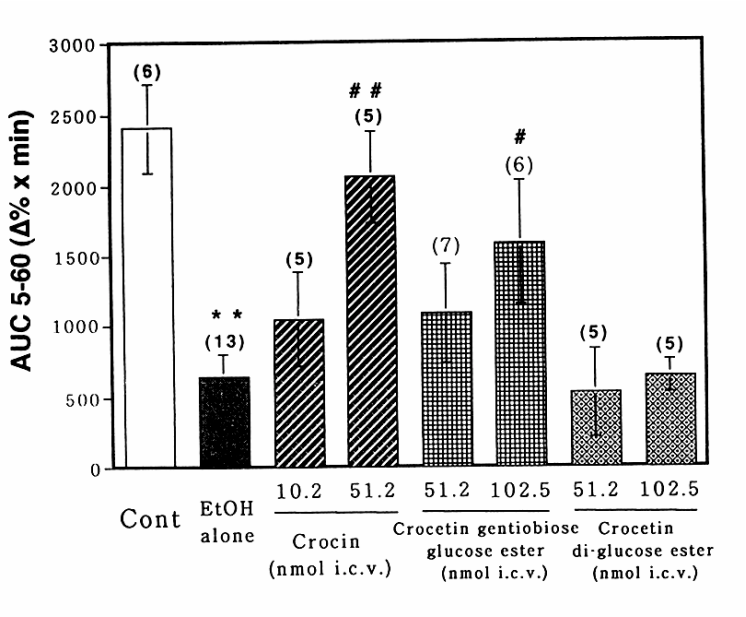

Fig. 6. Effects of crocin and related compounds (i.c.v.) on LTP inhibited by Ethanol (i.v.)

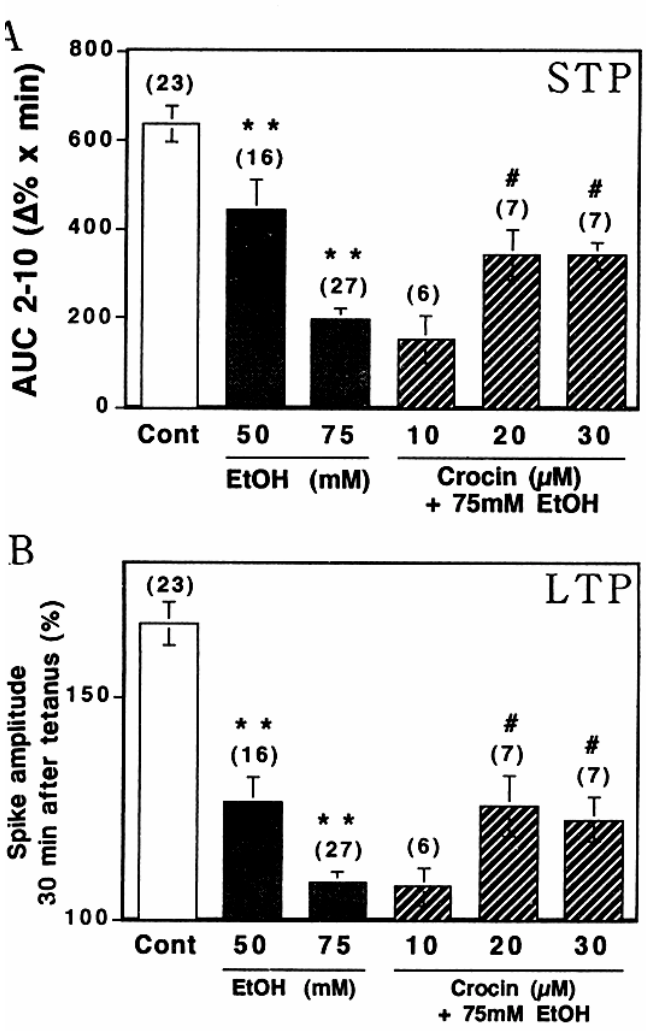

Fig. 7. Preventive Effect of Crocin on LTP inhibited by ethanol in rat hippocampal slices.

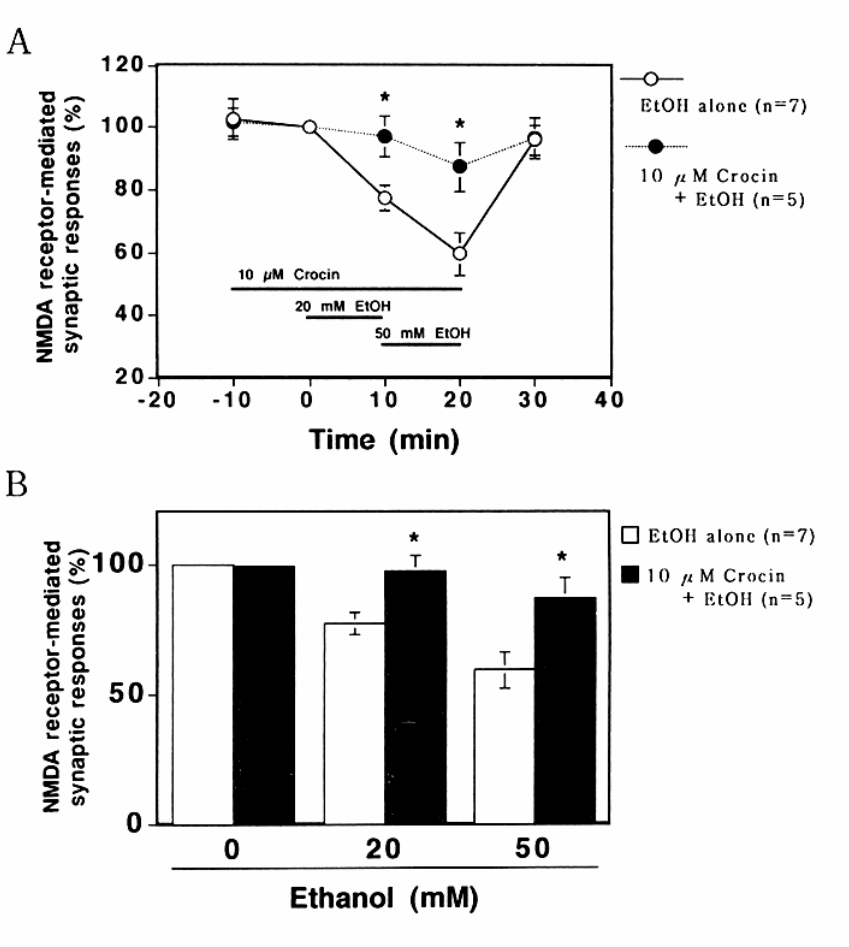

Fig. 8. Antagonizing effect of crocin on ethanol induced inhibition on NMDA-receptor mediated synaptic response

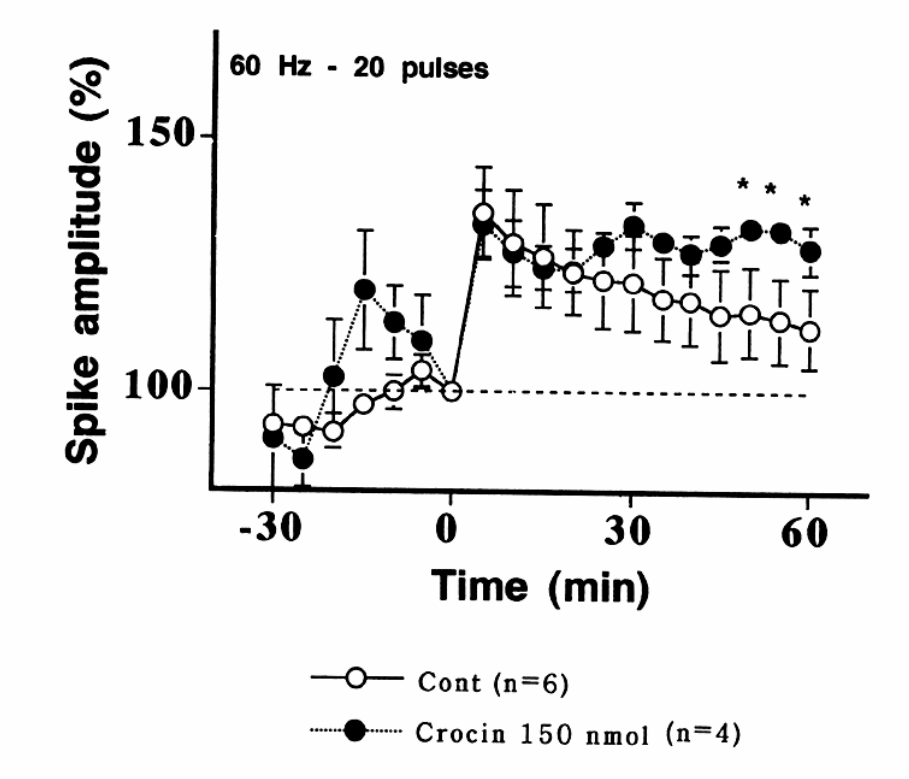

Fig. 9. Effect of high-dose of crocin (i.c.v.) on short-term potentiation

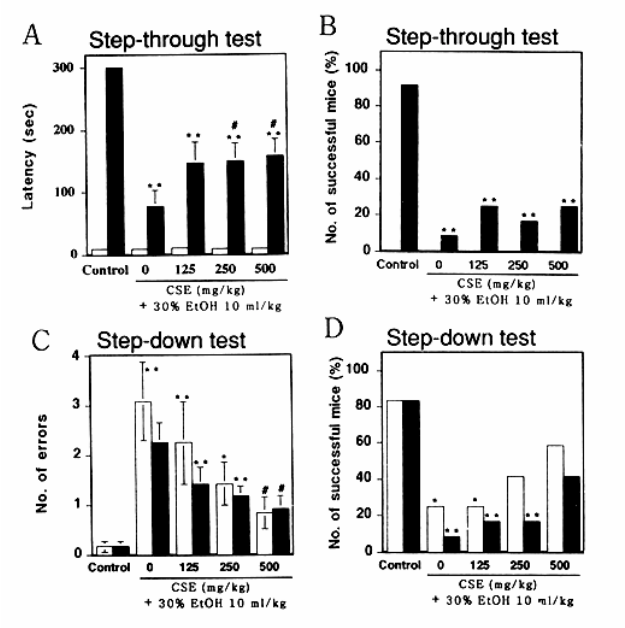

Fig. 10. Effect of CSE on ethanol-induced memory acquisition deficit

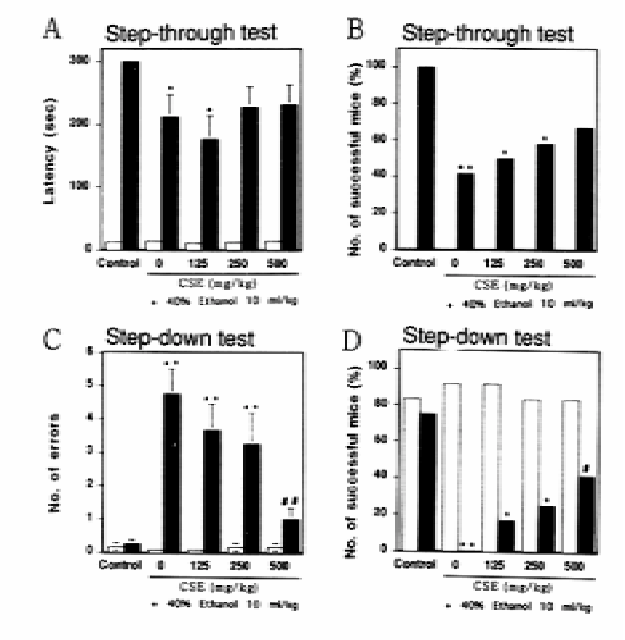

Fig. 11. Effect of CSE on ethanol-induced memory retrieval deficit.

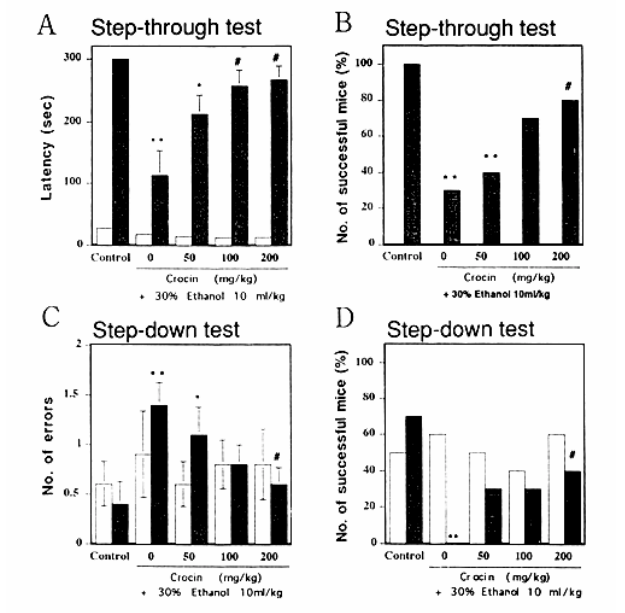

Fig. 12. Effect of crocin on ethanol-induced memory acquisition deficit

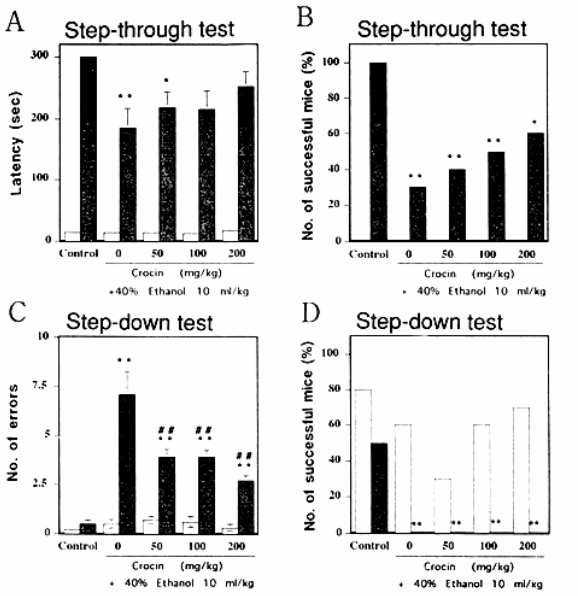

Fig. 13. Effect of crocin on ethanol-induced memory retrieval deficit