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FOOD & FUNCTION

Crocin promotes non-rapid eye movement sleep in mice

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Crocus sativus L. (saffron) has been traditionally used for the treatment of insomnia and other diseases of the nervous systems. Two carotenoid pigments, crocin and crocetin, are the major components responsible for the various pharmacological activities of *C. sativus* L. In this study, we examined the sleep-promoting activity of crocin and crocetin by monitoring the locomotor activity and electroencephalogram after administration of these components to mice. Crocin (30 and 100 mg/kg) increased the total time of non-rapid eye movement (non-REM) sleep by 60 and 170%, respectively, during a 4-h period from 20:00 to 24:00 after its intraperitoneal administration at a lights-off time of 20:00. Crocetin (100 mg/kg) also increased the total time of non-REM sleep by 50% after the administration. These compounds did not change the amount of REM sleep or show any adverse effects, such as rebound insomnia, after the induction of sleep.

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A variety of natural substances have been used for the management of sleep or wakefulness for many centuries, although most of them have not been chemically or pharmacologically characterized. The plant *Crocus sativus* L., commonly known as saffron, is used in the traditional Chinese medicine as an analgesic, antispasmodic, antidepressant and nerve sedative ingredient [1–3]. Saffron is now used worldwide in folk medicine and is reported to be useful in treating various central and peripheral diseases such as depression [4, 5].

Crocetin and crocin (crocetin-di-(β-D-digentiobiosyl)-ester, as shown in Fig. 1, are carotenoid pigment of saffron. Crocin is a major natural form of saffron and a number of pharmacological studies have demonstrated that crocin and also crocetin have a wide range of activities [6–10]. Notably, the neuroprotective activities of crocin have been demonstrated using various experimental models of brain disorders, such as Alzheimer's disease [11], depression [12] and memory impairment [13–18]. Crocetin is effective in rat model of Parkinson's disease [19].

These observations suggest that crocin and crocetin also contains hypnotic activities. However, the effects of crocin and crocetin on sleep/wake regulation have not yet been evaluated. Here, we examined the effects of crocin and crocetin on the sleep/wake regulation in mice and found both compounds to promote sleep.

We administered crocin orally to mice and quantified the locomotor activity of the animals (for detailed information on methods, see Supporting Information). Figure 2A shows the locomotor activity every hour after the administration of crocin at a dose of 80 mg/kg. The locomotor activity was significantly decreased for 8 h from 2 to 10 h after the administration. The suppressive effect of crocin on the locomotor activity peaked, with 57% suppression, at 5 h after the administration.

Crocin at a dose of 40 mg/kg did not affect the total amount of locomotor activity during a dark period for 12 h after the crocin administration (vehicle: 13 500 ± 510 counts/12 h, crocin: 13 300 ± 900 counts/12 h). However, at upper

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Abbreviation: REM, rapid eye movement

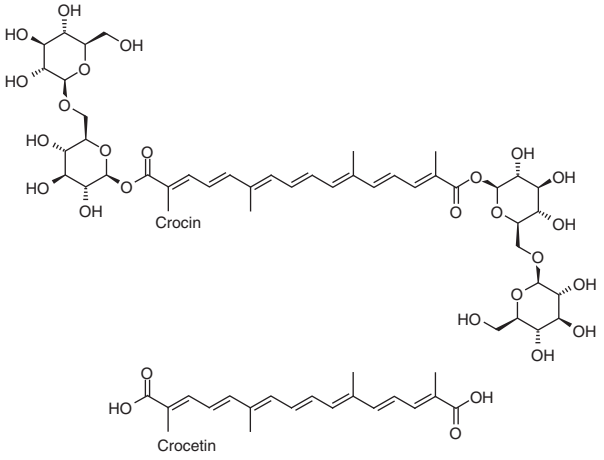

Figure 1. Chemical structures of crocin and crocetin.

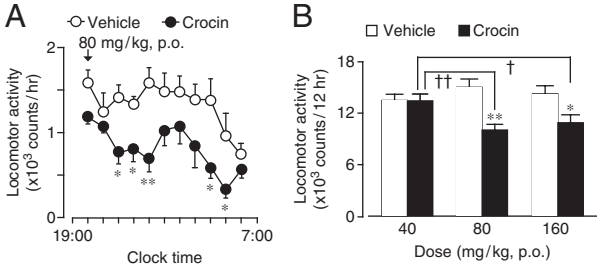

Figure 2. Suppression of spontaneous locomotor activity by crocin. (A) Time courses of the change in spontaneous locomotor activity every hour after oral administration (a vertical arrow) of vehicle (○) or crocin (●) at a dose of 80 mg/kg ($n = 8$). (B) Cumulative count of locomotor activity for 12 h after the administration of vehicle (open column) or crocin (closed column) at a dose of 40, 80 or 160 mg/kg. Each value presents mean ± SE ($n = 8$). $^{*}p < 0.05$, $^{**}p < 0.01$ versus vehicle control by two-tailed paired *t*-test. $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$ versus 40 mg/kg by ANOVA followed by Scheffe's test.

doses of crocin it significantly suppressed the total amount of locomotor activity during the 12 h by 33% (vehicle: 15 100 ± 970, crocin 80 mg/kg: 10 000 ± 670 counts/12 h) and 20% (vehicle: 13 500 ± 510, crocin 160 mg/kg: 10 800 ± 1080 counts/12 h), respectively, as compared with the vehicle control and by 25 and 20%, respectively, compared with the activity at 40 mg/kg (Fig. 2B). However, crocetin did not change the locomotor activity of the animals even after the oral administration at doses up to 500 mg/kg (data not shown).

We examined the sleep-promoting effect of crocin and crocetin on mice after an intraperitoneal administration at 20:00 during the wake period. Figure 3A shows time courses of the hourly amounts of non-REM and REM sleep and wakefulness after the administration of vehicle or crocin (100 mg/kg). When 100 mg/kg of crocin was injected on the experimental day, the amount of non-REM sleep was increased immediately after the injection; and the effect was

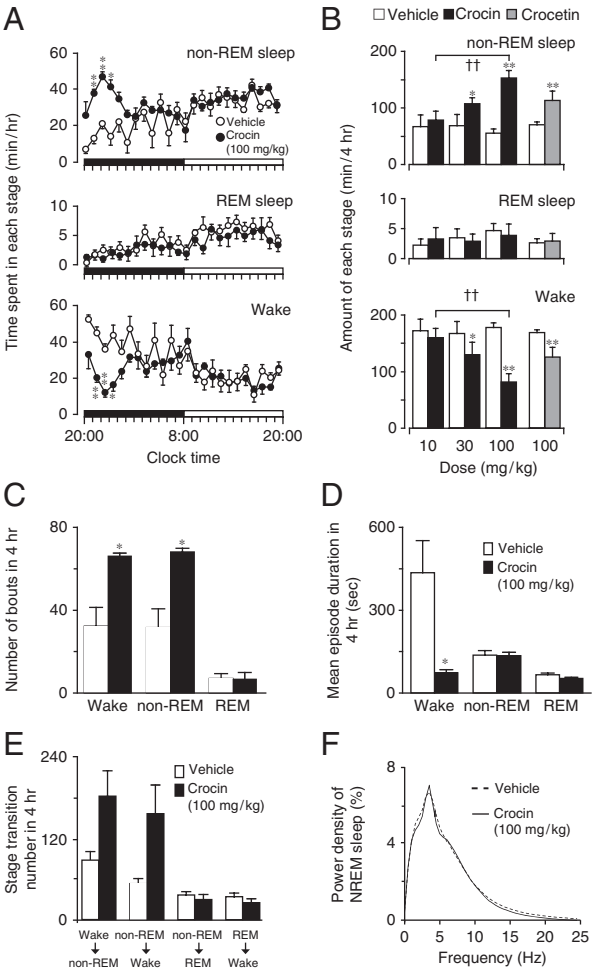

Figure 3. Increase in non-REM sleep by crocin. (A) Time courses of non-REM and REM sleep, and wakefulness after an intraperitoneal administration of vehicle (○) or crocin (●) at a dose of 100 mg/kg. Each circle represents the hourly mean ± SE of non-REM and REM sleep and wakefulness. Crocin was given at 20:00. The horizontal filled and open bars on the *X*-axis indicate the 12-h dark and 12-h light periods, respectively. $^{*}p < 0.05$, $^{**}p < 0.01$ versus vehicle control by two-tailed paired *t*-test. (B) Total time spent in non-REM and REM sleep and in wakefulness for 4 h after the administration of vehicle (open column), crocin (closed column) or crocetin (shaded column). Each value represents mean ± SE ($n = 4$–6). $^{*}p < 0.05$, $^{**}p < 0.01$, compared with vehicle control by two-tailed paired *t*-test. $^{\dagger\dagger}p < 0.01$ versus 10 mg/kg by ANOVA followed by Scheffe's test. (C–E) Number of bouts, mean episode duration and stage transition number during 4 h after the administration of vehicle (open column) or crocin (closed column) at a dose of 100 mg/kg. Each value represents mean ± SE ($n = 8$). $^{*}p < 0.05$, versus vehicle control by two-tailed paired *t*-test. (F) EEG power density of non-REM sleep after the administration of crocin. There was no essential difference in EEG power density during non-REM sleep between crocin treatment and vehicle treatment.

statistically significant from 2 to 4 h after the administration. However, crocin did not change the REM sleep after the administration. The increase in non-REM sleep and

decrease in wakefulness lasted more than 4 h after the injection. There was no further disruption of the sleep architecture during the subsequent period (8:00 to 20:00). These data indicate that crocin induced non-REM sleep without occurrence of adverse effects, such as rebound insomnia, after the sleep induction.

We calculated the total time spent in non-REM and REM sleep and wakefulness for 4 h after the crocin or crocetin injection (Fig. 3B). Crocin at 10 mg/kg did not affect the cumulative amounts of non-REM or REM sleep or wakefulness for 4 h after the injection. Crocin given at 30 and 100 mg/kg statistically significantly increased the total amount of non-REM sleep by 60% (from 69 ± 20 to 110 ± 21 min) and 170% (from 56 ± 7 to 153 ± 14 min), respectively, and decreased the total amount of wakefulness by 20% (from 168 ± 21 to 130 ± 22 min) and 50% (from 178 ± 9 to 82 ± 14 min), respectively, without changing the amount of REM sleep during this 4-h period as compared with the vehicle control. The increase in non-REM sleep and the decrease in wakefulness were statistically significant between the 2 doses of 10 and 100 mg/kg. Crocetin at a dose of 100 mg/kg significantly increased the total amount of non-REM sleep by 60% (from 70 ± 5 to 113 ± 17 min) and decreased the total amount of wakefulness by 25% (from 169 ± 5 to 126 ± 17 min) without changing the REM sleep amount during the 4-h period as compared with the vehicle control.

We determined the number of non-REM sleep and wake bouts, mean episode duration, and stage transition number after the administration of vehicle or crocin (100 mg/kg, Fig. 3C). Compared with the vehicle-treated control, the number of non-REM sleep bouts increased by 2.2-fold (from 32 ± 9 to 68 ± 2) and also those of wake bouts by 2.0-fold (from 32 ± 9 to 66 ± 2) for 4 h after the crocin treatment. On the other hand, the number of REM sleep bouts did not change after the crocin treatment. Crocin significantly decreased the mean episode duration of wake episode by 83% (from 435 ± 116 to 73 ± 11 s) after the crocin administration, but the mean episode duration of non-REM and REM sleep was not affected during this 4-h period after the injection (Fig. 3D). Crocin increased the number of stage transitions from wakefulness to non-REM sleep and from non-REM sleep to wakefulness by 110% (from 86 ± 13 to 179 ± 40) and 190% (from 52 ± 8 to 153 ± 45), respectively. The number of transitions from non-REM to REM sleep or from REM sleep to wakefulness was not changed by crocin (Fig. 3E). We then determined the EEG power spectra during non-REM sleep. As shown in Fig. 3F, there was no significant difference in EEG power density of non-REM sleep between the crocin treatment and the vehicle control, indicating crocin did not affect the EEG power density of NREM sleep.

In the present study, we demonstrated that crocin and crocetin increased the amount of non-REM sleep in mice.

Crocin decreased the number of mean episode duration of wakefulness without affecting the mean episode duration of non-REM and REM sleep and increased the number of wake and non-REM bouts, suggesting that crocin decreased the maintenance of wakefulness. And crocin did not change the EEG power density of NREM sleep. Crocin may be considered to induce sleep very similar to physiological sleep, suggesting its potential use for the treatment of sleep disorder.

Both crocin and crocetin, carotenoid pigments of *C. sativus* L., increased non-REM sleep after their intraperitoneal administration. In comparison of their non-REM sleep-promoting effects, crocetin at a dose of 100 mg/kg had almost the same effect as crocin at a dose of 30 mg/kg. As the molecular weight of crocetin (328.4) is about 1/3 of that of crocin, i.e. crocetin digentiobiose ester (977.1), crocin had an approximately 10-hold higher efficacy to induce non-REM sleep than crocetin. In a case of the oral administration, crocetin at a dose of 500 mg/kg did not affect the locomotor activity, although crocin at a dose of 80 mg/kg showed a significant sedative effect. Orally administered crocin are hydrolyzed to crocetin in the gastrointestinal lumen before absorption or in the intestinal mucosa during absorption, but not easily hydrolyzed after absorbed [20]. As shown in the present study, crocin is the major ingredient of saffron to induce non-REM sleep and the gentiobiose modification is important to improve bioavailability or stability of crocetin. Further modification of crocin with gentiobiose or other sugars might result in improved efficacy to induce non-REM sleep.

Crocin increases the intracellular glutathione level and prevents cell death in serum-deprived and hypoxic PC12 cells, a cell culture model for brain ischemia, owing to its antioxidant property [21]. The antioxidant activities implicated in these neuroprotective potentials of crocin and crocetin [4, 8, 22, 23]. Such neuroprotective events and learning and memory function are enhanced during sleep [24, 25]. On the other hand, neuronal oxidative response, glutathione metabolisms and nitric oxide production have been shown to regulate sleep in rats or mice [26–28]. Therefore, the increase in non-REM sleep caused by crocin may, at least in part, have multiple beneficial roles in neural survival and synaptic plasticity.

We recently reported that non-REM sleep induction with a decreased duration of wakefulness episodes and an increased number of wake and non-REM bouts, similar to crocin-induced sleep, was caused by diazepam, a common benzodiazepine derivative drug used for insomnia. The sleep-promoting effect of diazepam is partially explained by the down-regulation of acetylcholine release [29]. We examine the molecular mechanism of crocin-induced sleep by using various gene-manipulated mice, e.g. knock-out (KO) mice of receptors for adenosine, histamine, or dopamine, and found that crocin-induced sleep was markedly reduced in histamine H1 receptor KO mice (unpublished result). These results suggest that crocin may modulate the histaminergic or cholinergic arousal system to induce non-REM sleep.

Crocin is considered to induce non-REM sleep that is very similar to physiological sleep, suggesting its potential

use for the treatment of insomnia. In conclusion, although further research will be needed to examine their bioavailability and safety, both crocin and crocetin may be useful for the promotion of sleep in human.

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The authors have declared no conflict of interest.

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