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SHORT COMMUNICATION

Effects of the active constituents of *Crocus sativus* L., crocins, in an animal model of anxiety

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Abstract

Crocus sativus L. is a plant cultivated in various parts of the world. Crocins are among the active components of *Crocus sativus* L. The present study was designed to investigate in the rat whether or not crocins possess anxiolytic properties. For this aim, the light/dark test was selected. Either crocins, at a dose which did not influence animals' motor activity (50 mg/kg), or diazepam (1.5 mg/kg), significantly increased the latency to enter the dark compartment and prolonged the time spent in the lit chamber in the rats. Conversely, lower doses of crocins (15–30 mg/kg) did not substantially modify animals' behaviour. The present results indicate that treatment with these active constituents of *Crocus sativus* L. induce anxiolytic-like effects in the rat.

Keywords: Crocins; Anxiety; Light/dark test; Motility; Rat

Introduction

Crocus sativus L., commonly known as saffron, is a plant cultivated in various parts of the world such as Iran, China, Spain, India and Greece. In Greece, cultivation takes place in Krokos, Kozani area, in North Greece. Chemical analysis of its stigmas has shown the presence of water soluble carotenoids (crocins), small amounts of monoterpene aldehydes (picrotoxin and saffranal) and flavonoids (quercetin and kaempferol) (Tarantilis et al., 1995). The pistils of *Crocus sativus* L. are used in folk medicine as an anticatarrhal, eupeptic, expectorant and emmenagogue (Rios et al., 1996). Modern pharmacological studies have demonstrated that its crude extracts and purified chemicals possess anti-tumour effects (Nair et al., 1991; Salomi et al., 1991; Tarantilis et al., 1994), anti-inflammatory properties (Hosseinzadeh and Younesi, 2002), counteract atherosclerosis (Gainer and Jones, 1975) and hepatic damage (Wang et al., 1991).

Saffron and its active constituents affect a number of different neural processes, e.g. antagonized memory impairments in rodents (Pitsikas and Sakellaridis, 2006; Pitsikas et al., 2007; Sugiura et al., 1995; Zhang et al., 1994) conferred neuroprotection in a rat model of Parkinson disease (Ahmad et al., 2005) and expressed antioxidant properties in an *in vitro* model of Alzheimer disease (Papandreou et al., 2006). Finally, in studies performed in humans, the antidepressant properties of *Crocus sativus* L. and its extracts were revealed (Akhondzabeh et al., 2004; Noorbala et al., 2005).

In traditional medicine saffron was also used as an antispasmodic and nerve sedative (Rios et al., 1996). Recently, it has been reported that saffranal, an active component of saffron, exhibited anticonvulsant properties in animals (Hosseinzadeh and Khosravan, 2005; Hosseinzadeh and Sadeghnia, 2007).

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Taken the above evidences into account, the aim of the present work was to investigate the therapeutic potential of crocins in anxiety. For this purpose the light/dark test was selected. This test is based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behaviour of rodents in response to mild stressors, that is, novel environment and light (Crawley and Goodwin, 1980). In addition, in an attempt to distinguish between specific and unspecific changes in animals' activity due to the drug treatment, the effects of crocins on rats' motility were assessed in a locomotor activity test.

Material and methods

Animals

Male, 3-month-old Wistar rats (Hellenic Pasteur Institute, Athens, Greece) weighing 250–300 g were used in this study. The animals were housed in Makrolon cages (45 cm long × 35 cm high × 20 cm wide) three per cage, in a regulated environment (21 ± 1 °C; 50–55% relative humidity; 12/12 h light/dark cycle, lights on at 07:00 h), with free access to food and water. Experiments were conducted in the room where only these animals were housed, and took place between 10:00 and 13:00 h. Behavioural observations and evaluations were performed by experimenters who were unaware of the pharmacological treatment.

Procedures involving animals and their care were conducted in conformity with the international guidelines, in compliance with National and International laws and policies (EEC Council Directive 86/609, JL 358, 1, December 12, 1987; *NIH Guide for Care and Use of Laboratory Animals*, NIH publication no. 85-23, 1985).

Light/dark test

The test apparatus consisted of a wooden box (48 cm long × 24 cm high × 27 cm wide) divided into two equal-size compartments by a barrier possessing a doorway (10 cm high × 10 cm wide). One of the compartments was painted black and covered with a lid, whereas the other was painted white and illuminated with a 60-W light bulb set 40 cm above the box. On the day of the test, rats were transported to the darkened testing room and left in their home cages for 2 h. Animals were then placed in the middle of the lit compartment, facing away the dark chamber. Rats were allowed to freely explore the box for 5 min. The latency to enter (with all four paws) the dark compartment, the number of transitions and the time spent in the light and dark compartments were video-recorded.

Motor activity test

Spontaneous motor activity was assessed in an activity cage (Ugo Basile, Varese, Italy). The rats subjected to locomotor activity experiment were tested only once. On the day of the test, rats were transported to the darkened testing room and left in their home cages for 2 h. Then, each animal was placed in the middle of the apparatus. Thereafter, motor activity was monitored for 10 min (Pitsikas et al., 2001).

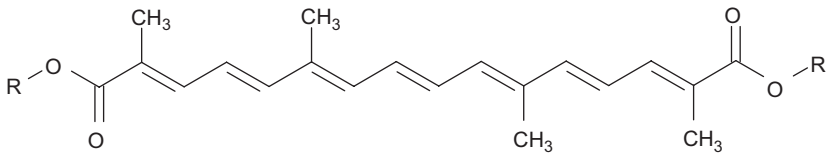
Chemicals

Crocin were isolated from the red dried stigmas (saffron) of *Crocus sativus* L., as described previously (Tarantilis et al., 1995) (Fig. 1). The compound was dissolved in saline (NaCl 0.9%) and was then administered intraperitoneally (i.p.). Diazepam (Valium, Roche) was used as a reference drug.

Experimental protocol

Light/dark test

Rats were randomly divided into four experimental groups (10 rats per group) as follows: vehicle; crocins 15 mg/kg; crocins 30 mg/kg; crocins 50 mg/kg; and diazepam (1.5 mg/kg). Dose of diazepam was selected based on prior study in which it was found active in the same behavioural paradigm and did not produce adverse side effects (Zanoli et al., 2002). Vehicle and crocins were injected i.p., 60 min before testing. Diazepam was administered i.p., 20 min before undertaking the experiment.



Crocin: R = β-Gentiobiosyl (Crocetin-di-β-D-gentiobiosylester)

Fig. 1. Structure of crocin.

Motor activity test

Rats were randomly divided into two experimental groups (10 rats per group) as follows: vehicle; and crocins 50 mg/kg. Vehicle and crocins were injected i.p., 60 min before testing.

Statistical analysis

Data are expressed as mean ± s.e.m. and were analyzed by using the one-way analysis of variance (ANOVA) test followed by Tukey's *post-hoc* test.

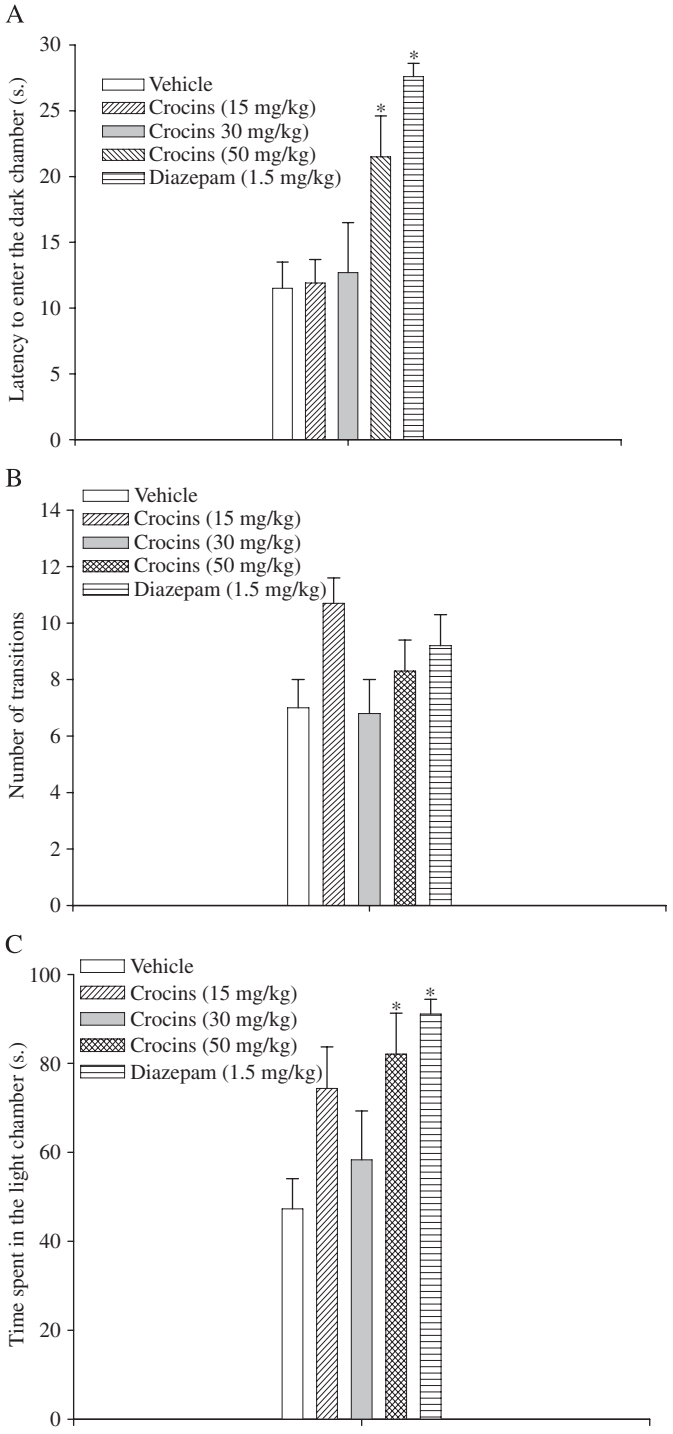


Fig. 2. Vehicle and crocins were injected i.p., 60 min before testing. Diazepam (1.5 mg/kg) used as a reference drug was administered i.p., 20 min before testing. Results are expressed as mean ± s.e.m. (A) Latency to enter the dark chamber. (B) Number of transitions. (C) Time spent in the light chamber. $*p < 0.05$ vs. the vehicle group.

Table 1. Effects of crocins on spontaneous motility in rats

Group	*N*	Cumulative scores (counts/10 min)
Vehicle	10	175.4 ± 17
Crocins (50 mg/kg)	10	194.8 ± 33.4

N = number of rats.
Vehicle and crocins (50 mg/kg) were injected i.p., 60 min before testing.
Results are expressed as mean ± s.e.m.

Results

Administration of crocins and diazepam significantly affected the duration of the first stay in the lit box [$F(4, 45) = 8$, $p < 0.01$]. Post-hoc comparisons indicated that the 50 mg/kg crocins and the diazepam-treated rats spent more time in the lit chamber before their first entrance to the dark compartment with respect to the vehicle-treated animals ($p < 0.05$, Fig. 2A). Analysis of number of transitions results (Fig. 2B) did not display a significant main effect of treatment [$F(4, 45) = 2.3$, $p = 0.072$, not significant]. Finally, analysis of the data relative to the time spent in the light compartment of the apparatus also evidenced a main effect of treatment [$F(4, 45) = 4.5$, $p < 0.01$]. Post-hoc comparisons demonstrated that the 50 mg/kg crocins and the diazepam-treated rats spent more time in the lit chamber before with respect to the vehicle-treated animals ($p < 0.05$, Fig. 2C).

The effects of crocins (50 mg/kg) on rats' spontaneous motility were evaluated in the motor activity test (Table 1). Analysis of motility results did not reveal any difference among the two experimental groups.

Discussion

The present results show that crocins administered at 50 mg/kg induced a significant increment of the latency for the first transition to the dark side and of the amount of time spent by rats in the illuminated chamber of the light/dark box. This dose did not affect the number of transitions between the lit and the dark area of the box. These results were not statistically different from those displayed by the reference compound diazepam under our experimental conditions. Crocins, given at lower doses (15 and 30 mg/kg), did not influence animals' behaviour. It has been demonstrated that the time spent in light compartment is a more sensitive parameter to indicate the anxiolytic action of drugs than the number of transitions between the lit and dark compartments of the apparatus (Kilfoil et al., 1989; Young and Johnson, 1991). It has also been indicated that the number transfers reflect both anxiety and exploration (Lepicard et al., 2000). Our findings seem to be in agreement with these observations.

The light/dark test is a useful procedure to predict anxiolytic-like or anxiogenic-like activity in rodents (Crawley and Goodwin, 1980). This paradigm is limited by its ability to yield false positive results due to a drugs' ability to affect general activity. This, however, can probably be ruled out, since locomotor activity levels displayed by the 50 mg/kg crocins-treated rats were not different from those exhibited by the vehicle-treated animals. In support of this view, in a prior study of ours, we have found that crocins (15–30 mg/kg) did not affect rats' motor activity (Pitsikas et al., 2007). In addition, the number of rearings displayed by rats in the lit area of the light/dark box was not different among the various groups of animals (data not reported). Based on the present results it could be concluded that crocins reduced the anxiety of animals exposed to the light/dark procedure without influencing rodents' motor activity.

The pharmacological mechanism(s) that might account for the anxiolytic effect of crocins has yet to be determined. Further studies will be required to assess the generality of the present findings to other species and behavioural paradigms. Finally, the current findings, for the first time, to our knowledge, demonstrate a possible implication of these active constituents of saffron in anxiety.

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