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Effects of the active constituents of *Crocus Sativus* L., crocins, in an animal model of obsessive–compulsive disorder

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HIGHLIGHTS

- Excessive self-grooming is considered as an animal model of obsessive–compulsive disorder (OCD).
- mCPP induced excessive self-grooming in the rat.
- Crocins attenuated this effect of mCPP.
- Crocins effects cannot be attributed to changes in locomotor activity.
- Crocins might play a role in OCD.

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ABSTRACT

Crocins are among the active components of the plant *Crocus Sativus* L. *C. Sativus* L. and its constituents were effective in different models of psychiatric disorders including anxiety and depression. Obsessive–compulsive disorder (OCD) is a common psychiatric disorder defined by the presence of obsessive thoughts and repetitive compulsive actions. The non selective serotonin (5-HT) receptor agonist mCPP is known to induce OCD-like behavior (excessive self-grooming) in rodents and exacerbate symptoms in patients with OCD. The present study investigated whether or not crocins were able to counteract excessive self-grooming induced by mCPP (0.6 mg/kg, i.p.) in rats. Crocins (30 and 50 mg/kg, i.p.) attenuated mCPP-induced excessive self-grooming. The present results also indicate that these effects of crocins on an animal model of OCD cannot be attributed to changes in locomotor activity. Our findings suggest that the active constituents of *C. Sativus* L. crocins might play a role in compulsive behavior and support a functional interaction between crocins and the serotonergic system.

1. Introduction

Crocus Sativus L. is a plant cultivated in many countries such as Iran, India, Italy, Spain and Greece. Its product is the well known spice called saffron. Saffron is the dried red stigmas of the flower. The main substances of saffron are crocins, picrocrocin and safranal. Crocins, glucosyl esters of crocetin, are unusual water-soluble carotenoids and are responsible for its characteristic color. Picrocrocin, glycoside of safranal, is responsible for the bitter taste of the spice and is precursor of safranal. Safranal, the main component of the distilled essential oil, is a monoterpene aldehyde, responsible for its characteristic aroma [9,19].

The stigmas of *C. Sativus* L. are used in folk medicine as an anticatarrhal, eupeptic, expectorant and emmenagogue (for review, see [17]). Modern pharmacological studies have demonstrated that its crude extracts and purified chemicals possess anti-tumor effects display anti-inflammatory properties, counteract atherosclerosis and hepatic damage [17].

Saffron and its active constituents affect a number of different neural processes, e.g. antagonized memory impairments in rodents [13,14,18,20], conferred neuroprotection in a rat model of Parkinson disease [1] and expressed antioxidant properties in an in vitro model of Alzheimer disease [12]. In this context, it has been previously demonstrated that treatment with *C. Sativus* L. and its constituents induced an anxiolytic-like effect in rodents [8,15]. Further, in studies performed in humans, the antidepressant properties of saffron and its extracts were evidenced [2,11].

Obsessive–compulsive disorder (OCD) is a common psychiatric disorder defined by the presence of obsessive thoughts and repetitive compulsive actions, and it often encompasses anxiety and depressive symptoms [5]. Excessive grooming behavior

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in animals is regarded similar to the symptoms of OCD and other obsessive–compulsive (OC)-spectrum disorders in humans including trichotillomania [6,7]. The non-selective serotonin (5-HT) receptor agonist mCPP is known to induce excessive self-grooming in rats [3,4] and exacerbate symptoms in patients with OCD [21].

Taken the above evidences into account, the aim of the present work was to evaluate the therapeutic potential of crocins in an animal model of compulsive behavior. Therefore, the ability of crocins to antagonize mCPP-induced excessive self-grooming was investigated in rats.

2. Materials and methods

2.1. Animals

Male, 3-month-old Wistar rats (Hellenic Pasteur Institute, Athens, Greece) that weighed 250–300 g were used in this study. The animals were housed in Makrolon cages three per cage, in a regulated environment (21 ± 1 °C; 50–55% relative humidity; 12 h:12 h light/dark cycle, lights on at 7 am) with free access to food and water.

The procedures that involved animals and their care were in accordance with international guidelines and 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).

2.2. Behavioral assay

Spontaneous animals' behavior was assessed in an activity cage (Ugo Basile, Varese, Italy). The apparatus consisted of a box made of Plexiglas (41 cm length × 33 cm height × 41 cm width). For assessing in rats grooming behavior, a procedure modified from a previous work [4] was utilized. Each animal was placed individually into the apparatus. After 10 min of habituation period in the test cage, each animal received the assigned pharmacological treatment. Thereafter, the rat was placed again into the apparatus and the number and duration of grooming events were recorded for 20 min. Vibration, face and head washing, body grooming, scratching, paw licking, head shaking and genital grooming were included as components of grooming behavior [4]. In addition, locomotor activity, expressed as total counts over 20 min was recorded.

2.3. Chemicals

Crocins were isolated from the red dried stigmas (saffron) of *C. Sativus* L., using a slightly modified method described previously [10]. They were purified from stigmas after successive and exhaustive extraction by: (a) petroleum ether 40–60 °C; (b) diethyl ether (Et_2O); and (c) methanol (MeOH) 80% using ultrasound assisted extraction. The ultrasound extraction was performed in a Sonorex, Super RK 255H type (300 mm × 150 mm × 150 mm internal dimensions) ultrasound water bath (indirect sonication), at the fixed frequency of 35 kHz. The temperature of the sonicated water was 25 °C. Procedures (a) and (b) took place in order for the stigmas to be free of the picrocrocin and safranal respectively. The methanol extract, after evaporation (condensed to dryness) under vacuum at room temperature, provided crocins which are dark red powder residue.

Crocins and 1-(3-chlorophenyl)piperazine hydrochloride (mCPP) (Sigma, St. Louis, MO, U.S.A.) were dissolved in saline (NaCl 0.9%). The crocins doses (30 and 50 mg/kg) were selected based on the results of a previous study in which the same dose range was found to display anxiolytic-like effects in the light/dark test in the rat [15]. The dose of mCPP (0.6 mg/kg) was chosen based on a prior study in which this dose caused excessive self-grooming in rats without producing side effects [4]. All solutions were freshly prepared on the day of testing and were administered intraperitoneally (i.p.) in a volume of 1 ml/kg. Specifically, the concentrations of crocins were 30 or 50 mg/ml respectively. Control animals received isovolumetric amounts of the vehicle (NaCl 0.9%).

2.4. Experimental protocol

The experiments were conducted between 10 am and 2 pm in a room where only these animals where housed. Animals' behavior was video-recorded. Data evaluation was subsequently performed by experimenters who were unaware of the pharmacological treatment of each subject.

Rats were randomly divided into six experimental groups (8 rats per group) as follows: vehicle + vehicle; vehicle + crocins 30 mg/kg; vehicle + crocins 50 mg/kg; mCPP 0.6 mg/kg + vehicle; mCPP 0.6 mg/kg + crocins 30 mg/kg; and mCPP 0.6 mg/kg + crocins 50 mg/kg.

2.5. Statistical analysis

All data are expressed as mean ± S.E.M. Results were analyzed by using the two-way analysis of variance (ANOVA) test. Post hoc comparisons were made by the Tukey *t*-test. A p value < 0.05 was considered statistically significant.

3. Results

The statistical analyses of self-grooming duration data (Fig. 1) showed a significant main effect of mCPP [$F(1, 47) = 55.3$, $p < 0.01$], of crocins [$F(2, 47) = 6$, $p < 0.01$] and a significant mCPP × crocins two-way interaction [$F(2, 47) = 4.6$, $p < 0.01$]. The post hoc comparisons revealed an increase in the duration of grooming events in the mCPP + vehicle-treated animals as compared to the other experimental groups, including the rats that received either mCPP + crocins 30 mg/kg or mCPP + crocins 50 mg/kg ($p < 0.05$). The duration of self-grooming exhibited by animals that received both mCPP + crocins was longer compared to that displayed by their cohorts that received crocins + vehicle ($p < 0.05$).

The statistical analyses of the number of grooming events evidenced a significant main effect of mCPP [$F(1, 47) = 28.4$, $p < 0.01$], of crocins [$F(2, 47) = 3.77$, $p < 0.05$] and a significant two-way interaction between mCPP and crocins [$F(2, 47) = 3.6$, $p < 0.05$, Fig. 2]. The post hoc comparisons revealed that the mCPP + vehicle-treated animals displayed a higher number of grooming episodes compared to the other experimental groups,

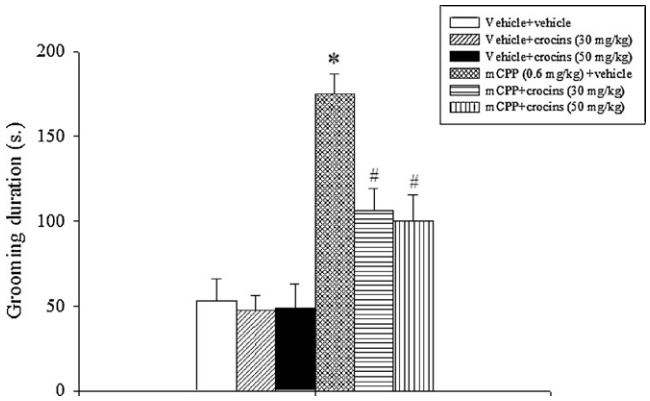

Fig. 1. Grooming duration. Vehicle, mCPP and crocins were injected i.p., just before testing. Results are expressed as mean ± S.E.M. *$p < 0.05$ vs. all the other groups; #$p < 0.05$ vs. the vehicle and the respective control groups.

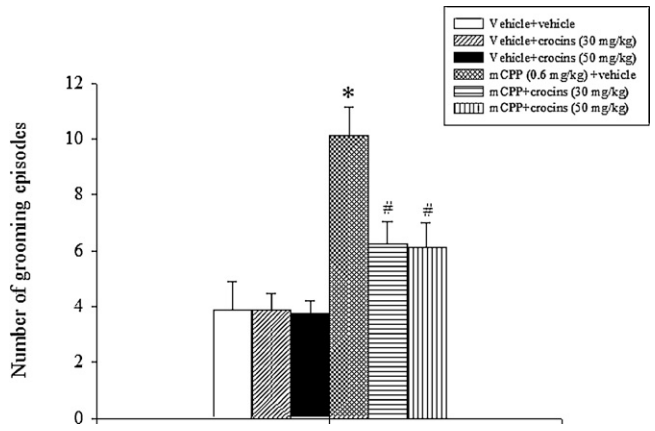

Fig. 2. Number of grooming episodes. Vehicle, mCPP and crocins were injected i.p., just before testing. Results are expressed as mean ± S.E.M. $^{*}p < 0.05$ vs. all the other groups; $^{\#}p < 0.05$ vs. the vehicle and the respective control groups.

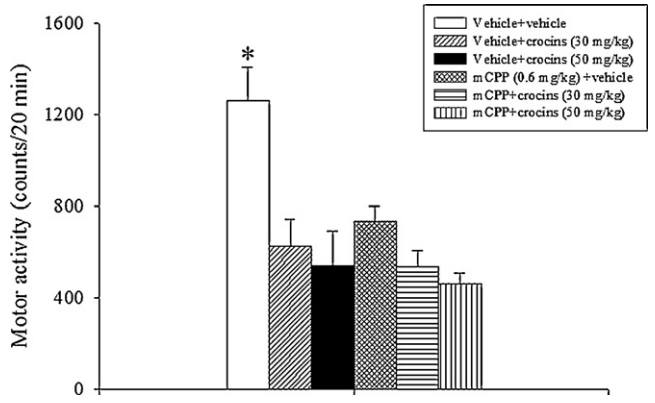

Fig. 3. Locomotor activity. Vehicle, mCPP and crocins were injected i.p., just before testing. Results are expressed as mean ± S.E.M. $^{*}p < 0.05$ vs. all the other groups.

including the mCPP + crocins 30 mg/kg and the mCPP + crocins 50 mg/kg-treated animals ($p < 0.05$).

A two-way ANOVA conducted on motor activity results demonstrated a significant main effect of mCPP [$F(1, 47) = 7.1$, $p < 0.05$], of crocins [$F(2, 47) = 12.3$, $p < 0.01$] but not a significant two-way interaction between mCPP and crocins [$F(2, 47) = 2.8$, $p = 0.07$, Fig. 3]. The post hoc comparisons indicate that animals that received mCPP and crocins displayed lower motility levels compared to their vehicle-treated counterparts ($p < 0.05$).

4. Discussion

In line with previous results administration of mCPP induced excessive self-grooming in rats [3,4]. A per se effect of crocins on grooming behavior was not observed since treatment with these carotenoids did not affect grooming activity at any of the doses tested. Interestingly, crocins reduced mCPP-induced excessive self-grooming. Grooming activity of rats that received mCPP and crocins however, was higher than that displayed by their control counterparts.

Locomotor activity was assessed as an independent control for direct drug effects on physical activity that could confound the interpretation of results from self-grooming. Animals that received mCPP and/or crocins showed similar levels of motility which however, were lower compared to those displayed by the vehicle + vehicle-treated rats. This pattern of results implies that the effects of compounds on rats' performance were unrelated to the extent of motor activity.

The current findings appear to be in contrast with the results of a previous study in which a sedative effect of crocins was revealed because these compounds reduced both grooming and motility in mice [8]. These discrepant findings with regard to the effects of crocins may be attributable to difference in the experimental settings (type of animal, pharmacological design, behavioral procedure). Specifically, in that study [8] the effects of crocins were tested in the mouse, and were administered 30 min before testing, at a higher dose regimen (50–600 mg/kg) than that used in our experimentation.

mCPP is a non selective 5-HT receptor agonist displaying affinity for the 5-HT_2 family receptors which consists of three subtypes, namely the 5-HT_{2A}, the 5-HT_{2B} and the 5-HT_{2C} receptor. Functional characterization on recombinant human receptors showed that, compared to 5-HT, mCPP has a higher affinity for the 5-HT_{2C} receptor with respect to the other receptors of the 5-HT_2 family receptors [16]. Excessive self-grooming induced by mCPP was reversed by the selective 5-HT_{2C} receptor antagonist SB-242084, but not by the selective 5-HT_{2B} receptor antagonist SB-215505. These results provide evidence that the mCPP-induced exaggerated grooming is mediated, at least, by activation of the 5-HT_{2C} receptor [4]. In this context, the present findings suggest, that crocins might alleviate the mCPP-induced excessive self-grooming by an antagonistic action at the 5-HT_{2C} receptor site.

The pharmacological mechanism(s) that might account for the effect of crocins on compulsive behavior has yet to be determined. Further studies will be required to assess the generality of the present findings to other species and behavioral paradigms. Finally, the current findings demonstrate an implication of these active constituents of saffron in compulsive behavior and support a functional interaction between crocins and 5-HT.

Conflict of interest statement

The authors declare no potential conflicts of interest with respect to authorship and/or publication of this article.

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