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The effect of acute crocin on behavioral changes and BDNF expression level in socially isolated rats

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Abstract

Social isolation is a reliable method used for the induction of depression and psychiatric disorders in rodents. It has been suggested that social isolation can lead to hyperlocomotion, as a schizophrenic-like symptom in rodents. On the other hand, crocin (the major constituent of *Crocus sativus*) induces a wide-range of neuroprotective and mood enhancer effects. In the present study, we aimed to investigate the effect of acute crocin on social isolation-induced behavioral changes and BDNF expression in the hippocampus. Novelty-suppressed feeding test, open field test, marble burying test, hot plate, forced swim test, and the shuttle box were used to assess anxiety-like behavior, locomotor activity, obsessive–compulsive-like (OCD-like) behavior, pain threshold, depressive-like behavior, and passive avoidance memory, respectively. Real-time PCR was used to assess BDNF hippocampal expression level. The results showed that social isolation decreased anxiety- and depressive-like behavior, pain threshold, and BDNF expression, and induced OCD-like behavior and hyperlocomotion. Crocin dose-dependently restored the effect of social isolation on pain threshold, locomotor activity, depressive-like behavior, OCD-like behavior, and BDNF expression. Passive avoidance memory performance was also unaffected. In conclusion, we showed a hyperlocomotion profile and OCD-like behaviors, and a robust decrease in pain threshold in socially isolated rats. It can be suggested that social isolation from adolescence induces a "hyperlocomotion state" that affects all the behavioral functions of rats. Also, the function of BDNF can be related to a hyperlocomotion state and OCD-like symptom. It seems that BDNF expression level can be related to the therapeutic effect of crocin.

Keywords Social isolation · Crocin · Hyperlocomotion · Pain · Brain-derived neurotrophic factor (BDNF)

Introduction

Social isolation is one of the most common methods used for the induction of depression and psychiatric disorders in rodents (Begni et al. 2020). Social isolation from early life significantly leads to the induction of long-lasting changes in molecular factors and behavioral functions (Begni et al. 2020; Cattaneo and Riva 2016). The hyperlocomotion is an

early onset and significant observation in socially isolated rats and is a criterion for the so-called isolation syndrome (Heidbreder et al. 2000). Also, increased anxiety- and depressive-like behaviors have been observed following social isolation (Djordjevic et al. 2012). Previous studies have shown that social isolation can lead to impaired neurogenesis, long-term potentiation (LTP), and neural plasticity (Roberts and Greene 2003; Fone and Porkess 2008; Silva-Gomez et al. 2003), all involved in cognitive and behavioral deficits. Many studies shown the role of social isolation in the induction of depression and anxiety (Si et al. 2023; Zarebavani et al. 2023). Social isolation may also be related to the symptoms of obsession and checking (Timpano et al. 2014). In addition, it has been shown that social isolation reduces the expression level of brain-derived neurotrophic factor (BDNF) (Borges et al. 2023).

BDNF is an important neurotrophin that is well studied and is one of the most well-characterized neurotrophic

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factors in the central nervous system (CNS) (Colucci-D'Amato et al. 2020). BDNF is significantly involved in mediating cognitive and behavioral function. BDNF and hippocampal neurogenesis have a close relationship (Numakawa et al. 2018). Also, BDNF modulates synaptic plasticity and neural survival (Li et al. 2017; Lu et al. 2013). Decreased level of BDNF expression has been reported in a wide-range of neuropsychiatric disorders and cognitive impairments. Previous study has shown that BDNF plays a crucial role in antidepressant-induced hippocampal neurogenesis (Li et al. 2008). It seems that the antidepressant effect of BDNF may be mediated by the improvement of neurogenesis (Scharfman et al. 2005). In addition, social isolation significantly reduces the expression of BDNF. It has been shown that social isolation for 14 days significantly reduces BDNF expression level in rats (Aswar et al. 2022). Other study has shown that BDNF expression level in the hippocampus is decreased in isolated reared rats (Galal et al. 2021).

On the other hand, crocin (an active and therapeutical component of *Saffron*) has pro-cognitive effects and can increase the expression and function of BDNF. There are many studies showing the pro-cognitive effects of crocin (Azmand and Rajaei 2021; Looti Bashiyan et al. 2021; Song et al. 2022). Furthermore, crocin induces antidepressant and anxiolytic effects. Previous research has shown that crocin attenuates depressive-like behavior in rats exposed to chronic unpredictable mild stress (Wang et al. 2022). It has also been revealed that crocin significantly reduces depressive- and anxiety-like behaviors in rats exposed to chronic unpredictable mild stress (Abbaszade-Cheragheali et al. 2022). Furthermore, crocin can increase the protein level of BDNF in the hippocampus of sleep-deprived rats, leading to the induction of pro-cognitive effects (Looti Bashiyan et al. 2021). It has also been shown that crocin induces neuroprotective effects via increasing BDNF expression in the hippocampus of rats (Mozaffari et al. 2019).

Thus, in the present study, we aim to explore the potential role of crocin in the improvement of mood state and BDNF function in socially isolated rats.

Material and method

Animals

Sixty male Wistar rats (180-200g, 8-9 weeks old) were used in this study. The rats were housed six per Plexiglas cage (25*50*20cm). Also, a 12h:12h light/dark cycle (lights on at 7:00h) and stable temperature (22±1°C) were provided. Each experimental group consisted of six rats, based on previous research (Wang et al. 2023; Al-Dmour et al. 2023; Rajkumar et al. 2023). All the rats were born and bred in Cognitive Neuroscience Lab, Medicinal Plants Research Center, Institute of Medicinal Plants, ACECR, Karaj, Iran. Also, all the rats had free access to food and water and all the experiments were done during the light hours (9:00 a.m. to 3:00 p.m.). Our experimental protocol was designed in accord with National Institutes of Health Guide for the Care and Use of Laboratory Animals (2011).

Experimental groups

This study consisted of 10 groups (n=6 for each group):

Group 1- Control: The rats received no injection.

Group 2- Sham: The rats (at PND 60) received saline (1 mL/rat) injection.

Group 3: Crocin 25 mg/kg: The rats (at PND 60) received crocin (25 mg/kg) injection.

Group 4: Crocin 50 mg/kg: The rats (at PND 60) received crocin (50 mg/kg) injection.

Group 5: Crocin 100 mg/kg: The rats (at PND 60) received crocin (100 mg/kg) injection.

Group 6- Social isolation: The rats (at 30 PND) were socially isolated for 30 days (from 30 to 60 PND).

Group 7- Social isolation + Saline: The rats (at 30 PND) were socially isolated for 30 days (from 30 to 60 PND), and saline (1 mL/rat) was injected at the day 60, 1 h before the tests.

Group 8- Social isolation + Crocin 25 m/kg: The rats (at 30 PND) were socially isolated for 30 days (from 30 to 60 PND), and crocin (25 mg/kg) was injected at the day 60, 1 h before the tests.

Group 9- Social isolation + Crocin 50 mg/kg: The rats (at 30 PND) were socially isolated for 30 days (from 30 to 60 PND), and crocin (50 mg/kg) was injected at the day 60, 1 h before the tests.

Group 10- Social isolation + Crocin 100 mg/kg: The rats (at 30 PND) were socially isolated for 30 days (from 30 to 60 PND), and crocin (100 mg/kg) was injected at the day 60, 1 h before the tests.

The order of the experiments was as follows: Novelty-suppressed feeding test, open field test, marble burying test, hot plate, forced swim test, shuttle box (train), shuttle box (test), real-time PCR. There was an interval of 30 minutes between novelty-suppressed feeding test (NFST) and open field test (OFT) to allow the rats to eat enough food. NFST, OFT, and marble burying test were conducted at 60 PND. Hot plate, forced swim test (FST), and shuttle box (training) were conducted at 61 PND. Shuttle box (test) and sacrifice were done at 62 PND. The timeline of the study has been provided in Fig. 1 (Fig. 1).
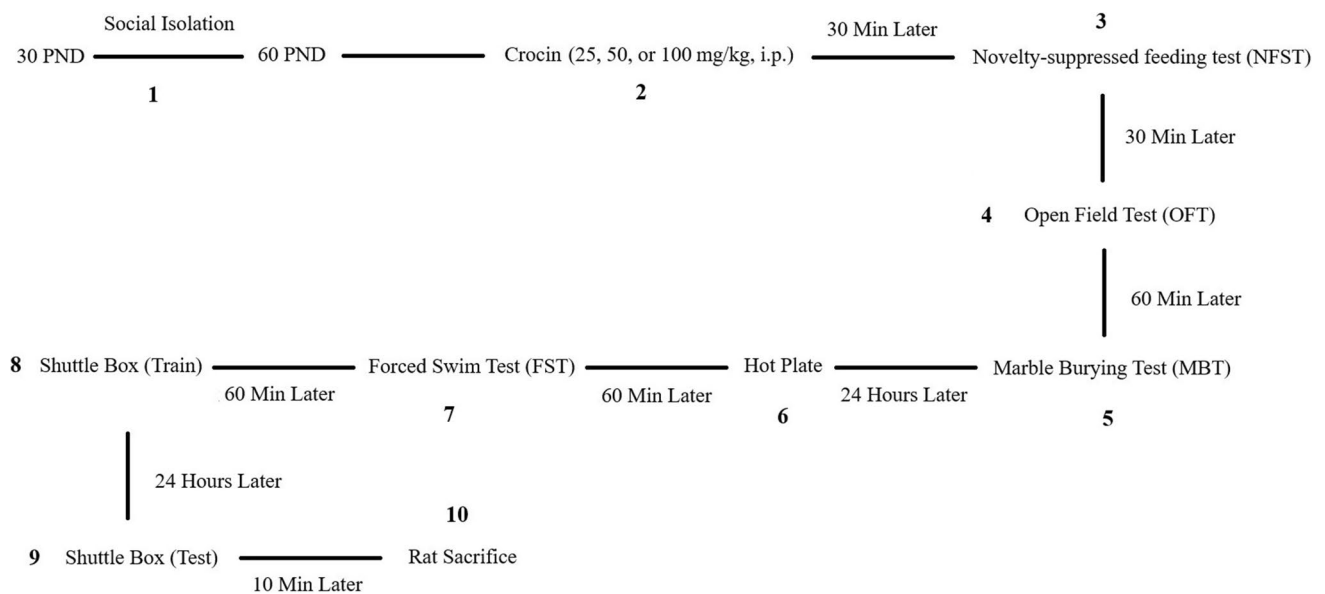
Fig. 1 The order of the experiments and the timeline of the study (24 h before novelty-suppressed feeding test (NFST), food deprivation started)

Social isolation

All the rats were socially isolated at the age of 30 days (30 postnatal day "PND"). During isolation, each rat was placed in a separate cage and food and water were provided. The duration of social isolation was 30 days (Guven et al. 2022). All the tests were performed when the rats were at the age of 60 days (60 PND).

Crocin

Crocin was purchased from *Pouyesh Darou Sina, Samisaz Co., Mashhad, Iran*. Crocin was dissolved in normal saline and was injected intraperitoneal at the doses of 25, 50, and 100 mg/kg. Crocin doses were chosen according to previous studies (Li et al. 2022; Azmand and Rajaei 2021; Sun et al. 2020).

Novelty-suppressed feeding test

Novelty-suppressed feeding test (NFST) evaluates anxiety-like behavior in rodents. Twenty-four hours before the test, all the rats were deprived only from food. During the test day, the rat was placed in one corner of a plastic box (100×100×40 cm) with wooden bedding covered floor and had ten minutes to explore the environment. A food pellet was placed on a circular filter paper in the center of the arena. Each rat was placed in a corner of the box and the time taken to bite food, not simply sniff or touch the pellet was recorded as feeding latency. If a rat did not bite food during ten minutes, the feeding latency was recorded as 10 min (Laaziz et al. 2022).

Open field test

The open field test (OFT) (Tajhiz-Gostar Omid Iranian Co, Tehran, Iran) is a reliable method to measure locomotor activity. This apparatus consists of clear perspex container box (height: 30×30×40cm) divided into 16 equal-sized squares. Locomotor activity is evaluated as the number of crossings from one square to another during 300s (Rezaie et al. 2021; Mahdavi et al. 2021).

Marble burying test

Marble burying test evaluates obsessive-compulsive disorder (OCD)-like behavior in rodents. In this test, standard glass toy marbles (assorted styles and colors, 15 mm diameter, 5-6 g in weight) gently put on the surface of the bedding in 2 rows of 5 marbles (10 marbles). Note that, marble burying test involves the placement of any number of marbles (usually between 4 and 25, depending on the zone configuration of the marble-burying arena) (de Brouwer et al. 2019). The duration of the test was 30 minutes. OCD-like behavior was evaluated by recording the number of buried marbles.

Hot plate

Pain threshold was assessed using hot plate apparatus. Hot plate was a sheet getting hot by electric current (Tajhiz-Gostar Omid Iranian Co, Tehran, Iran). At first, hot plate sheet was cleaned by ethanol 70% and each rat was placed on the hot sheet. The start time was determined and as soon as the rats started to lick their paws or change their steps, the pain threshold was recorded. The temperature of the apparatus was set at 50 °C. The cut-off time was 100 seconds (Mahdavi et al. 2021).

Forced swim test

Forced swim test (FST) is an approved test to evaluate depressive-like behavior in rats. FST consists of a cylindrical transparent container that is filled with water (20-22°C) up to 2/3 of it. Each rat was float inside the cylindrical transparent container. FST duration was 5 minutes. During 5 minutes, immobility and climbing time were measured. The duration of immobility was considered as depressive-like behavior (Kordestani-Moghadam et al. 2020).

Passive avoidance memory

Shuttle box apparatus was used to assess passive avoidance memory performance. Shuttle box had two equal-sized compartments (25×25×25 cm), including a light and a dark compartment with a grid floor. The two compartments were separated by a guillotine door. This test had two sessions: training and test. In the training session, each rat was placed into the light compartment for 60 seconds. After opening the guillotine door and entrance of the rat into the dark compartment, the door was closed and a 0.5 mA with 50 Hz foot electric shock was delivered for 2 seconds via the grid floor. 20 seconds later, the rat was delivered to its cage. In the test session (24h after training), rats were placed in the light compartment. The step-through latency to enter the dark compartment was measured, to a maximum of 300 seconds (Rezaie et al. 2020).

Real-time PCR

Total RNA extraction and preparation of cDNA

Ten minutes after the shuttle box test, the brain sample of each rat was obtained after euthanasia and decapitation. Following the decapitation, the hippocampus samples were dissected from the brain; snap-frozen in liquid nitrogen; and stored at -80 ◦C before sample preparation. For gene expression measurement, total RNA was extracted from 100 mg of the hippocampus by Qiazol (Qiazol lysis reagent, USA) in a sterilized RNase-free tube. NanoDrop ND-100 spectrophotometer (Thermo Scientific, Waltham, MA, USA) evaluated the concentration and purity of RNA by the ratio of the absorbance at 260 nm and 280 nm (A 260 /A 280). RNA was converted into complementary DNA (cDNA) by DNase I first strand synthesis system for RT-PCR (Fermentase, Germany), according to the manufacturer's recommendations.

Real-time PCR reactions were done using Takara SYBR Premix Ex Taq II (Tli RNaseH Plus) (2X conc.) in a final volume of 20 μl on StepOnePlus Real-Time PCR System (Applied Biosystems). 2 μl of the synthesized cDNA was used in all reactions. The annealing temperature optimized for primers pairs was 64 °C. For quantification of target gene, standard curve method was applied. All the samples were loaded in duplicate and the mean data were used for further analysis. The specificity of PCR products was verified by observing a single peak in melting curve analysis. The data obtained were normalized against the housekeeping gene (encoding glyceraldehyde 3-phosphate dehydrogenase, GAPDH). Relative quantification according to the $\Delta\Delta$Ct-method (Livak and Schmittgen 2001) was used for data analysis. For complementary length verification, PCR products were visualized on 2.5% agarose gel (Malboosi et al. 2020).

Oligonucleotide set design

GAPDH was used as the housekeeping gene to normalize target gene expression. The primers that were used for the real-time PCR was BDNF (Table 1).

Statistical analyses

SPSS software (V.26) was used to analyze data. The Shapiro-Wilk test was used to assess normality of distribution (The Shapiro-Wilk results were not significant for all the tests). Furthermore, two-way and one-way ANOVA, and post hoc Tukey's were used to compare groups. Spearman correlation test was also used to assess potential correlations between BDNF expression level and each behavioral function. Data were expressed as mean $\pm$ SD and $P<0.05$ was considered as the level of statistical significance.

Results

Anxiety

The results of one-way ANOVA revealed that there was no significant difference between control rats ($F_{4,29} = 1.52$, $P>0.05$). Also, the results of one-way ANOVA revealed that there was no significant difference between socially isolated rats ($F_{4,29} = 0.31$, $P>0.05$) (Fig. 2).

Table 1 The primers used for the Real-time PCR

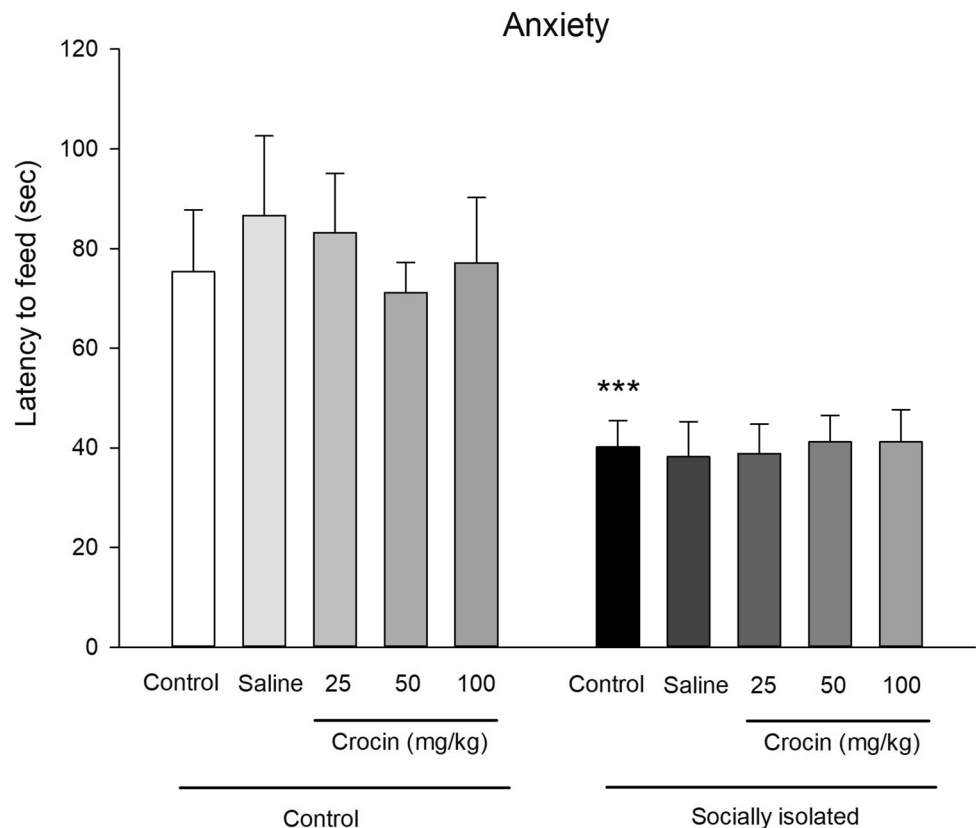| Primer	Sequence ($5´\rightarrow3´$)
BDNF	F: 5'-GGACAT ATCCATGAC CAGAAAGA-3' R: 5'-GGCAAC AAACCACAA CATTATCG-3'
GAPDH	F: 5'-CATTCT TCCACCTTT GATGCTG-3' R: 5'-TGGTCC AGGGTTTCT TACTCC-3'

The results of two-way ANOVA revealed that only the effect of isolation was significant ($F_{1,50} = 239.96$, P<0.001), while the effect of crocin ($F_{4,50} = 0.79$, P>0.05) and crocin*isolation ($F_{4,50} = 0.15$, P>0.05) was not significant. Post hoc Tukey test showed that social isolation significantly decreased anxiety-like behavior (P<0.001), however, crocin at all doses had no effect (Fig. 2).

Locomotor activity

The results of one-way ANOVA revealed that there was no significant difference between control rats ($F_{4,29} = 0.66$, P>0.05). Also, the results of one-way ANOVA revealed that there was a significant difference between socially isolated rats ($F_{4,29} = 19.66$, P<0.001). Post hoc Tukey test showed that crocin (100 mg/kg) decreased locomotor activity in socially isolated rats (P<0.001) (Fig. 3).

The results of two-way ANOVA revealed that the effect of isolation ($F_{1,50} = 294.39$, P<0.001), crocin ($F_{4,50} = 10.10$, P<0.001), and crocin*isolation ($F_{4,50} = 9.55$, P<0.001) was significant. Post hoc Tukey test showed that social isolation significantly increased locomotor activity (P<0.001), and crocin at the dose of 100 mg/kg reversed this effect (P<0.001) (Fig. 3).

Marble burying test

The results of one-way ANOVA revealed that there was no significant difference between control rats ($F_{4,29} = 0.18$, P>0.05). Also, the results of one-way ANOVA revealed that there was a significant difference between socially isolated rats ($F_{4,29} = 16.31$, P<0.001). Post hoc Tukey test showed that crocin (100 mg/kg) decreased OCD-like behavior in socially isolated rats (P<0.001) (Fig. 4).

The results of two-way ANOVA revealed that the effect of isolation ($F_{1,50} = 149.04$, P<0.001), crocin ($F_{4,50} = 9.11$, P<0.001), and crocin*isolation ($F_{4,50} = 11.08$, P<0.001) was significant. Post hoc Tukey test showed that social isolation significantly induced OCD-like behavior (P<0.001), and crocin at the dose of 100 mg/kg reversed this effect (P<0.001) (Fig. 4).

Pain threshold

The results of one-way ANOVA revealed that there was a significant difference between control rats ($F_{4,29} = 12.01$, P<0.001). Post hoc Tukey test showed that crocin (100 mg/kg) increased the pain threshold (P<0.001). Also, the results of one-way ANOVA revealed that there was a significant difference between socially isolated rats ($F_{4,29} = $

Fig. 2 shows anxiety-like behavior in control and socially isolated rats, received no injection, saline, or crocin at the doses of 25, 50, and 100 mg/kg (***P < 0.001 in comparison with control group) (n = 6 for each group)
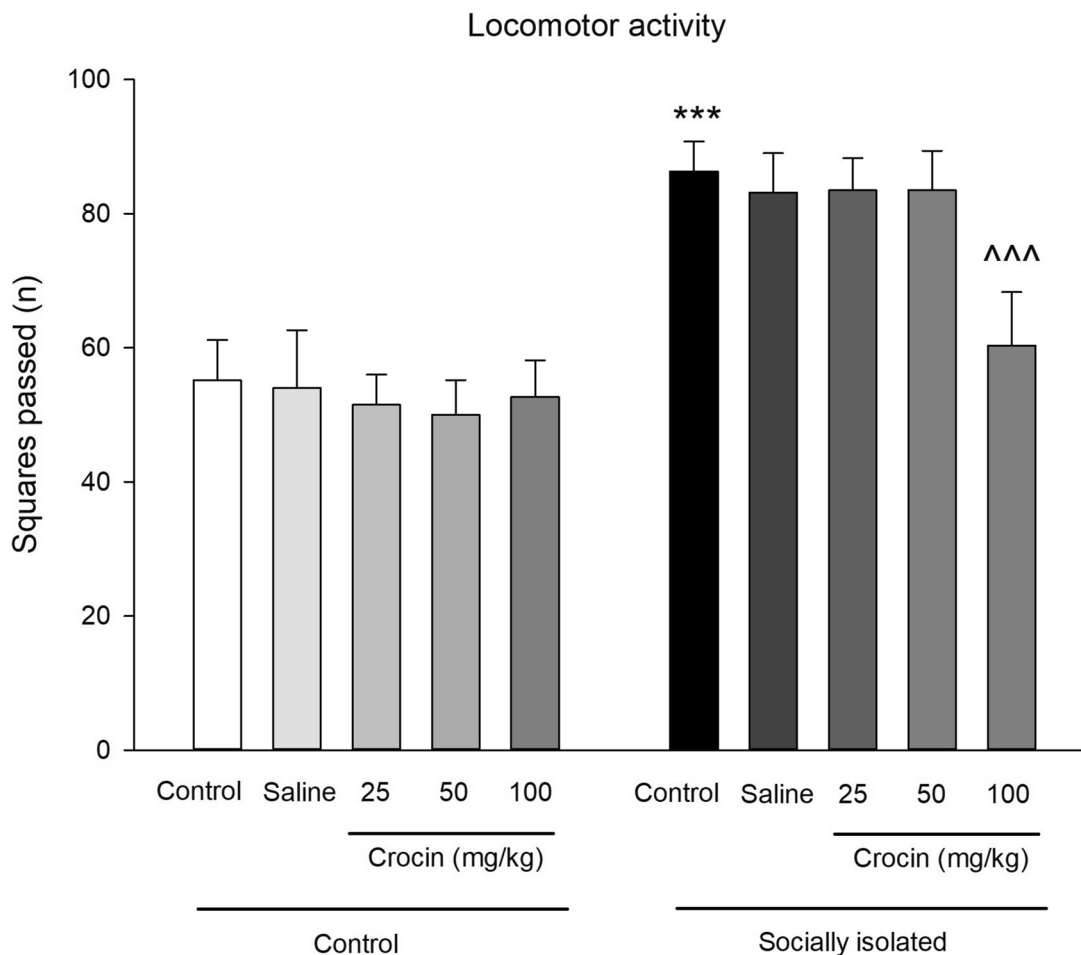
Fig. 3 shows locomotor activity in control and socially isolated rats, received no injection, saline, or crocin at the doses of 25, 50, and 100 mg/kg (***$P < 0.001$ in comparison with control group; ^^^$P < 0.001$ in comparison with social isolation group) (n = 6 for each group)

86.38, $P<0.001$). Post hoc Tukey test showed that crocin (100 mg/kg) increased pain threshold in socially isolated rats ($P<0.001$) (Fig. 5).

The results of two-way ANOVA revealed that the effect of isolation ($F_{1,50} = 495.00$, $P<0.001$), crocin ($F_{4,50} = 64.37$, $P<0.001$), and crocin*isolation ($F_{4,50} = 5.67$, $P<0.001$) was significant. Post hoc Tukey test showed that social isolation significantly decreased pain threshold ($P<0.001$), and crocin at the dose of 100 mg/kg reversed this effect ($P<0.001$) (Fig. 5).
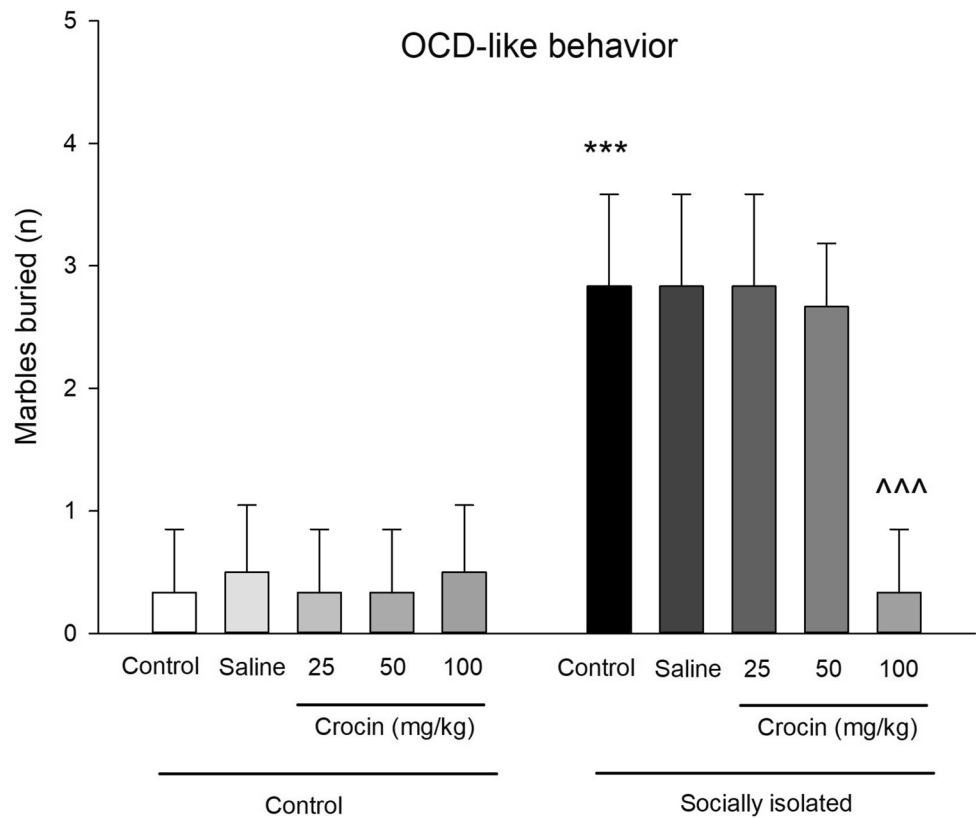
FST

Climbing- The results of one-way ANOVA revealed that there was no significant difference between control rats ($F_{4,29} = 0.52$, $P>0.05$). Also, the results of one-way ANOVA revealed that there was no significant difference between socially isolated rats ($F_{4,29} = 0.14$, $P>0.05$). The results of two-way ANOVA revealed that only the effect of isolation

was significant ($F_{1,50} = 51.86$, $P<0.001$), while the effect of crocin ($F_{4,50} = 0.27$, $P>0.05$) and crocin*isolation ($F_{4,50} = 0.52$, $P>0.05$) was not significant. Post hoc Tukey test showed that social isolation significantly increased climbing ($P<0.05$), however, crocin at all doses had no effect (Fig. 6).

Immobility- The results of one-way ANOVA revealed that there was no significant difference between control rats ($F_{4,29} = 0.97$, $P>0.05$). Also, the results of one-way ANOVA revealed that there was a significant difference between socially isolated rats ($F_{4,29} = 11.87$, $P<0.001$). Post hoc Tukey test showed that crocin (100 mg/kg, $P<0.001$; and 50 mg/kg, $P<0.01$) increased immobility in socially isolated rats. The results of two-way ANOVA revealed that the effect of isolation ($F_{1,50} = 54.14$, $P<0.001$), crocin ($F_{4,50} = 6.88$, $P<0.001$), and crocin*isolation ($F_{4,50} = 4.47$, $P<0.01$) was significant. Post hoc Tukey test showed that social isolation significantly decreased immobility ($P<0.001$), and crocin at the doses of 50 and 100 mg/kg reversed this effect ($P<0.05$) (Fig. 6).

Fig. 4 shows OCD-like behavior in control and socially isolated rats, received no injection, saline, or crocin at the doses of 25, 50, and 100 mg/kg (***$P < 0.001$ in comparison with control group; ^^^$P < 0.001$ in comparison with social isolation group) (n = 6 for each group)

Passive avoidance memory

The results of one-way ANOVA revealed that there was no significant difference between control rats ($F_{4,29} = 0.04$, $P > 0.05$). Also, the results of one-way ANOVA revealed that there was no significant difference between socially isolated rats ($F_{4,29} = 0.27$, $P > 0.05$) (Fig. 7).

The results of two-way ANOVA also showed that the effect of isolation ($F_{1,50} = 0.65$, $P > 0.05$), crocin ($F_{4,50} = 0.22$, $P > 0.05$), and crocin*isolation ($F_{4,50} = 0.13$, $P > 0.05$) was not significant (Fig. 7).

BDNF

The results of one-way ANOVA revealed that there was a significant difference between control rats ($F_{4,29} = 39.84$, $P < 0.001$). Post hoc Tukey test showed that crocin (100 mg/kg) increased BDNF expression level in the hippocampus of control rats. Also, the results of one-way ANOVA revealed that there was a significant difference between socially isolated rats ($F_{4,29} = 54.03$, $P < 0.001$). Post hoc Tukey test showed that crocin (100 mg/kg, $P < 0.001$; and 50 mg/kg, $P < 0.01$) increased BDNF expression level in the hippocampus of socially isolated rats (Fig. 8).

The results of two-way ANOVA revealed that the effect of isolation ($F_{1,50} = 20.92$, $P < 0.001$), crocin ($F_{4,50} = 91.98$, $P < 0.001$), and crocin*isolation ($F_{4,50} = 4.78$, $P < 0.01$) was significant. Post hoc Tukey test showed that social isolation significantly decreased BDNF expression level ($P < 0.001$), and crocin at the doses of 50 ($P < 0.01$) and 100 ($P < 0.001$) mg/kg reversed this effect (Fig. 8).

Correlations

Spearman correlation test showed that there was a significant correlation between BDNF expression level and pain threshold in control rats (rho = 0.442, $P < 0.05$), and between BDNF expression level and locomotor activity (rho = -0.353, $P < 0.05$), pain threshold (rho = 0.398, $P < 0.05$), and immobility (rho = 0.786, $P < 0.001$) in socially isolated rats. Therefore, results showed a direct correlation between BDNF expression level and pain perception in control rats received crocin 100 mg/kg, and between BDNF expression level and pain perception or immobility in socially isolated rats received no injection or crocin 100 mg/kg. Also, there was an inverse (opposite) correlation between BDNF expression level and locomotor activity in socially isolated rats received no injection or crocin 100 mg/kg (Table 2).
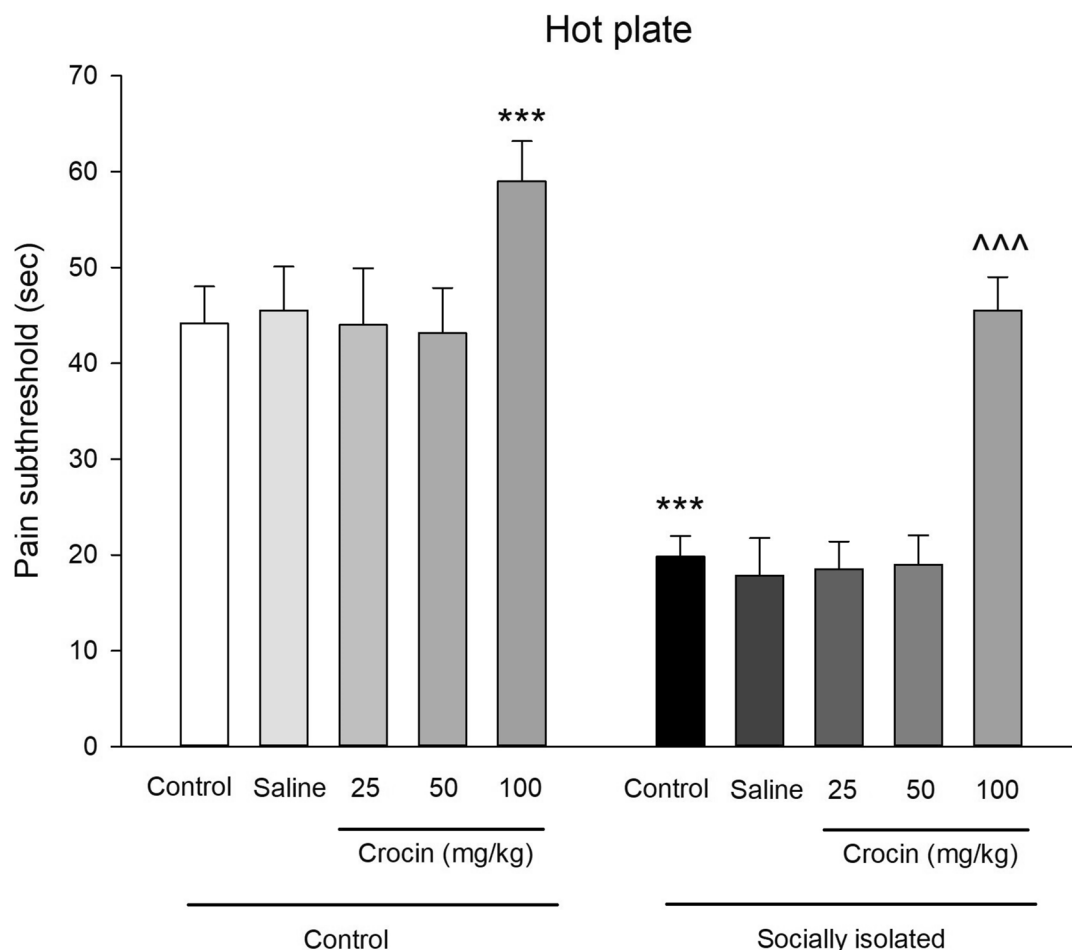
Hot plate

Fig. 5 shows pain threshold in control and socially isolated rats, received no injection, saline, or crocin at the doses of 25, 50, and 100 mg/kg (***$P < 0.001$ in comparison with control group; ^^^$P < 0.001$ in comparison with social isolation group) ($n = 6$ for each group)

Discussion

As the results showed, social isolation decreased anxiety- and depressive-like behavior, pain threshold, and BDNF expression, and induced hyperlocomotion and OCD-like behavior. However, crocin dose-dependently reversed the effect of social isolation on pain threshold, OCD-like behavior, locomotor activity, depressive-like behavior, and BDNF expression. Both social isolation and crocin did not affect passive avoidance memory performance. Also, crocin had no effect on climbing and anxiety-like behavior in socially isolated rats. Pearson correlation test also showed significant correlations between BDNF expression level and locomotor activity, pain perception, and immobility (depressive-like behavior) in control rats received crocin 100 mg/kg and in socially isolated rats received no injection or crocin 100 mg/ kg (Table 2).

Social isolation is one of the most effective stressors that significantly disrupts cognitive functions and behavioral performance (Reinwald et al. 2018). Chronic social isolation is

a highly stressful condition that induces a wide-range of neural and behavioral deficits in humans, non-human primates, and rodents (Harlow et al. 1965; Fone and Porkess 2008; Brenes et al. 2020). Social isolation has been considered as one of the most reliable preclinical models of schizophrenia, because it induces the core features of mental disorders, cognitive impairments, hyperactivity, and sensory gating deficits (Fone and Porkess 2008). It has been reported that social isolation in males and females, and restraint in males during adolescence lead to dynamic changes in the hippocampus, prefrontal, and amygdala (Pattwell et al. 2016). Importantly, increased locomotor activity induced by social isolation is a significant symptom observed in isolated rats, and has been considered as a criterion for the "isolation syndrome" (Heidbreder et al. 2000). It has been suggested that animals isolated during adulthood show hyperactivity, while returning to a social context during adolescence normalized the behavioral phenotype (Begni et al. 2020). A recent study has shown that learning and memory networks centered on the amygdala and hippocampus are significantly vulnerable to
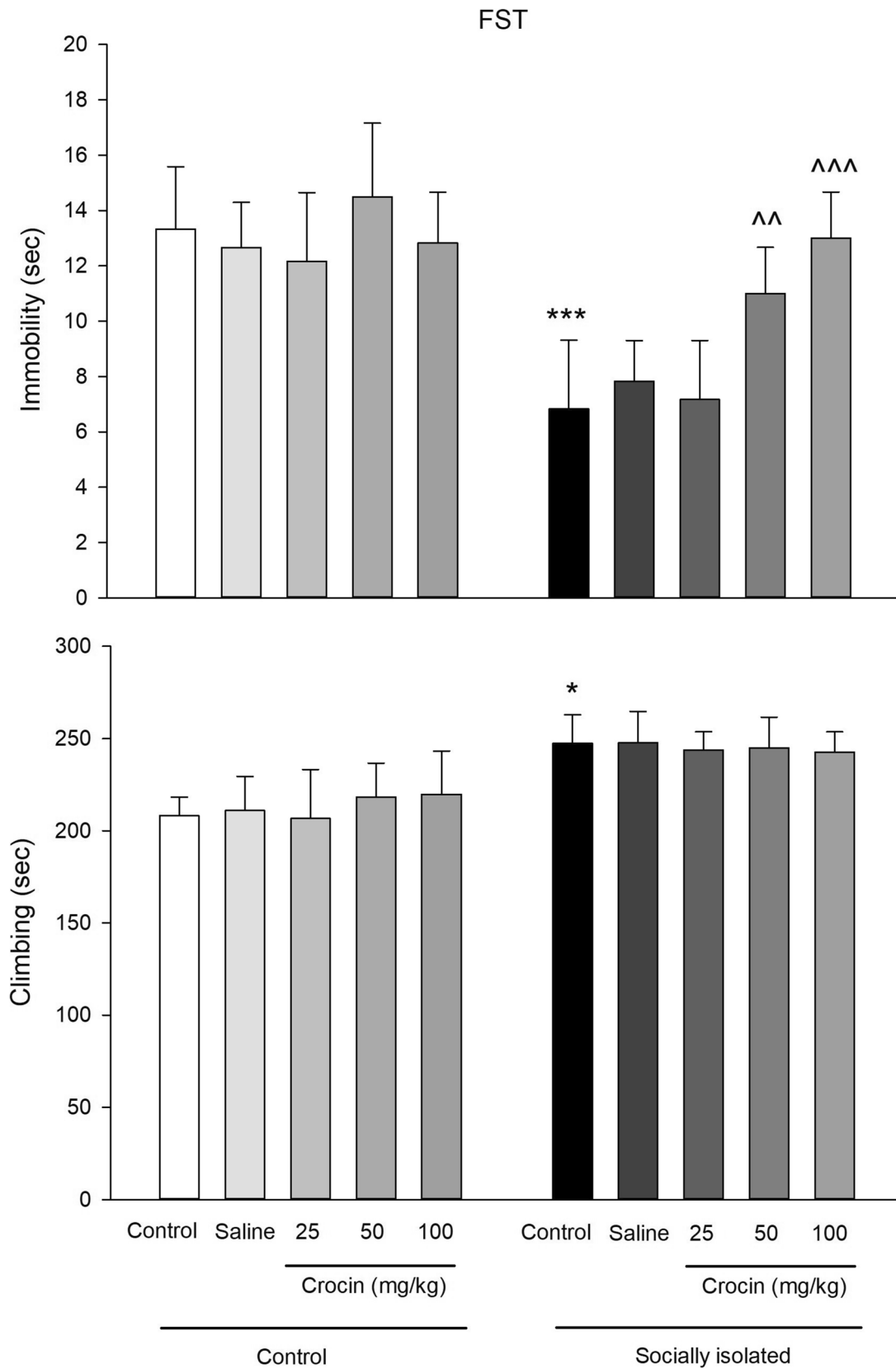
Fig. 6 shows immobility and climbing in control and socially isolated rats, received no injection, saline, or crocin at the doses of 25, 50, and 100 mg/kg (***$P < 0.001$ and *$P < 0.05$ in comparison with control group; ^^^$P < 0.001$ and ^^$P < 0.01$ in comparison with social isolation group) (n = 6 for each group)
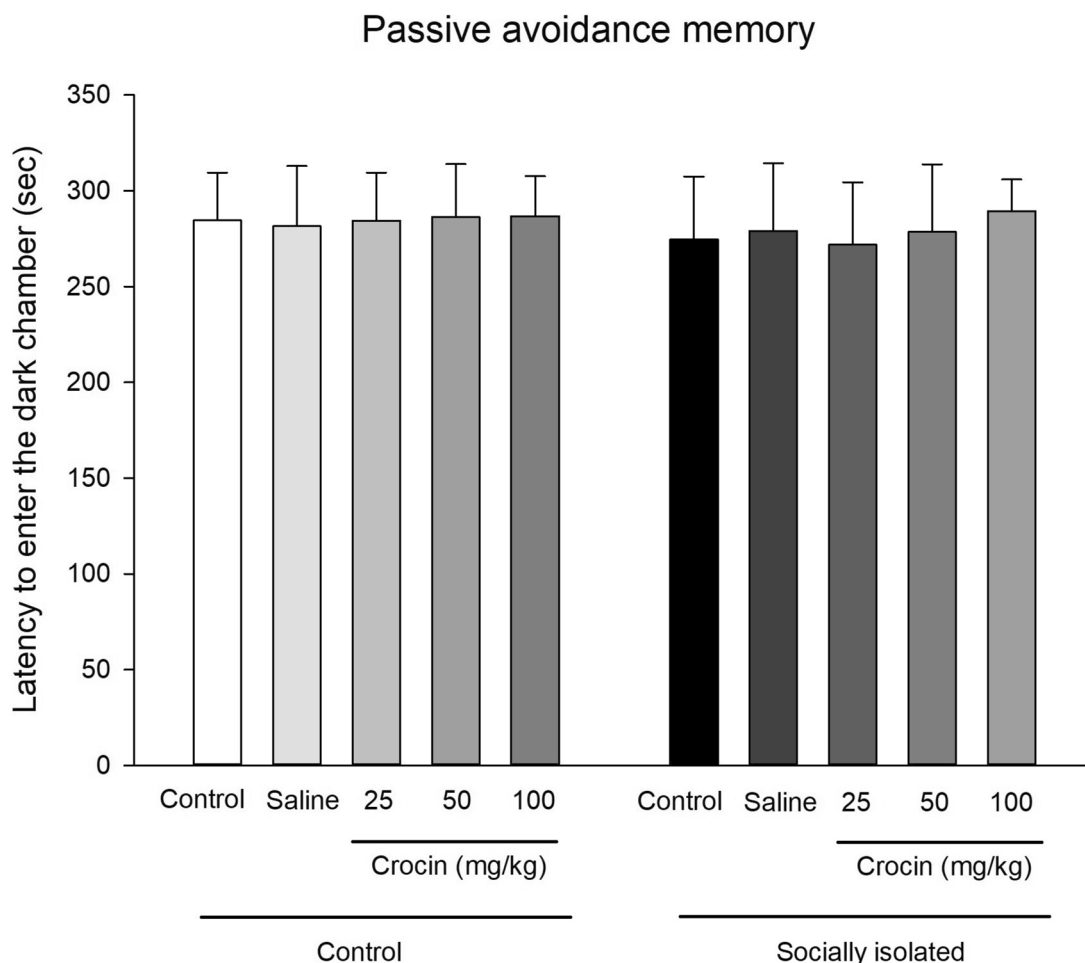
Passive avoidance memory

Fig. 7 shows passive avoidance memory performance in control and socially isolated rats, received no injection, saline, or crocin at the doses of 25, 50, and 100 mg/kg (n = 6 for each group)

the negative impacts of maternal social isolation (McDonald et al. 2023). It has also been shown that socially isolated rats have high level of anxiety, and depressive- and psychotic-like symptoms (Mncube and Harvey 2022). Previous research has reported that social isolation increases locomotor activity and induces social anxiety and depressive-like behavior, that is not responsive to fluoxetine (Mncube et al. 2021). Social isolation for three weeks also leads to anhedonic-type behavior and an anxiogenic profile in adolescent male rats (Acero-Castillo et al. 2021). Furthermore, chronic social isolation may induce OCD-like behavior in rats (Perić et al. 2021, 2018).

However, our data did not show a depressive or anxiogenic effect following social isolation, but on the contrary, we showed that immobility and feeding latency were decreased, along with a hyperlocomotion profile and a robust decrease in pain threshold. Although our findings are almost unexpectable, some studies have shown similar results. Previous research has shown that initiating isolation-housing in adulthood (PND 66 or PND 116) actually increases both open-field center entries and defensive burying, suggesting

that adult social isolation may lead to reduced anxiety-like behavior (Arakawa 2005, 2007). Other study has shown that isolation has no effect on anxiety-like behavior in the elevated plus maze when tested on PND 62 (Brenes and Fornaguera 2009). Furthermore, a significant anxiolytic effect of social isolation for male rat in the elevated plus maze and open field has been shown, when social isolation is initiated in later adolescence (PND 45, mid-adolescence) (Thorsell et al. 2006). It has been suggested that inconsistent reports in relation to the expression of anxiety-like behaviors may be a result of varying adversities inherent in the different test paradigms (Hall et al. 2000), differing isolation onset and duration, or may represent strain differences between Lister Hooded rats (Einon et al. 1978) vs. Sprague-Dawley and Wistar rats (Lukkes et al. 2009b, 2009a, 2009c). We also suggest that hyperlocomotion induced by social isolation may affect the rats' performance in the NFST and FST. Social isolation from adolescence can lead to the induction of a "hyperlocomotion state" that affects all the behavioral functions of rats. Therefore, antidepressant- and anxiolytic-like effects of social isolation may be related to
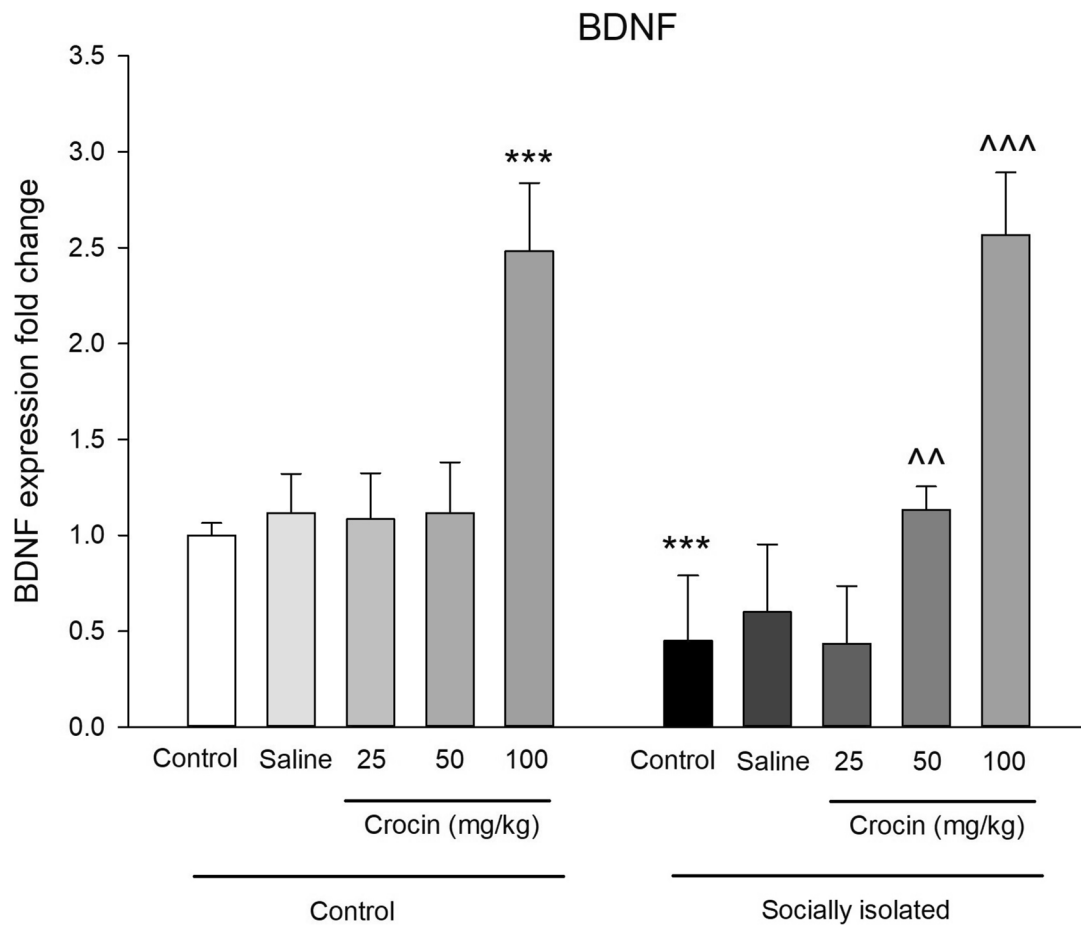
BDNF

Fig. 8 shows BDNF expression level in the hippocampus in control and socially isolated rats, received no injection, saline, or crocin at the doses of 25, 50, and 100 mg/kg (***$P < 0.001$ in comparison with control group; ^^^$P < 0.001$ and ^^$P < 0.01$ in comparison with social isolation group) (n = 6 for each group)

Table 2 The direction of the indicators' changes has been provided. The results showed a direct relation between BDNF expression level and pain perception in control rats received crocin 100 mg/kg, and between BDNF expression level and pain perception or immobility in socially isolated rats received no injection or crocin 100 mg/kg. Also, there was an inverse (opposite) relation between BDNF expression level and locomotor activity in socially isolated rats received no injection or crocin 100 mg/kg

Rats	BDNF	Locomotor activity	Pain threshold	Immobility
Socially isolated	↓	↑	↓	↓
Socially isolated + Crocin 100 mg/kg	↑	↓	↑	↑
Control + Crocin 100 mg/kg	↑	-	↑	-

the induction of hyperlocomotion, and social isolation by itself may have not an antidepressant or anxiolytic effect. It seems that the effect of social isolation on locomotor activity is a very important outcome that can alter the mood state of rats. Of note, hyperlocomotion has been considered as a symptom of schizophrenia in rodents (van den Buuse 2010). It has been suggested that increased motor activity is an important feature of current pharmacological models of schizophrenia, because hyperlocomotion is related to the positive symptoms of schizophrenia (van den Buuse 2010).

On the other hand, social isolation can lead to the induction of schizophrenia-like profile in rodents (Stevens et al. 1997). Social isolation has been considered as a reliable method for the induction of schizophrenia (Jones et al. 2011). Previous research has shown that adolescent social isolation can lead to schizophrenia-like behaviors in male rats (Sun et al. 2017). Although in the present research, we did not provide a rat model of schizophrenia, but isolation-induced hyperlocomotion was the most important feature in socially isolated rats.

Social isolation can alter pain perception. Previous study has shown that social isolation increases pain perception and leads to a nociceptive response in juvenile female Sprague Dawley rats (O'Sullivan et al. 2020). However, it has been reported that short-term social isolation from littermates is associated with analgesia in rats (Kozlov et al. 2013). A previous study has also shown that rats' performance in the hot plate test was not changed following four weeks of social isolation (Woodworth and Johnson 1988). Therefore, the effects of social isolation on pain perception and pain threshold are inconsistent. In addition, it has been shown that social isolation can alter the expression level of BDNF. Previous study has shown that social isolation reduces BDNF level in the hippocampus of male Sprague-Dawley rats (Scaccianoce et al. 2006). It has been shown that social isolation significantly decreases BDNF level in the prefrontal cortex of rats (Begni et al. 2020). Also, other study has shown that hippocampal BDNF level is markedly decreased in socially isolated rats (Kim et al. 2020). On the other hand, it seems that hyperlocomotion can be related to the function of BDNF. It has been shown that methylphenidate leads to hyperactivity and decreases BDNF, while crocin significantly reverses both effects (Ebrahimzadeh et al. 2019). Other study has shown that ketamine leads to hyperlocomotion (schizophrenia-like symptom in rats) and decreases BDNF level, while treatment with vinpocetine reverses both (Xu et al. 2019). Consistent with previous findings, we showed hyperlocomotion and decreased BDNF level in socially isolated rats, although socially isolated rats showed a decrease in anxiety-like behavior. This can be a challenging finding, because most previous studies have shown increased anxiety level following a decrease in BDNF expression level in socially isolated rats (Pisu et al. 2011; Murinova et al. 2017; Sun et al. 2013; Ma et al. 2016). However, it has been shown that anxiety-like behavior does not change following a decrease in BDNF protein expression in the central amygdala and the hippocampus (Ravenelle et al. 2014), or in the frontal cortex, striatum, hippocampus and cerebellum (Simpson et al. 2012) in socially isolated rats. Other study has shown an increase in anxiety-like behavior in socially isolated rats, with no changes in BDNF expression level (Evans et al. 2012). A previous study has shown a decrease in anxiety-like behavior with no changes in BDNF mRNA level in socially isolated male rats (Weintraub et al. 2010). This study (Weintraub et al. 2010) also showed that BDNF mRNA level was decreased in the CA3 region of the hippocampus of socially isolated female rats, with no changes in anxiety-like behavior.

Furthermore, crocin only at the highest dose significantly reversed isolation-induced hyperlocomotion. Also, it reversed the effect of social isolation on pain threshold, OCD-like behavior, depressive-like behavior, and BDNF expression. Crocin is an active and therapeutic component

of *Saffron* (Ahmed El-Sheikh et al. 2023). Large evidence has shown the pro-cognitive effects of crocin (Abbaszade-Cheragheali et al. 2022; Looti Bashiyan et al. 2021). Also, crocin can induce antidepressant and anxiolytic effects (Ghalandari-Shamami et al. 2019; Zhang et al. 2022). It seems that crocin can also affect locomotor activity. Previous study has shown that crocin (50 mg/kg) attenuates ketamine-induced hypermotility, stereotypies, and ataxia in rats (Georgiadou et al. 2014). Previous research has also shown that crocin significantly reduces hyperlocomotion induced by MK-801 in a rat model of schizophrenia (Sun et al. 2020). Furthermore, it has been shown that crocin at the dose of 400 mg/kg suppresses locomotor activity in rats exposed to morphine (Tamaddonfard and Hamzeh-Gooshchi 2010). On the other hand, crocin can induce analgesic effect. It has been shown that crocin decreases thermal hyperalgesia and mechanical allodynia in a rat model of neuropathic pain (Vafaei et al. 2020). Crocin significantly reduces the level of pain-related factors and glial activation in rats (Wang et al. 2020). In line with previous findings, our data showed that crocin increased pain threshold and decreased locomotor activity in socially isolated rats.

In addition, crocin can improve the function of BDNF. Previous study has shown that crocin enhances the protein level of BDNF in the hippocampus of sleep-deprived rats (Looti Bashiyan et al. 2021). It has been shown that crocin increases the expression of BDNF in rats exposed to chronic unpredictable mild stress (Abbaszade-Cheragheali et al. 2022). Furthermore, crocin significantly enhances CA3 BDNF level in the stressed rats (Ghalandari-Shamami et al. 2021). Our data also showed that crocin increases BDNF expression level in both control and socially isolated rats. On the other hand, BDNF expression level may be related to OCD-like behaviors. It has been shown that treatment with L. casei Shirota and fluoxetine significantly improves OCD-like behavior and increases the expression level of BDNF in a rat model of OCD (Sanikhani et al. 2020). Also, it has been suggested that lower BDNF level is related to OCD (Suliman et al. 2013). It has also been suggested that BDNF may serve as a potential biomarker of OCD (Hao et al. 2022). In the present study, crocin significantly restored BDNF expression level in socially isolated rats. This effect may be related to the improvement of OCD-like behavior in socially isolated rats received crocin (100 mg/kg). However, more detailed studies are needed to better investigate the effect of social isolation and crocin on locomotor activity and OCD-like behavior, and subsequent behavioral functions. In addition, the potential relationship between BDNF function, locomotor activity, and OCD-like behaviors can be an important research topic in preclinical studies. Of note, we showed that crocin increased BDNF expression level and pain threshold in control rats. Although there is sparse evidence on the

effect of crocin on BDNF expression level in control animals, previous research has shown the same finding. This study (Vahdati Hassani et al. 2014) has shown that crocin (25 and 50 mg/kg) increases BDNF protein level in the hippocampus of rats, using western blotting method. This study has also shown that crocin (12.5 mg/kg) increases BDNF gene expression level in the hippocampus of rats (Vahdati Hassani et al. 2014). However, the effect of crocin on pain perception in control rats has not well investigated. We can relate this finding to the analgesic effect of crocin (Hashemzaei et al. 2020; Tamaddonfard et al. 2013). It should be noted that, observed decrease in BDNF gene expression without evaluation of BDNF protein expression in the hippocampus is a limitation of the present study. We did not evaluate BDNF protein level using molecular methods particularly western blotting, and this could be a limitation for the present research.

Conclusion

In the present research, we found that social isolation decreased anxiety- and depressive-like behavior, pain threshold, and BDNF expression, and induced OCD-like behavior and hyperlocomotion. Crocin dose-dependently reversed the effect of social isolation on pain threshold, OCD-like behavior, locomotor activity, depressive-like behavior, and BDNF expression. It can be concluded that hyperlocomotion is the core symptom of social isolation that also can affect the mood state. But given that hyperlocomotion can affect not only mood state but also emotion-related behaviors, OCD-like behavior, and pain threshold assessment, we can suggest that hyperlocomotion may have an impact on the manifestation of many behavioral functions.

Declarations

Consent to participate N/A.

Consent to publish N/A.

Competing interests The authors declare no competing interests.

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