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Research Paper

The Protective Effects of Crocin on Input-Output Functions and Long-term Potentiation of Hippocampal CA1 Area in Rats Exposed to Chronic Social Isolated Stress

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ABSTRACT

Introduction: The lack of social communication is associated with the primary risk of proper brain functions. It is reported that crocin helps relieve this problem. The present study examined the protective effect of two doses of crocin on Long-term potentiation (LTP) of hippocampal cornu ammonis 1 (CA1) area as a cellular mechanism in rats exposed to chronic social isolated stress.

Methods: Rats were assigned to the control, sham, isolation stress, and two stress groups (receiving 30 and 60 mg/kg crocin). Chronic isolation stress (CIS) was induced 6 h/d, and crocin was administrated for 21 days. The field excitatory postsynaptic potential (fEPSP) slope and amplitude were measured by input/output functions and LTP induction in the CA1 area of the hippocampus. Also, the corticosterone and glucose levels were assayed in the hippocampus and frontal cortex.

Results: The slope and amplitude of fEPSP severity were impaired in both input/output and LTP responses in the CIS group. Crocin at a dose of 30 and particularly 60 mg/kg improved input/output and LTP responses in the CIS group. Also, the corticosterone levels significantly increased in the frontal cortex and especially the hippocampus. In contrast, only a high dose of crocin decreased hippocampal corticosterone levels in the CIS condition. Finally, the glucose levels did not change in the hippocampus and frontal cortex in all experimental groups.

Conclusion: The chronic isolation stress impaired neural excitability and Long-term plasticity in the CA1 area due to elevated corticosterone in the hippocampus and probably the frontal cortex. The low and high doses of crocin improved excitability and Long-term plasticity in the chronic isolation stress group by only decreasing corticosterone levels in the hippocampus, but not the frontal cortex.

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Highlights

- Neuronal excitability and long-term plasticity of CA1 were impaired by chronic isolation stress.
- The memory was protected by low and particularly high doses of crocin in the chronic isolation stress condition.
- Crocin decreased the corticosterone levels in hippocampus, but not frontal cortex.

Plain Language Summary

The lack of social communication (isolation stress) is associated with the primary risk of brain functions. On the other hand, crocin as one of effective components of saffron is helpful for improvement of memory. Therefore, the protective effect of two doses of crocin on cellular mechanism of memory in rats exposed to chronic social isolated stress was investigated in present study. Chronic isolation stress (CIS) was induced 6h/day, and crocin was administrated for a period of 21 days at two doses of 30 and 60 mg/kg. The electrophysiological and cellular mechanism of memory in the CA1 area of the hippocampus were investigated. Also, the corticosterone and glucose levels were assayed in the hippocampus and frontal cortex. It was concluded that the chronic isolation stress impaired neural excitability and long-term plasticity in the CA1 area due to elevated corticosterone in the hippocampus and probably the frontal cortex. The low and high doses of crocin improved excitability and long-term plasticity in the chronic isolation stress group by only decreasing corticosterone levels in the hippocampus, but not the frontal cortex. Also, the corticosterone levels significantly increased in the frontal cortex and especially the hippocampus. Also, the glucose levels did not change in the hippocampus and frontal cortex in all experimental groups.

1. Introduction

Vrain functions are sensitive to alterations in environmental factors (Friederici, 2006; Grossman, Churchill, McKinney, Kodish, Otte, & Greenough, 2003) such as stressful conditions and daily usage of some supplementary materials, including various herbal plants (Dashti-R, Zeinali, Anvari, & Hosseini, 2012). Stress affects the endocrine and nervous systems (Figueiredo, Bodie, Tauchi, Dolgas, & Herman, 2003). It mainly affects neural, hormonal, and biochemical mechanisms for brain functions such as memory processing (Kosten, Kim, & Lee., 2012). For instance, stress changes the hypothalamic-pituitary-adrenal (HPA) axis activity (Ranjbar, Radahmadi, Alaei, Reisi, & Karimi, 2016). On the other hand, the hippocampus and frontal cortex are two main memory regions in the brain.

Memory impairments can be caused by changes in the hippocampus and frontal cortex functions via alterations of stress mediators under emotionally stressful conditions (Holtzer et al., 2017; Hosseini Dastgerdi, Radahmadi, & Pourshanazari, 2021; Mao, Huang, Zhong, Xian, & Ip, 2014). Therefore, the HPA axis affects Long-term potentiation (LTP) as a significant type of synaptic plasticity via glucocorticoid secretion (Cazakoff & Howland, 2010; MacDougall & Howland, 2013; Mrdalj et al., 2013) and glucose levels (as another critical memory and stress mediator) in a different region of the brain (Nugent et al., 2013; Persson, Sim, Virding, Onishchenko, Schulte, & Ingelman-Sundberg, 2014; Radahmadi et al., 2017). High glucose levels in the frontal cortex and hippocampus damage these structures and their involved neurotransmitters in LTP (Detka et al., 2015).

Some herbal plants have been suggested to enhance brain functions (Persson, Sim, Virding, Onishchenko, Schulte, & Ingelman-Sundberg, 2016). These herbal plants are usually available and have low side effects on humans (Bandegi, Rashidy-Pour, Vafaei, & Ghadrdoost, 2014). It is worth noting that the cognitive capacity and function of the nervous system can be affected by the positive saffron (Crocus sativus) effects and its practical components, such as crocin, through biochemical changes in the body (Bandegi et al., 2014; Papandreou, Tsachaki, Efthimiopoulos, Cordopatis, P., Lamari, & Margarity, 2011; Rajabian, Hosseini, Hosseini, & Sadeghnia, 2019; Roustazade, Radahmadi, & Yazdani, 2022).

Nowadays, many people are involved in social isolation stress that was already indicated as intense stress (Marčinko, Jakovljević, Jakšić, Bjedov, & Mindoljević Drakulić, 2020). Social isolation can induce memory deficits (Wood, Dudchenko, Robitsek, & Eichenbaum,

2000) and predispose people to severe disorders such as Alzheimer disease (Rothman & Mattson, 2010). Also, social isolation stress is a risk factor for mortality independent of health behavior (Cacioppo et al., 2015). The various stressors affect the physiologic system via differences in the type and even time of stress (Radahmadi et al., 2017). Therefore, there may need to be additional treatment protocols against various stresses. Although some research has been performed on the multiple aspects of crocin, there are no available reports on the effect of chronic isolation stress on some cellular mechanisms of memory and its effective biochemical factors in memory regions of the brain. The present study examined the protective effect of crocin (at two doses) on input/output functions and LTP of hippocampal CA1 area, as a cellular mechanism, and two memory mediators (levels of glucose and corticosterone in the frontal cortex and hippocampus) in rats exposed to chronic social isolated stress.

2. Materials and Methods

Study animals

All experiments were performed on 40 male Wistar rats (250-300 g) obtained from the Pasteur Institute, Tehran, Iran. The rats were housed under controlled light conditions (12/12 h light/dark; lights on between 07:00 and 19:00) and humidity (50%±5%). The animal's room temperature was 23±2°C. Water and food were available ad libitum. The Ethics Committee of Animal Use at the Isfahan University of Medical Sciences approved the study (Code: IR.MUI.REC.1394.3.934). The rats were randomly divided into five groups (n=8 in each group): (control group (Co); rats did not receive special treatment for 21 days), (sham group (Sh); rats received saline as a drug vehicle during 21 days), (chronic isolation stress (CIS); rats maintained individual housing for 6 h/d for 21 days), and chronic isolation stress-crocin 30 group (CIS-Cr30) and chronic isolation stress-crocin 60 group (CIS-Cr60)

Experimental procedures

Drugs

The intraperitoneally (IP) injection of 30 and 60 mg/kg of crocin, a critical component of saffron (Sigma Company, the USA), dissolved in sterile normal saline and injected for 21 days. The sham group received only equal volumes of sterile normal saline (Khani, Radahmadi, Alaei, & Jafari,, 2018).

Induction of chronic isolation stress

To induce chronic isolation stress, the rats were kept in individual cages 6 hours a day (8:00 to 14:00) alone and without any other neighbors for 21 consecutive days (Khani et al., 2018). Then, they were placed back in their communal home cage.

Surgical procedures

Input/output functions (neural excitability) and LTP (Long-term plasticity) were investigated in the present study. The rats were anesthetized with urethane (1.5 g/kg; Sigma Company, America; IP) dissolved in normal saline (on day 22). Sometimes an extra urethane dose (about 0.2 g/kg) was injected. Then, their heads were fixed to a stereotaxic apparatus (Stoelting Co., USA). Also, body temperature was maintained at 36.5°C±0.5°C with a suitable pad and covered during the experiment to record better signals.

Two small holes were drilled on the exposed skull for the stimulating and recording electrodes site to induce input/output functions and LTP. A bipolar stainless steel stimulating electrode with 0.125 mm diameter (Advent Co., UK) was placed in the Schaffer collateral (Sch) pathway (anterior-posterior=−4.2 mm; medial-lateral=3.8 mm; dorsal-ventral=2.3–2.7 mm). Also, a stainless-steel recording electrode was lowered into the CA1 area (anterior-posterior=−3.4 mm; medial-lateral=1.5 mm; dorsal-ventral=4.4−5.1 mm; at an angle of 52.5 degrees). The electrodes were lowered very slowly to the brain (at 2 mm/min) to minimize the brain tissue trauma. Finally, the electrophysiological and histological standards are used to determine the correct location of the electrodes.

Electrophysiological study

Input/Output functions and LTP induction

The electrophysiological recording (in-vivo input/output functions and LTP) procedures from the hippocampal CA1 area were based on Hosseini Dastgerdi, et al. (2021) study in urethane-anesthetized rats (Dastgerdi, Radahmadi, & Reisi, 2020). Therefore, following stimulation of the Sch collateral pathway, extracellularly-evoked responses were obtained in this study. Extracellular field potentials were amplified and filtered (×1000 and 1-3000 Hz bandpass, respectively). Signals were passed through an analog to digital interface (Science Beam-D3111, Iran) into a computer, and then all data were analyzed using custom software (eProbe software). For evaluating the synaptic potency, input/output (I/O)

or stimulus-response functions were acquired by systematic variation (1000-1000 μA) of the stimulus current before induction of LTP.

The LTP indicates activity-dependent synaptic plasticity that may underlie brain function (learning and memory) (Heo, Kim, Shin, Kim, Kim, & Shin, 2004). To determine any changes in the synaptic response of the CA1 neurons, some baseline recordings were taken 30 min before LTP induction and 120 min after that. LTP was induced using high-frequency stimuli (HFS) protocols of 100 Hz (4 bursts of 50 stimuli, 0.15 ms archive stimulus duration, 10 s inter-burst interval). However, all potentials employed as the baseline and high-frequency stimuli were evoked at an intensity that produced 50% of the maximal field excitatory postsynaptic potential (fEPSP) (Dehghani & Reisi, 2015).

Assessment of hippocampal and frontal cortical corticosterone and glucose levels

On day 22 of the experiments, the animals were sacrificed from 14:00 to 16:00 in deep anesthesia by decapitation. Then, their brains were instantly removed from the skull. The hemi-frontal cortex and the hemi-hippocampus were immediately dissected on dry ice, subsequently immersed in the ProBlock™-50, EDTA-free (Gold Bio Co., USA), and a phosphate buffer solution (0.01 M, pH=7.4), separately. This solution contained a complete protease inhibitor cocktail (Dastgerdi, Radahmadi, Pourshanazari, & Dastgerdi, 2017). The frontal cortex and hippocampus were homogenized and centrifuged in a cooled centrifuge (4°C, 10000 g) for about 20 min. Then, the supernatant of these tissues was collected and stored at −80°C until the assessment was used to assess levels. Also, the frontal cortical and hippocampal glucose and corticosterone levels were measured by the glucose oxidase (Pars Azmun Co., Tehran, Iran) and ELISA methods (Zellbio Co., Marburg, Germany), respectively.

Statistical analysis

All biochemical data were analyzed by 1-way analysis of variance followed by Tukey's post hoc test between groups. Data from I/O and induction and maintenance of LTP were analyzed statistically using the repeated measures analysis of variance followed by Tukey's post hoc test. All data were reported as Means±SEM. P-values less than 0.05 were statistically considered significant.

3. Results

None of the data showed significant differences between the Co and the Sh groups, indicating that the injection did not show significant changes in all variables. Therefore, all comparisons were performed with the control group.

Electrophysiological results

Input/Output functions

The slope and amplitude of the I/O curve had significant decreases in the CIS group compared to the Co group (both $P<0.05$) (Figure 1A and 1B). It indicated that chronic isolation stress reduced the CA1 area responsiveness.

In Figure 1, parts A and B, the slope and amplitude of fEPSP showed significant enhancements in the CIS-Cr30 and CIS-Cr60 groups compared with the CIS group ($P<0.01$ in slope and amplitude of CIS-Cr30 and $P<0.001$ in those of CIS-Cr60).

LTP responses

The fEPSP slope and amplitude in the CIS group after LTP induction had significant (in both $P<0.001$) reductions compared to the Co group, indicating chronic isolation stress caused a weaker induction of LTP in the CA1 hippocampus (Figure 2A, 2B).

As shown in Figure 2, parts A and B, the fEPSP slope and amplitude after LTP induction significantly increased in the CIS-Cr30 and CIS-Cr60 groups compared to the CIS group ($P<0.001$ in slope and amplitude).

Assessment of hippocampal and frontal cortical corticosterone levels

As shown in Figure 3, the hippocampal and frontal cortical corticosterone levels significantly increased in the CIS group compared with the Co ones ($P<0.01$ and $P<0.05$, respectively). Also, only the hippocampal corticosterone level significantly ($P<0.05$) decreased in the CIS-Cr60 group compared to the CIS group (Figure 3).

Assessment of hippocampal and frontal cortical glucose levels

As shown in Figure 4, parts A and B, the hippocampal and frontal cortical glucose levels did not show significant ($P>0.05$) changes in chronic isolation stress and crocin-receiving groups compared to the Co group.

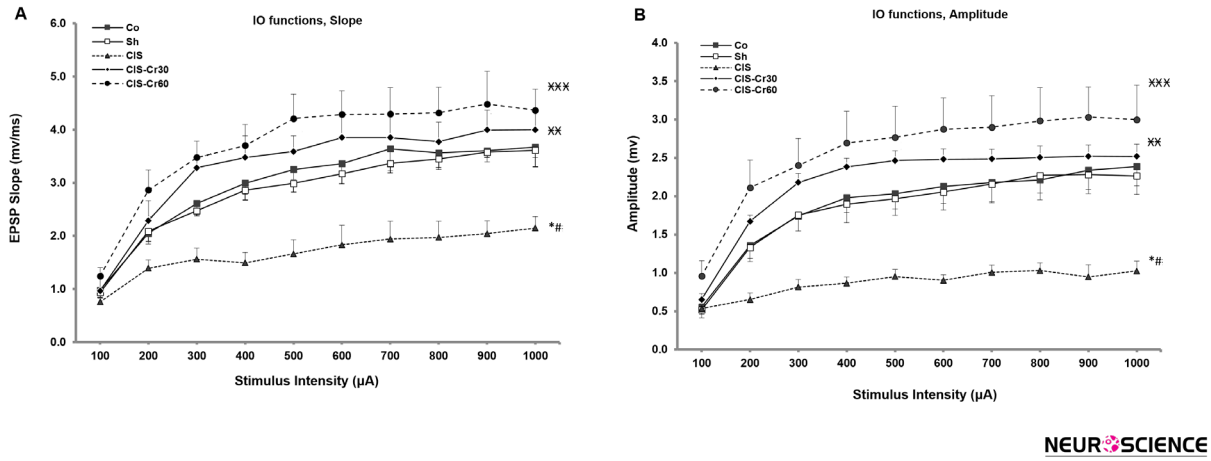

Figure 1. Results are reported as Means±SEM (repeated measures of ANOVA followed by Tukey's post-hoc test).

(A) Input/Output (I/O) Curves of the Field Excitatory Postsynaptic Potential (fEPSP) Slope, (B) the I/O Curves of fEPSP Amplitude in the Hippocampal CA1 for the Different Experimental Groups (n=8)

*P<0.05 compared with the control group, #P<0.05 compared with the sham group, XXP<0.01, and XXXP<0.001 compared with the CIS group

4. Discussion

This study investigated the effects of crocin at doses of 30 and 60 mg/kg on electrophysiological responses (by input/output and LTP induction) and two memory indicators, including the glucose and corticosterone levels in the frontal cortex and hippocampus for evaluating neural excitability and plasticity or memory in rats exposed to chronic social isolated stress.

According to the current findings, chronic isolation stress severity impaired neural excitability and Long-term potentiation (as a cellular memory mechanism) in the CA1 area. Also, chronic isolation stress increased the corticosterone levels in both the frontal cortex and hippocampus, whereas the glucose levels did not change in these regions. In this way, some previous reports demonstrated the role of stress on excitability impairments and Long-term plasticity deficit in other hippocampal areas (Artola, 2008; Radahmadi, Hosseini, & Nasimi, 2014; Sun et al., 2020) and even with other types of stress (Hosseini Dastgerdi et al., 2021). Moreover, different factors affect brain functions in stress conditions, such as the severity, period and pattern, and nature of stress-

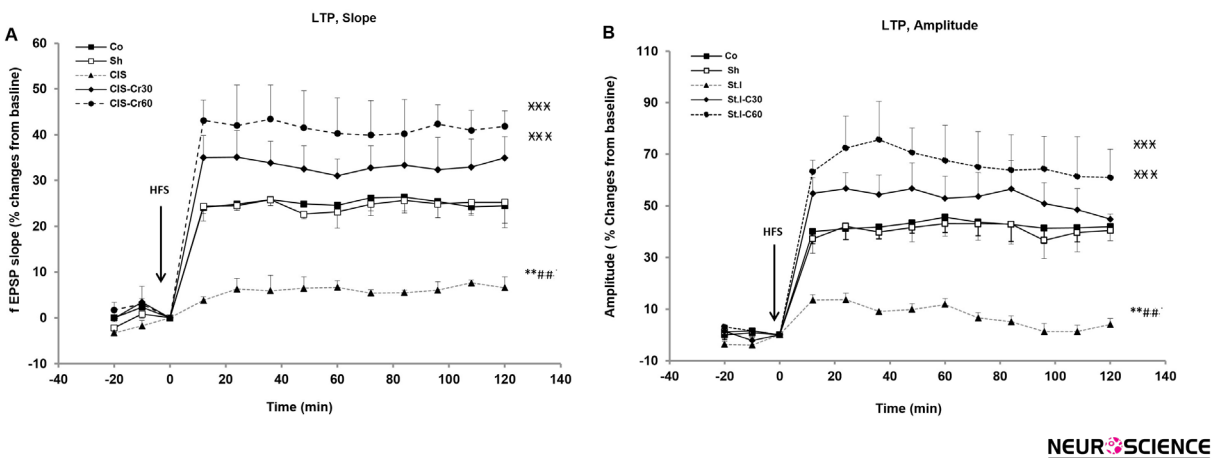

Figure 2. Results are reported as Means±SEM (Repeated measures of ANOVA followed by Tukey's post-hoc test).

(A) Long-term Potentiation (LTP) Curves of the Field Excitatory Postsynaptic Potential (fEPSP) Slope (B) LTP Curves of fEPSP Amplitude in the Hippocampal CA1 for the Different Experimental Groups (n=8)

**P<0.01 compared with the control group, ##P<0.01 compared with the sham group, XXXP<0.001 compared with the CIS group.

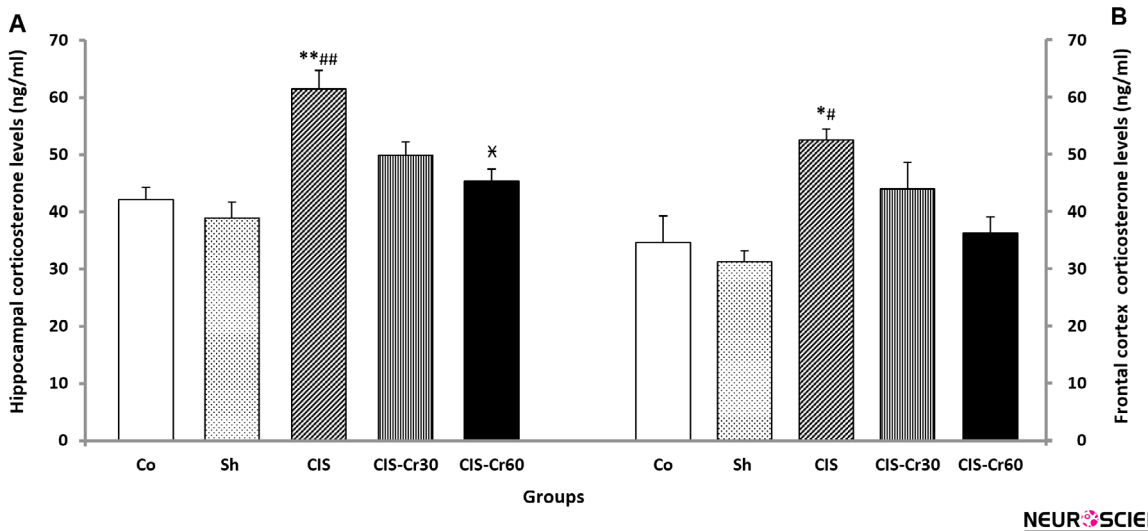

Figure 3. Results are reported as Means±SEM (ANOVA test Tukey's post hoc test).

(A) Hippocampal Corticosterone Levels (ng/mL) (B) Frontal Cortical Corticosterone Levels (ng/ml) in the Different Experimental Groups (n=8)

*P<0.05 and **P<0.01 compared with the control group, #P<0.05 and ##P<0.01 compared with the sham group, ⁎P<0.05 compared with the CIS group.

ors (Radahmadi, Alaei, Sharifi, & Hosseini, 2015). In the current study, the stress-related memory impairments could be mainly due to changes in hippocampal corticosterone levels that were similar to some prior studies (Radahmadi et al., 2015; Sato, Takahashi, T., Sumitani, Takatsu, & Urano, 2010). Contrary to the present findings, Loganathan, and Rathinasamy. (2016) reported that noise stress did not induce any significant changes in the serum corticosterone (Loganathan & Rathinasamy, 2016). It seems that glucocorticoids probably affect hippocampal synaptic plasticity through changes in glutamate, gamma-aminobutyric acid, glucocorticoid receptors, neural metabolism, and morphology of cells in the hippocampus (Hu, Zhang Czéh, Flügge, & Zhang, 2010; Krugers et al., 2010; McLaughlin et al., 2007; Popoli, Yan, McEwen, & Sanacora 2012; Tsai et al., 2014). Moreover, some studies reported that social stresses are very destructive and associated with poor health (Epel et al., 2018), as isolation stress is associated with more cortisol and inflammatory secretions. Also, multiple brain

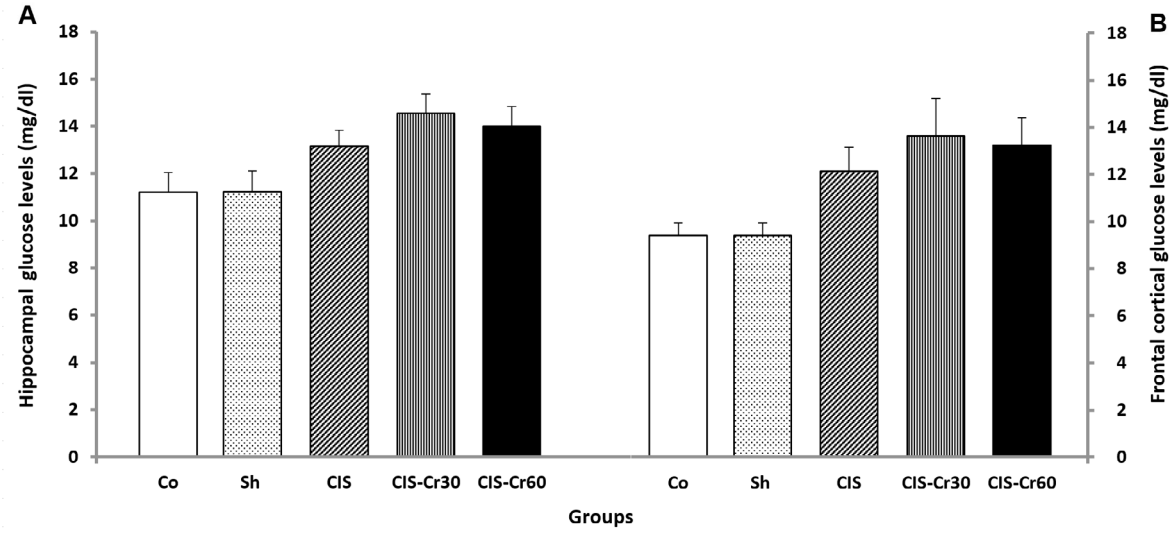

Figure 4. Results are reported as means±SEM (ANOVA test Tukey's post hoc test).

(A) Hippocampal Glucose Levels (mg/dL) (B) Frontal Cortical Glucose Levels (mg/dL) in the Different Experimental Groups

functions via different brain areas involved in stress containing the neural reward system (e.g., ventral striatum), social stress aversion (e.g., anterior cingulate, anterior insula, amygdala), and attention to one's self-preservation in a social context (e.g., orbitofrontal cortex, temporal-parietal junction, superior temporal sulcus, medial prefrontal cortex) (Cacioppo et al., 2015).

Another finding revealed that daily chronic administration of crocin (doses of 30 and 60 mg/kg) improved the destructive effects of chronic isolation stress on excitability and Long-term plasticity. However, Hosseini Dastgerdi et al. (2021) reported that low doses of crocin were more beneficial for memory improvement due to restraint stress which was indicated as emotional stress (Hosseini Dastgerdi et al., 2021). Also, a dose of 30 mg/kg of crocin improved spatial memory by more than 15 mg/kg in a behavioral test in subjects with 6 h/d restraint stress (Ghadrdoost et al., 2011). Therefore, it seems that brain response to crocin depends on not only the dose of crocin but also the type of stress. The comparison between the present study and Hosseini Dastgerdi et al. (2021) study confirmed that isolation stress was more destructive than emotional stress induced by restraint stress on memory (Hosseini Dastgerdi et al., 2021). Because a high dose of crocin was necessary for the improvement of memory in isolation stress with respect to restraint stress. Therefore, there is a possible beneficial interaction between crocin and hippocampal synaptic plasticity. Although, the mechanisms of action underlying the role of crocin on brain function such as memory is not still clear. It was reported that crocin improved impairment of brain function due to stress by a variety of memory behavioral tasks and also against ethanol-induced inhibition of hippocampal LTP (Khani et al., 2018; Sugiura et al., 1995). It seems that multiple mechanisms may be involved in the positive effect of crocin on cognitive functions, such as its antioxidant and anti-inflammatory properties, modulation of the antioxidant enzyme synthesis, enhancement of brain dopamine and glutamate concentration (Bandegi et al., 2014; Ettehadi et al., 2013; Papandreou et al., 2011; Soeda et al., 2007). In the current study, only the high dose of crocin acted on memory and hippocampal corticosterone levels. The comparison between Khani et al. (2018) study and the present study (with the same protocol) demonstrated that elevated serum corticosterone level was more than the hippocampal and frontal cortical corticosterone level in chronic isolation stress conditions. Also, the serum corticosterone level crossed the blood-brain barrier, although this barrier limited the access of corticosterone to the brain (Nishi & Kawata, 2000). Therefore, this evidence confirmed the lower levels of hippocampal corticosterone with respect to corticosterone levels in the serum. Also, a prior study reported that crocin reduced serum corticosterone levels (Khani et al., 2018). Therefore, the hippocampal corticosterone levels decrease by crocin with different mechanisms such as modulation of the HPA axis activity through decreasing CRH and glucocorticoid receptor gene expression in the hypothalamus and pituitary glands, reducing synaptic glutamate transfer, changes of NMDA receptors activity (Asalgoo, Tat, Sahraei, & Pirzad Jahromi, 2017; Georgiadou et al., 2014; Hadipour et al., 2018; Lechtenberg et al., 2008; Popoli et al., 2012; Rajabian et al., 2019).

5. Conclusion

In conclusion, chronic isolation stress severely affects neuronal excitability and Long-term plasticity. Low and particularly high doses of crocin improved the destructive effects of CIS on them. In contrast, only the high dose of crocin acted well on memory improvement and hippocampal corticosterone levels. It seems that the preservation of psychological health depends on public interaction. Therefore, lack of social communication is associated with a primary risk of neuronal disturbances. Hence, a high dose of crocin as a potential pharmacological agent may have beneficial properties for nearly all aspects of brain function in chronic isolation stress. Accordingly, evaluating the other factors and gene expression that are probably involved in the retention is highly recommended. Also, the underlying mechanisms of decreasing the effect of crocin on corticosterone levels are unknown and require further investigation.

Ethical Considerations

Compliance with ethical guidelines

The Ethics Committee of Animal Use at the Isfahan University of Medical Sciences approved the study (IR.MUI.REC.1394.3.934).

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Authors' contributions

Conceptualization and Supervision: Maryam Radahmadi; Methodology: Maryam Radahmadi, Hojjatalah Alaei; Investigation, Writing – original draft, and Writing – review & editing: All authors; Data collection: Fate-

meh Khani; Data analysis: Maryam Radahmadi; Funding acquisition and Resources: Maryam Radahmadi.

Conflict of interest

The authors declared no conflict of interest.

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