Original Article

Comparing the Efficacy of Escitalopram with and without Crocin in Restoring I/O Functions and LTP within the Hippocampal CA1 Region of Stressed Rats

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Abstract

Background: Escitalopram, a pharmacological compound, and crocin, the active compound of saffron, influence brain functions and serotonin levels. This study examined the efficacy of escitalopram with and without crocin in restoring the input-output (I/O) functions and long-term potentiation (LTP) within the hippocampal cornu ammonis 1 (CA1) region of stressed rats.

Materials and Methods: Rats were divided into six groups: control (Co), sham (Sh), stress-recovery (St-Rec), stress-escitalopram (St-Esc), stress-crocin (St-Cr), and stress-escitalopram-crocin (St-Esc-Cr) groups. They underwent 14 days of restraint stress (6 h/day). After being subjected to stress, they received 14 days of escitalopram (20 mg/kg) and crocin (30 mg/kg), as well as co-administration of these two compounds during the next 14 days. The field excitatory postsynaptic potential (fEPSP) slope and amplitude were measured using I/O functions and LTP induction in the CA1 region. Corticosterone (CORT) levels were also evaluated.

Results: The fEPSPs slope and amplitude in the I/O functions and LTP induction significantly decreased in stressed rats without therapeutic intervention. These variables in the I/O functions declined in rats with escitalopram administration alone. All electrophysiological parameters showed an increase in rats treated with crocin alone compared to stressed subjects without any treatment. Serum CORT levels decreased only with crocin treatment for stressed rats.

Conclusion: Neural excitability and memory within the CA1 region were severely disrupted among stressed rats without any treatment. Furthermore, administering crocin alone improved neural excitability and memory post-chronic stress. Treatment with escitalopram alone also impaired neural excitability within the CA1 region. The use of escitalopram with and without crocin did not enhance memory under chronic stress.

Keywords: Crocin, escitalopram, long-term potentiation, memory, stress

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Introduction

Learning and memory are complex processes that involve the brain's ability to store and retrieve information.[1] Accordingly, long-term potentiation (LTP) has a pivotal role in synaptic plasticity and memory in specific brain regions.[2] Changes in neurochemical factors, neurogenesis, the number of receptors at the postsynaptic sites, neural energy, neurotoxicity, excitability, plasticity, neural morphology, and even cell death have been recognized as some adverse effects of stress on the brain, especially impacting the hippocampus as an integral center related to learning and memory.[3] Moreover, stress is often associated with brain dysfunctions and disruptions in LTP due to corticosterone (CORT) secretion from the adrenal glands.[4]

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Currently, various medications are employed in the treatment of stress-related disorders.[5] As an example, serotonin reuptake inhibitors (SSRIs) like escitalopram are used as the primary therapeutic methods for alleviating symptoms of stress-related disorders, including anxiety.[6] Reports suggest that escitalopram can enhance memory and neuroplasticity by elevating serotonin levels in the hippocampus.[7]

Moreover, crocin as an active component of the saffron plant could improve memory, and LTP induction,[8–10] by affecting the synthesis of serotonin in the nervous system.[11] Since herbal drugs typically exhibit fewer side effects in comparison with chemical drugs, it is important to consider specific aspects of combination therapy. Additionally, crocin can be regarded as a viable supplement for memory improvement.[12] Despite the existing literature regarding the impact of exclusive administration of either crocin or escitalopram alone on brain activities in stressed subjects, no studies have investigated the comparative efficacy of escitalopram with and without crocin in restoring input-output (I/O) functions and LTP in the hippocampal cornu ammonis 1 (CA1) region of rats subjected to chronic stress.

Materials and Methods

Experiment subjects

Forty-eight male Wistar rats (200–250 g) were procured from the Isfahan University of Medical Sciences for the study. The rats were accommodated in an environment with controlled temperature and humidity, following a 12-hour light/dark cycle (23 ± 2 °C, 50 ± 5%, lights on 07:00–19:00). The rats had *ad libitum* access to food and water, except during the stress induction period. They were housed in groups of four for 28 days. Approval for the study was granted by the Animal Use Ethics Committee of Isfahan University of Medical Sciences (IR.MUI.MED.REC.1398.606). Following a one-week acclimatization period, the study subjects were randomly allocated into six groups (n = 8).

– *Control (Co) group:* Rats underwent standard procedures without any special treatment during the entire study.

– *Sham (Sh) group:* Rats were administered equal volumes of a drug vehicle over the following 14 days.

– *Stress-recovery (St-Rec) group:* Rats were placed in a restrainer for 14 days. Subsequently, they were reintroduced to their home cages for the following 14 days.

– *Stress-crocin (St-Cr) group:* Rats were placed in a restrainer for 14 days. Subsequently, they were administered daily injections of crocin for the following 14 days.

– *Stress-escitalopram (St-Esc) group:* Rats were placed in a restrainer for 14 days. Subsequently, they were administered daily injections of escitalopram for the following 14 days.

– *Stress-escitalopram-crocin (St-Esc-Cr) group:* Rats were placed in a restrainer for 14 days. Subsequently, the rats were administered daily injections of crocin and escitalopram for the following 14 days [Figure 1].

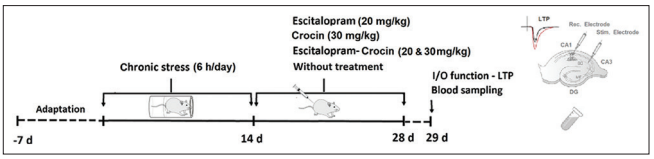

Figure 1: A schematic diagram illustrating the experimental design across all groups

Chronic stress paradigm

Rats were confined in restrainers that immobilized them for 14 days (6 h/day, from 08:00 to 14:00).[13] After the stress induction, the stressed rats underwent the experimental protocol.

Chemical agents

Stressed rats were administered intraperitoneal (i.p.) injections with a 20 mg/kg dose of escitalopram oxalate (Sobhan Darou, Iran) and a 30 mg/kg dose of crocin (Sigma-Aldrich, the United States). Both medications were dissolved in a normal saline solution.

Surgical and electrophysiological studies

Prior to surgery, the rats underwent anesthesia through a urethane injection (1.5 g/kg, i.p., Sigma-Aldrich, the United States). The absence of a pedal reflex was indicative of achieving deep anesthesia.[14] After placing each rat in a stereotaxic apparatus (Stoelting, the United States), two holes were drilled inside the skull for placement of the stimulation and recording electrodes (diameter: 125 µm, stainless steel coated with Teflon; Advent, United Kingdom). These electrodes were surgically implanted into the rat's brain. The bipolar stimulation electrode was positioned in the right Schaffer collateral pathway (anteroposterior = −4.2 mm, mediolateral = 3.8 mm, and dorsoventral = 2.7–3.8 mm). Also, the unipolar recording electrode was placed in the right CA1 region, positioned at an angle of 52.5° from the upper left side (anteroposterior = −3.4 mm; mediolateral = 1.5 mm; dorsoventral = 4.4–5.1 mm). The electrodes were carefully and slowly inserted into the intended regions to avoid any brain tissue damage. The experiments were performed on day 29.

A prevalent approach for investigating LTP and/or synaptic plasticity, both integral to learning and memory, involves the extracellular recording of the field excitatory postsynaptic potential (fEPSP) waveforms. The fEPSP slope and amplitude are the primary measures for assessing synaptic plasticity.[15] The fEPSP slope was assessed as an absolute value of the maximum slope in the descending fEPSP phase between 10% and 90% of the negative peak response. Also, the fEPSP amplitude was evaluated by calculating the voltage difference between the baseline and maximum negative deflection of the fEPSP waveform.[8,12] The CA1 region was stimulated at a frequency of 0.1 Hz, eliciting fEPSP waves. Subsequently, the fEPSPs were then amplified by a factor of 1000 and filtered to pass through a frequency range of 1–3 kHz. The digital signals were then conveyed to a computer for analysis through eTrace (Science Beam, Parto Danesh, Iran). The

effects of interventions on basal circuitry properties and neural excitability in the CA1 region were assessed by I/O functions. A range of current stimuli (100–1000 μA) were applied prior to LTP induction. Following the confirmation of baseline fEPSP stability, recordings were obtained 30 minutes pre-LTP and 60 minutes post-LTP induction. High-frequency stimulation (HFS), comprising 4 bursts of 50 stimuli, each lasting for 0.15 ms and delivered at 10-second intervals, induced an enduring enhancement in synaptic strength. This synaptic strength is referred to as LTP. According to the current experimental protocol, it was induced by setting the stimulus intensity at 50% of the maximum fEPSP slope, derived from the I/O curves. The LTP magnitude was measured as the percentages of alteration in the initial baseline fEPSP slope and amplitude as monitored over 60 minutes post-HSF.

Serum CORT assessment

On day 29, the rats were sacrificed by decapitation between 16:00 and 18:00 following the electrophysiological studies. Subsequently, blood serum samples were collected and subjected to centrifugation at 6000 rpm for 20 minutes. They were then preserved at a low temperature (−80 °C) for analysis. A commercial ELISA kit with an intra-assay coefficient of variation of less than 10% was used to measure the levels of CORT in the serum (ZellBio, Germany).

Histological verification of the brain in the CA1 region

To ensure accurate electrode placement, the animal brains were promptly extracted and submerged in a solution containing 10% formalin for at least three days. Moreover, a freezing microtome was used to cut the brain into 60-μm-thick slices without employing any staining protocol. As shown in Figure 2, these slices were examined under a microscope.

Statistical assessments

In this study, all data were reported as means ± standard error of the mean (SEM). To compare electrophysiological data in different groups, repeated-measures analysis of variance (ANOVA) and LSD post-hoc analyses were used. Also, the blood serum CORT levels obtained from different groups were subjected to a comparative analysis via one-way ANOVA, followed by a LSD post-hoc test. The calculations were executed using SPSS Statistics software (ver. 26). A P value less than 0.05 ($P < 0.05$) was considered statistically significant.

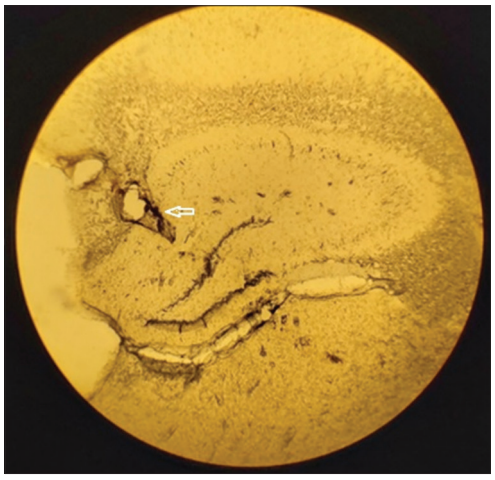

Figure 2: Photomicrograph of the hippocampal section displaying the recording electrode placement in the CA1 region for the fEPSP recordings (2.5 × 10 magnified)

Results

The Co and Sh groups did not show any significant differences regarding any of the measured variables. Therefore, the Co group was chosen as the reference against which all comparisons with other groups were made.

Assessment of the fEPSP slope and amplitude in the I/O curves

The repeated measures ANOVA, assigned different levels of significance regarding the I/O function slope [within groups, F (9, 378) = 240.661, P = 0.000; between groups, F (5, 42) = 2.724, P = 0.032] and amplitude [within groups, F (9, 378) = 304.490, P = 0.000; between groups, F (5, 42) = 2.936, P = 0.023]. The St-Rec group exhibited a significant decrease in both fEPSP slope and amplitude in the I/O curves compared to the Co group ($P < 0.01$ for both). These parameters decreased significantly in the St-Esc group compared to the Co group ($P < 0.05$ for both). Whereas they enhanced significantly in the St-Cr group compared to the St-Rec group ($P < 0.05$ for both). Notably, none of the therapeutic groups exhibited any statistically significant differences in terms of the fEPSP slope and amplitude of the I/O curves [Figure 3].

Assessment of the fEPSP slope and amplitude in the LTP

The repeated measures ANOVA assigned different levels of significance regarding the LTP slope [within groups, F (5, 210) = 1.431, P = 0.214; between groups, F (5, 42) = 1.628, P = 0.174] and amplitude [within groups, F (5, 210) = 7.212, P = 0.000; between groups, F (5, 42) = 2.105, P = 0.084]. The St-Rec group exhibited a significant reduction in both fEPSP slope and amplitude after LTP induction compared to the Co group ($P < 0.05$ for both). Therefore, the recovery period following chronic stress severely disrupted LTP induction among stressed subjects. Additionally, the fEPSP slope and amplitude after LTP induction were significantly higher in the St-Cr group compared to the St-Rec group ($P < 0.05$ for both). These results indicated that only therapeutic approaches involving crocin were effective for LTP induction. However, subsequent to LTP induction, these parameters were not significantly different in the St-Esc and St-Esc-Cr groups ($P > 0.05$ for both) in comparison with the St-Rec group. These findings suggest that only treatments with crocin were sufficiently effective in reversing the LTP deficits induced by chronic stress in the hippocampal CA1 region [Figure 4].

Evaluation of serum CORT levels

The ANOVA assigned different levels of significance regarding the CORT levels [F (5, 42) = 1.764, P = 0.141]. The St-Rec

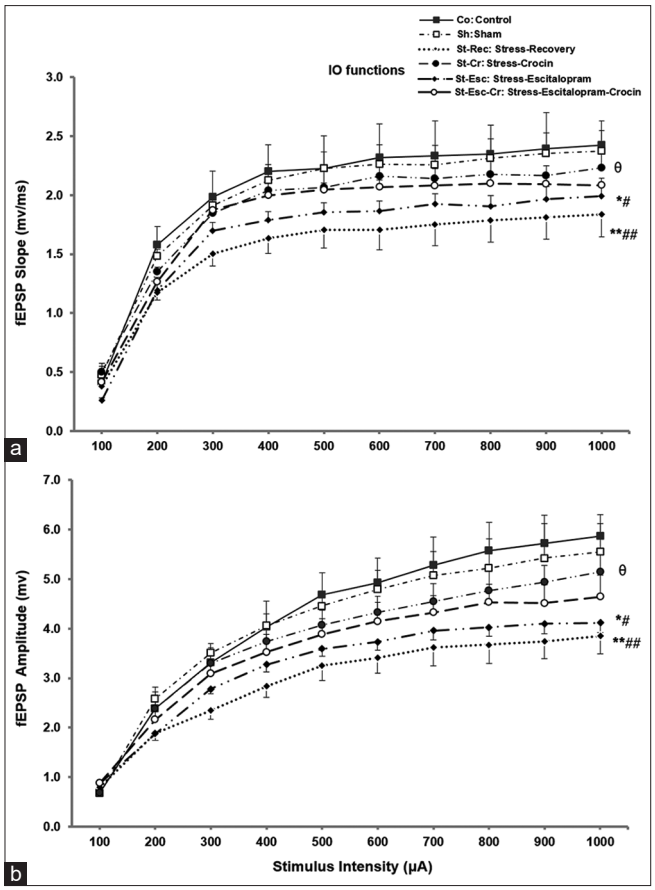

Figure 3: Input-output (I/O) curves of (a) the fEPSP slope and (b) amplitude in the CA1 region related to existing treatment groups. Results are expressed as means ± SEM (repeated measures ANOVA, followed by an LSD post-hoc analysis). *$P < 0.05$ and ** $P < 0.01$ compared to the Co group; $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ compared to the Sh group; $^{\Theta}P < 0.05$ compared to the St-Rec group

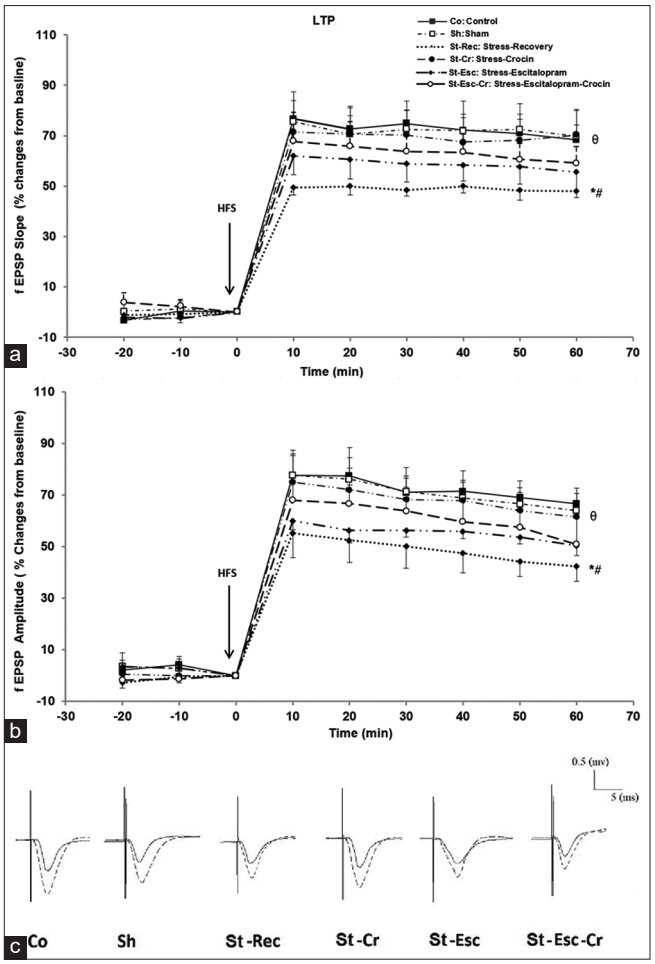

Figure 4: Different treatment protocols for LTP induction in the CA1 region employing 100 Hz tetanic stimulation. The fEPSP (a) slope and (b) amplitude are shown as the percentages of the baseline response for all experimental groups. (c) Traces of typically recorded fEPSP samples in the hippocampal CA1 neurons pre- and post-HFS for LTP induction among experimental groups. Results are expressed as means ± SEM (repeated measures ANOVA, followed by an LSD post-hoc analysis). *$P < 0.05$ compared to the Co group; $^{\#}P < 0.05$ compared to the Sh group; $^{\Theta}P < 0.05$ compared to the St-Rec group

group represented a significantly higher CORT level in comparison with the Co group ($P < 0.05$). As shown in Figure 5, the serum CORT level exhibited a significant decrease in the St-Cr group compared to the St-Rec group ($P < 0.05$).

Discussion

This study examined the therapeutic impact of escitalopram, crocin, the combination of escitalopram and crocin, as well as a recovery period after chronic stress on the I/O function and LTP induction within the hippocampal CA1 area in stressed rats.

The present findings indicated that stressed rats, receiving no treatments, to simulate a recovery period following chronic stress, exhibited decreased neural excitability and reduced intensity of LTP induction and maintenance in the hippocampal CA1 neurons. In addition, the elevated CORT levels confirmed the LTP changes in stressed subjects without treatment. Chronic stress can impair synaptic plasticity and memory.[12] Nevertheless, the recovery period following chronic stress did not revert chronic stress-induced memory impairments back to normal condition. It seems that chronic stress may lead to significant alterations in calcium flow, hyperpolarization, and glutamate synaptic transmission, all of which can severely affect neural excitability and LTP induction.[16] Moreover, additional mechanisms may contribute to memory impairment during chronic stress, encompassing glucocorticoids, neurotransmitters, brain-derived neurotrophic factors (BDNFs), glutamate and GABAergic receptors, as well as the nervous system morphology.[17,18] These factors play a crucial role in learning, memory, and the long-term changes in synapses.[19] Despite implementing a recovery period following chronic stress of a duration at least similar to that of the stress period, it proved inadequate in mitigating stress-induced plasticity and/or memory impairments by reversing the elevated CORT levels. Even following different conditions, such as exercise or the use of saffron extract, memory exhibited no discernible alterations during the recovery period.[20,21] In

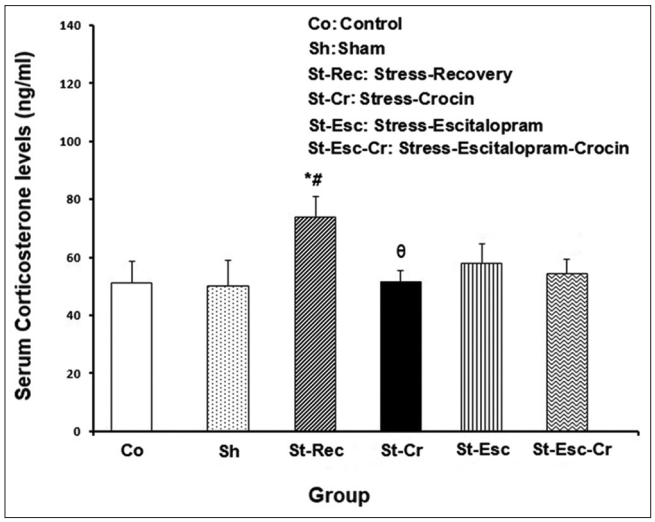

Figure 5: Changes in serum corticosterone (CORT) levels (ng/ml) among all experimental groups. Results are expressed as means ± SEM (one-way ANOVA, followed by an LSD post-hoc analysis). $^{*}P < 0.05$ compared to the Co group; $^{\#}P < 0.05$ compared to the Sh group; $^{\ominus}P < 0.05$ and compared to the St-Rec group

line with these observations, a prior study highlighted that chronic stress could induce changes in the expression of genes within the hippocampus, and these alterations do not fully revert during the recovery period.[22] Another study further reported that hippocampal proliferation fails to return fully during the recovery period.[23] On the contrary, a separate study proposed that the elimination of stressors might enhance neuroplasticity,[24] with this improvement potentially being linked to a reduction in glucocorticoid levels.[25] Supporting these observations, yet another study demonstrated a significant structural reorganization in the hippocampus and observed behavioral improvements in rats following a stress recovery period.[26] Moreover, cell proliferation exhibited improvement after a 21-day recovery period following stress induction.[27] These contradictions could be attributed to diverse recovery time frames and the dynamic changes in neurochemical factors within the hippocampus following stress induction.[28,29]

In this study, exclusively those therapeutic interventions involving the administration of crocin alone, subsequent to chronic stress, exhibited a notable enhancement in neural excitability, as well as in LTP induction and maintenance in the hippocampal CA1 region. The observed decreased levels of CORT served as confirmation of memory improvement among stressed subjects undergoing crocin therapy. Reports indicate that a 30 mg/kg dose of crocin has a protective effect on reversing hippocampal excitability and mitigating LTP impairments within the CA1 region among stressed subjects.[8,12] Moreover, there is suggestive evidence that crocin may lead to increased synaptic plasticity, synaptogenesis, and neurogenesis.[30] Various mechanisms have been proposed to explain the role of crocin in enhancing plasticity and memory; these mechanisms include changes in the activity levels of BDNFs, glucocorticoids, and neurotransmitters (e.g., acetylcholine and glutamate).[8,9,31–33] Furthermore, a study indicated that crocin elevated acetylcholine levels, improved memory, and prevented hippocampal neural apoptosis in a rodent model of cerebral ischemia.[34] Another study highlighted an interaction between crocin and the glutamatergic system, suggesting potential improvement in Aβ-induced LTP deficits.[9] In contrast, other researchers have reported that crocin does not affect the inhibition of cholinesterase by diazinon.[35]

Other therapeutic protocols, such as the administration of escitalopram alone and the combination of escitalopram-crocin following chronic stress, failed to reverse the disruptions in neural excitability, LTP induction, and maintenance in the CA1 region, as well as the serum CORT levels, when compared to stress subjects without any treatment. This indicated that escitalopram with and without crocin was not efficacious following a chronic stress period. The administration of escitalopram may be beneficial when concurrent with chronic stress periods, but not when initiated post-chronic stress. Several studies have reported that escitalopram does not affect memory functions.[36,37] However, those reported effects of escitalopram on reversing plasticity and memory deficits in rodents[38] are likely due to changes in serotonin, BDNF,[38,39] tumor necrosis factors,[40] antioxidants,[41] and glutamatergic synapses.[42] In addition, it was suggested that escitalopram could decrease CORT levels.[37,43] The observed discrepancies in findings may be attributable to various factors, including study design, type of stress induction, sample size, drug dose, gender, and race, as reported by different studies.[21,44] In the present study, the combination of escitalopram and crocin following chronic stress did not reverse memory impairments and elevated serum CORT levels in stressed subjects. However, some researchers have suggested that substances like escitalopram and crocin may operate effectively in modulating memory impairments.[9,38,45] Nevertheless, the exact mechanism involved in their impact on memory function has not been elucidated. Notably, crocin appears to have improved electrophysiological parameters and partially decreased serum CORT levels when used in combination with escitalopram. Therefore, crocin (a medicinal herb) demonstrated superior efficacy compared to escitalopram (a chemical drug) in ameliorating stress-induced memory impairments after the chronic stress period. One possible explanation for the enhanced performance of crocin over escitalopram in this study could be the higher level of decrease in CORT levels caused by crocin. Moreover, prior research has indicated that escitalopram not only failed to affect the NMDA receptors,[46] but also inhibited these receptors.[47] Additionally, studies have shown that crocin increases levels of cyclic adenosine monophosphate (cAMP) response element-binding (CREB) and BDNF proteins in the hippocampus.[31,48,49] Nevertheless, escitalopram reduced hippocampal CREB levels, according to a different study.[50] However, these factors were not assessed in the present investigation. The abovementioned factors may suggest a partial inhibitory role of escitalopram, in this

study, on crocin when co-administered following chronic stress. Hence, further in-depth explorations are essential to comprehending the impact of crocin and escitalopram, both in combination and separately, on alleviating brain dysfunctions. These explorations should delve into other biochemical, structural, cellular, and molecular mechanisms. Understanding these mechanisms holds the potential to shed light on the development of more effective treatments for a cascade of brain disorders, such as stress, anxiety, and depression.

Conclusion

In summary, the neural excitability and long-term plasticity in the CA1 region were severely disrupted among stressed rats with no treatment. Hence, following chronic stress, even a recovery period failed to revert neural excitability and memory impairments in stressed subjects. The administration of crocin alone demonstrated beneficial effects on neural excitability and memory in the CA1 region. However, the use of escitalopram, either alone or with crocin, did not exert any advantageous effects on memory improvement in the CA1 region. Thus, crocin surpassed escitalopram in alleviating stress-induced memory impairments after the cessation of the stress period. In the present study, it seems that various neurochemical mechanisms are implicated in the influence of escitalopram, exerting a partial inhibitory role on crocin during their co-administration following chronic stress.

Ethics approval and consent to participate

All the experiments were approved by the Research and Ethics Committee of the Isfahan University of Medical Sciences **(IR.MUI.MED.REC.1398.606)**.

It is not applicable, as this study was an animal study and we did not have any human participants.

Consent to publish

All of authors have consent to publish.

Availability of data and materials

Data availability are presented within the text of the manuscript as a graph.

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Conflicts of interest

There are no conflicts of interest.

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