Original Article

Effect of Crocin, Exercise, and Crocin-accompanied Exercise on Learning and Memory in Rats under Chronic Unpredictable Stress

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Abstract

Background: Stress affects brain functions and induces psychological disorders. Previous studies have indicated different effects of crocin and exercise on the improvement of memory in some types of stress. The present study investigated the effect of crocin, exercise, and crocin-accompanied exercise on learning, memory, and memory consolidation in rats under chronic unpredictable stress (CUS). **Materials and Methods:** Male rats were randomly allocated to different groups: control, sham, stress, stress-exercise, stress-crocin, and stress-crocin-accompanied exercise groups. The CUS and treadmill running were applied 2 h/day and 1 h/day, respectively, for 21 days. Crocin (30 mg/kg) was daily intraperitoneally injected to the rats and their behavioral variables were evaluated as a brain function using the passive avoidance test. **Results:** Results showed that the CUS significantly decreased learning and memory compared to the control group, while crocin alone and crocin-accompanied exercise significantly improved learning and memory compared to the stressed group. It was found that exercise alone caused learning but did not improve memory in unpredictable stress rats. **Conclusion:** The data indicated that unpredictable stress had very destructive effects on the brain functions. Furthermore, unlike exercise, crocin improved memory under unpredictable stress conditions. Overall, it seems that the beneficial effects of crocin-accompanied exercise on learning and memory were probably because of crocin, but not exercise.

Keywords: *Crocin, exercise, leaning, memory, passive avoidance, rat, unpredictable stress*

Introduction

Stress is known as one of the major factors that influence psychological health and brain activity such as learning, memory, and memory consolidation.[1-3] According to the type of stress, there are predictable and unpredictable stresses containing social stress, isolation stress, immobility stress, restraint stress, and many other types of stress that each influences the physiological system of the body through different neural circuits.[4,5] Previous studies have reported some types of stress impaired different kind of memory in a variety of behavioral tasks.[6-8]

In herbal medication, plant drugs have been used to elevate brain functions due to their availability and supposedly lower side effects.[9] Crocin is one of the main effective components of saffron (*Crocus sativus*) that can counteract memory deficits in the passive avoidance task in animals.[9] In addition, it has been reported that various types of exercise had different (helpful, harmful, and without role) effects on the physiologic system of the body. Moreover, exercise timing has shown various effects on memory in normal and restraint stress conditions in rodents.[10-13]

Since exercise and saffron (containing crocin) are available in the majority of human societies, this study was designed. Moreover, although some studies have been conducted on crocin and exercise alone on stress conditions, no published report is yet available on the effects of unpredictable stress (one of the most stressful conditions in human society) with each crocin and exercise alone as well as unpredictable stress with crocin-accompanied exercise on memory processing. The present study was, therefore, designed to investigate which one(s) of crocin, exercise and/or crocin-accompanied exercise is/are effective on learning, memory, and memory consolidation in rats under chronic unpredictable stress (CUS).

How to cite this article: Dastgerdi HH, Radahmadi M, Reisi P, Dastgerdi AH. Effect of Crocin, Exercise, and Crocin-accompanied Exercise on Learning and Memory in Rats under Chronic Unpredictable Stress. Adv Biomed Res 2018;7:137.

Received: June, 2018. **Accepted:** September, 2018.

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Access this article online

Website: www.advbiores.net

DOI: 10.4103/abr.abr_153_18

Quick Response Code:

Materials and Methods

Animals

Forty-eight male Wistar rats (200–250 g; n = 8) were obtained from the Pasteur Institute (Tehran, Iran). All the experimental protocols were approved by the Ethics Committee of Isfahan University of Medical Sciences (Isfahan, Iran) in compliance with the "Principles of Laboratory Animal Care" and the European Community Council Directive of 1986. Rats were housed (4 rats in each cage) under standard laboratory conditions with a 12 h light/dark cycle (lights on at 07:00–19:00.) at a constant temperature (22°C ± 2°C). Food and water were available *ad libitum*. Animals were allowed a 2-week period to adapt themselves to the environment. Behavioral experiments were carried out between 13:00 and 15:00.

Animals were randomly assigned to six different groups (n = 8 in each group) as follows:

1. Control group (Co): Rats were transferred to the laboratory where they received no special treatments and were handled the same as the experimental animals throughout the study period
2. Sham group (Sh): Rats received equal volumes of saline (drug solvent) for 21 days
3. Chronic unpredictable stress (CUS) group: Rats were under unpredictable stress for 2 h/day for 21 days
4. Chronic unpredictable stress-Crocin 30 group (CUS-C30): Rats were under unpredictable stress for 2 h/day and daily received 30 mg/kg of crocin before stress for 21 days
5. Chronic unpredictable stress-exercise group (CUS-Exe): Rats were under unpredictable stress for 2 h/day and had daily regular exercise after stress (1 h/day) for 21 days
6. Chronic unpredictable stress-Crocin30-exercise group: (CUS-C30 and Exe): Rats were under unpredictable stress for 2 h/day and daily received crocin 30 mg/kg (before stress) and were subjected to exercise (1 h/day, after stress) for 21 days [Figure 1].

Stress paradigms

In the current study, CUS was randomly induced in the rodents. Each day, rats were subjected to one of the below stressors in an unpredictable order. The stressors included different types of stress as follows: restraint stress (rats were placed in a restraining device made of Plexiglas), heat stress (rats were placed in glass cage in a warm environment 40°C–42°C), elevated platform (rats were placed on a platform elevated about 1 m above the ground), cold stress (rats were placed in plastic cage in a cold environment 2°C–4°C), water immersion stress (rats were placed in a Plexiglas filed with water temperature about 22°C–24°C), isolated stress (rats were moved to individual cages), and crowded stress (rats were housed 10 rats per a standard cage). The control rats were housed under similar conditions but left undisturbed during the stress period. The CUS was randomly induced in the rodent throughout the experimental period each day, at 8:00–10:00 am 2 h/day for 21 consecutive days.

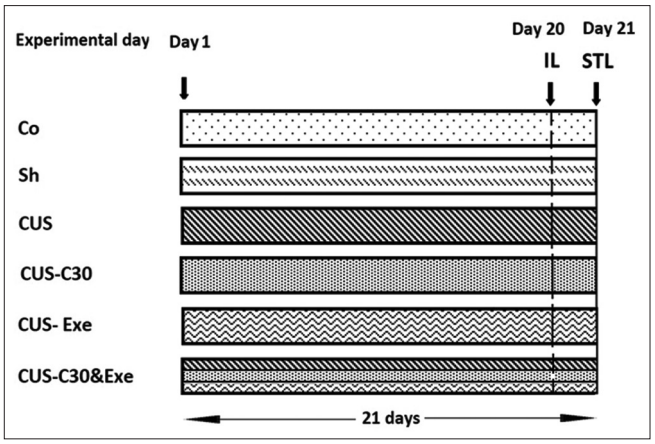

Figure 1: Experimental protocol for different groups and the days on which initial latency of entrance into the dark room and the step-through latency were tested. Co: Control group, Sh: Sham group, CUS: Chronic unpredictable stress group, CUS-C30: Chronic unpredictable stress-Crocin 30 group, CUS-Exe: Chronic unpredictable stress-exercise group, CUS-C30 and Exe: Chronic unpredictable-crocin-exercise group

Drugs

The animals received doses of 30 mg/kg of crocin (Sigma-Aldrich Co., USA) dissolved in saline, intraperitoneally injection for 21 consecutive days in the unpredictable stressed rats.[14]

Exercise protocol

Rats in the exercise group run on a treadmill at a speed of 20–21 m/min for 60 min daily (1 h/day) for 21 days.[11,15] To familiarize animals with the treadmill set up, its speed was increased from 10 to 21 m/min and over the course of 3 days.

Behavioral paradigms

In the current study, learning, memory, and memory consolidation were measured by the passive avoidance test as one of the behavioral tasks. The passive avoidance apparatus (64 cm × 25 cm × 35 cm) was divided into same size room (32 cm × 25 cm × 35 cm) that had a grid floor. The two rooms were separated by a sliding guillotine door. On day 19 of the experiment, each rat was placed in the apparatus for 300 s to habituate to it. On the day later (day 20), a single learning trial was performed. Then, on day 21, the memory trail of passive avoidance test was evaluated. Both habituation and memory trail were without any electrical foot shock. In the learning trial, rats were placed individually in the ligh troom for 60 s, then the guillotine door was raised. When the rat entered the dark room, the door was closed and a single foot electrical shock (0.5 mA, 2 s; once) was delivered through the grid

floor using an isolated stimulator.[14] The initial latency (IL) of entrance into the dark room was recorded before inducing an electrical shock. Also, in the memory trial, the step-through latency (STL) as the delay of entering to the dark room from lightroom (up to a maximum of 300 s) was measured. Furthermore, the trend between IL and STL indicated that learning was happened in animal experimental research.[14] The total dark stay (DS) time was recorded as memory consolidation and/or storage of novel information. The passive avoidance task determined the ability of the animal to remember the foot shock received. Avoiding entry into the dark compartment or a longer duration of stay in the light compartment was interpreted as a positive response.[16]

Statistical analysis

Since not all of the behavioral data in the passive avoidance test were normal, nonparametric tests were used. IL, STL, and DS time (between groups) were compared using a Kruskal–Wallis nonparametric one-way analysis of variance (ANOVA), followed by a two-tailed Mann–Whitney U-test with groups as the independent variable and performance in each session as the dependent variable. The comparisons of initial and step through latencies (within groups) were analyzed by Friedman test, followed by a Wilcoxon signed ranks test. All the data were reported as standard error of the mean $P < 0.05$ was considered statistically significant.

Results

The latency of entrance to the dark compartment

Figures 2 and 3, respectively, show the initial and stepthrough latency (IL and STL, respectively) of all groups in a single-trial passive avoidance test. Results showed that there were no statistically significant differences between control (Co) and sham (Sh) groups in the IL and STL, suggesting that the injection had no significant effect on these parameters. In addition, based on the other results, the IL did not show significant differences in all groups [Figure 2].

The STLs were significantly ($P < 0.01$ and $P < 0.05$, respectively) lower in the CUS and unpredictable stress-exercise (CUS-Exe) than the control group [Figure 2]. Whereas, the STLs show no significant decreases in unpredictable stress-crocin (CUS-C30) and CUS-crocin 30-exercise (CUS-C30 and Exe) compared to the control group [Figure 3].

As shown in Figure 3, the STL in the CUS-Exe had not significant enhancement compared to the CUS group, indicating that exercise did not have a considerable effect on memory deficit induced unpredictable stress. Moreover, the STL significantly ($P < 0.05$, in both them) increased in CUS-C30 and CUS-C30 and Exe groups when compared to the CUS group [Figure 3].

According to other present data, the STL was significantly ($P < 0.05$) lower in the CUS-Exe group than the CUS-C30 [Figure 3]. It indicated the positive effect of crocin compared to exercise in unpredictable stress conditions. Whereas, the STL did not significantly differ in the CUS-C30 and Exe compared to CUS-C30 group [Figure 3], indicating that exercise accompanied with crocin had not synergic effect on enhancement of memory in stress rats.

As presented in Figure 3, the STL was significantly ($P < 0.05$) higher in the CUS-C30 and Exe group than the CUS-Exe group. The results showed that chronic crocin treatment improved memory impairment due to unpredictable stress.

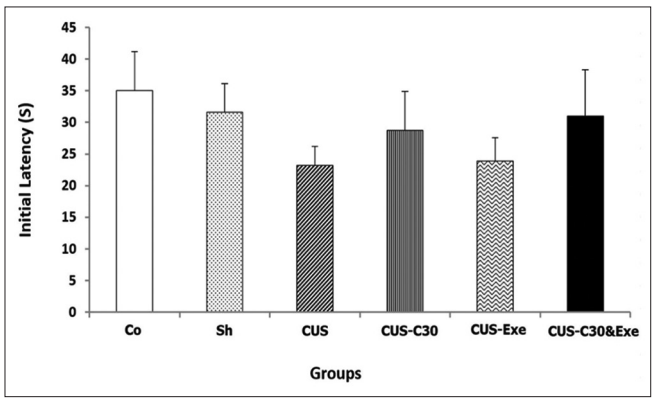

Figure 2: Initial latency to enter the dark room of the passive avoidance apparatus for all the groups before receiving a foot shock (n = 8). Results are expressed as means ± standard error of the mean (Kruskal–Wallis nonparametric one-way analysis of variance, followed by a two-tailed Mann-Whitney U test). No significant differences were observed among the groups. Co: Control group, Sh: Sham group, CUS: Chronic unpredictable stress group, CUS-C30: Chronic unpredictable stress-Crocin 30 group, CUS-Exe: Chronic unpredictable stress-exercise group, CUS-C30 and Exe: Chronic unpredictable-crocin-exercise group

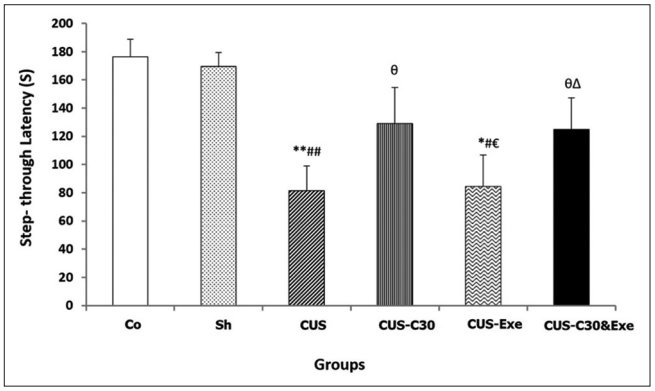

Figure 3: Step-through latency to enter the dark room of the passive avoidance apparatus for all the groups 1 day after receiving the foot shock (n = 8). Results are expressed as means ± standard error of the mean (Kruskal–Wallis nonparametric one-way analysis of variance, followed by a two-tailed Mann–Whitney U-test). *$P < 0.05$ and **$P < 0.01$ compared to the control, #$P < 0.05$ and ##$P < 0.01$ compared to the Sham, $^{\Theta}P < 0.05$ compared to the CUS group, $^{\triangle}P < 0.05$ compared to the CUS-Exe, $^{€}P < 0.05$ compared to the CUS-C30 group. Co: Control group, Sh: Sham group, CUS: Chronic unpredictable stress group, CUS-C30: Chronic unpredictable stress-Crocin 30 group, CUS-Exe: Chronic unpredictable stress-exercise group, CUS-C30 and Exe: Chronic unpredictable-crocin-exercise group

The IL and STL were analyzed by the two related samples test (Friedman test, followed by a Wilcoxon signed ranks test) to evaluate within-group latency differences. As shown in Figure 4, significant differences ($P < 0.05$) were detected between the IL and the STL in all the groups, indicating that learning occurred in all experimental groups at different levels.

Total dark compartment stay time

The Kruskal–Wallis nonparametric ANOVA and *post hoc* two-tailed Mann–Whitney U-test revealed no significant difference in the total dark compartment stay time (DS) between the Co group and Sh group [Figure 5].

As shown in Figure 5, the DS in the CUS group was significantly ($P < 0.05$) higher than that of the Co group [Figure 5], suggesting that exposure to the stress had interfered with ability consolidation of memory. In addition, in the CUS-Exe and CUS-C30 and Exe groups, the DS significantly ($P < 0.01$, in both them) increased compared to the Co group [Figure 5] while in the CUS-C30 group, the DS did not show significantly enhancement compared to the control group [Figure 5].

As can be seen from Figure 5, the DS had no significant differences in the CUS-C30, CUS-Exe, and CUS-C30 and Exe groups compared to the CUS group.

Discussion

The effects of crocin, exercise, and crocin-accompanied exercise on learning, memory, and consolidation of memory in rats under chronic unpredictable stress were investigated in the current study to determine which one(s) of crocin, exercise, and/or crocin-accompanied exercise are helpful for brain functions in rats under chronic unpredictable stress.

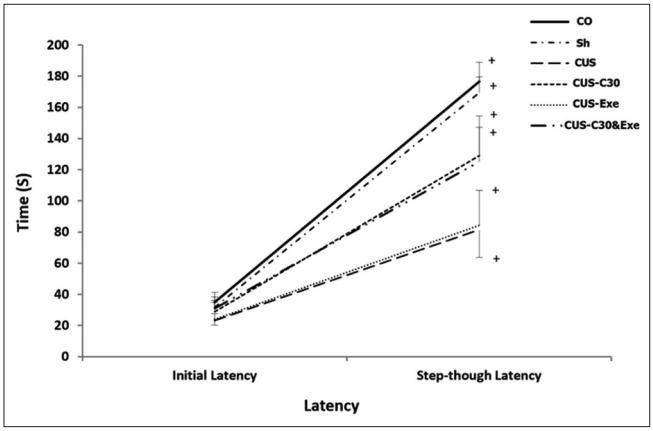

Figure 4: Initial latency and step-through latency after 1 day to enter the dark room of the passive avoidance apparatus before and after the foot shock (within groups) ($n = 8$). Results are expressed as means ± standard error of the mean (Friedman test, followed by a Wilcoxon signed ranks test) $^{+}P < 0.05$ initial latency relative to the step-through latency. Co: Control group, Sh: Sham group, CUS: Chronic unpredictable stress group, CUS-C30: Chronic unpredictable stress-Crocin 30 group, CUS-Exe: Chronic unpredictable stress-exercise group, CUS-C30 and Exe: Chronic unpredictable-crocin-exercise group

The results indicated that the CUS induced impairment of learning and memory similar to earlier studies on other chronic stress types. In this regard, many studies demonstrated the deleterious effects of different type of stress on brain functions such as memory, brain activity, gene expressions, and changes of stress biomarkers.[2,17-19] According to some previous reports, it seems that stress had various complex effects (impairing, neutral, and facilitating) on memory.[3,8,20-22] In addition, another result of this study is that the DS time increased in the CUS group, which may reflect impairment of the memory consolidation in unpredictable stress condition. In this way, some studies reported stress changed memory consolidation by adrenal hormonal changes such as glucocorticoid and catecholamine through activation of similar neural systems.[16,23] Furthermore, it is possible that the basolateral amygdala (as a key structure in a memory modulatory system) and interactions of the different brain regions could influence memory consolidation.[3]

Another finding of the present study is that chronic crocin treatment improved the severe harmful effect of the CUS on memory processing including learning, memory, and memory consolidation in the passive avoidance test, but not the same as the control group. Ghadrdoost *et al*. observed that crocin prevented spatial memory deficit induced by chronic restraint stress in the Morris water maze task.[14] In addition, saffron extract antagonized memory deficits due to scopolamine and streptozotocin-icv, as a model of Alzheimer's disease in the behavioral task.[24,25] However, it is possible that crocin as a potent antioxidant could improve altering, reversing, or forestalling the neuronal behavioral decrements.[26]

According to other data, the STL and DS values (as memory and memory consolidation indexes),

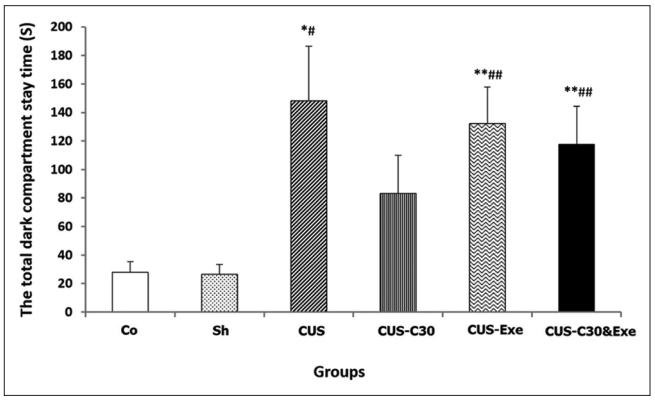

Figure 5: Total dark compartment stay time of the passive avoidance apparatus after electrical foot shock delivery in the different treatment groups ($n = 8$). Results are expressed as means ± standard error of the mean (Kruskal–Wallis nonparametric one-way analysis of variance, followed by a two-tailed Mann–Whitney U-test)). $^{*}P < 0.05$ and $^{**}P < 0.01$ when compared to the Co group, $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ when compared to the Sh group. Co: Control group, Sh: Sham group, CUS: Chronic unpredictable stress group, CUS-C30: Chronic unpredictable stress-Crocin 30 group, CUS-Exe: Chronic unpredictable stress-exercise group, CUS-C30 and Exe: Chronic unpredictable-crocin-exercise group

respectively, decreased and increased in the stress-exercise groups compared to the control group. In the stress-exercise group, the STL and DS values were nearly similar to the unpredictable stress group, suggesting that exercise could not improve memory deficit and memory consolidation in CUS condition. Many other and our previous studies demonstrated that exercise leads to improvements in the memory and DS time in normal and emotional stress conditions by changing some brain mediators.[10,11,13,27,28] Although some studies demonstrated beneficial effects of exercise on memory,[29,30] some researchers reported that forced exercise significantly impaired memory.[5,31] In this regard, our previous study demonstrated that synchronized exercise with emotional stress did not significantly improve short-, mid-, and long-term memory deficits in restraint-stressed rats.[32] However, despite a vast amount of research into the beneficial actions of exercise on memory, the present study found that exercise was not helpful in unpredictable stress situations. In other word, it seems that regular exercise acted as extra bad condition adjust unpredictable stress. In addition, it probably confirmed the very destructive effect of CUS on brain functions as memory. This opposite result may be related to type, duration, and time of stress and/or exercise. Notably, some studies reported that unpredictable stressors have a greater negative impact in humans than predictable ones, perhaps due to temporal uncertainty and inability to anticipate the event.[1] Based on our previous studies, it is possible that the balance of different mechanisms involved in the interaction of the psychical stress and exercise responses was disturbed. These mechanisms include the changes of different biomarkers, hormones, neurotransmitters, and radical generation as well as free antioxidant enzyme activities.[33-36]

Other present finding showed that crocin treatment was able to improve memory impairment due to unpredictable stress compared to the exercise group. It seems that crocin treatment, as herbal medicine, was more effective on memory deficit due to unpredictable stress than exercise. Contrary to our results, a number of studies have shown that exercise improves induced memory disorder by stress.[29] Despite little comparative studies conducted on the effect of crocin and exercise on the physiologic system, there is no study on brain functions in this regard. Ghorbanzadeh and *et al*. have shown that crocin combined with voluntary exercise improved insulin resistance and angiogenesis as well as reduction of glucose levels in diabetic rats.[37] Therefore, these differences are most probably related to the duration, intensity, and type of stress and exercise (e.g., forced vs. voluntary), task difficulty, gender, and other yet undefined variables.[11,38] Furthermore, based on the current study, other subjects were also probably involved in differences such as the induction time of stress and exercise, as well as the injection time of the drug.

According to the results of the present study, memory did not show any significant differences in crocin-accompanied exercise compared to the stress-crocin group. Moreover, crocin-accompanied exercise significantly improved memory deficit but not memory consolidation due to unpredictable stress, compared to the stress-exercise in passive avoidance test. It indicated that exercise accompanied with crocin did not have a synergic effect on enhancement of memory in stressed rats. Accordingly, it seems that memory improvement in the crocin-accompanied exercise was probably due to crocin, but not exercise, in unpredictable stress conditions. Also, it should be noted that although improvement of learning and memory were observed in the crocin-accompanied exercise and crocin groups in unpredictable stress condition, but the level of memory did not reach to the control group. It probably confirms the type of stress is very important in contrast to the response to different treatment. In addition, unpredictable stress was found as one of the destructive stress types.

Conclusion

To sum up, unpredictable stress had very devastating effects on memory and memory consolidation. The chronic crocin treatment improved the severe harmful effect of the CUS on memory processing, but not the same as the control group. Whereas, exercise could not improve memory deficit in the CUS conditions. Moreover, exercise accompanied with crocin had not a synergic effect on enhancement of memory in unpredictable stress. Hence, it seems that memory improvement in the crocin-accompanied exercise was probably due to crocin, but not exercise, in unpredictable stress conditions. Further research is, however, required to shed more light on the possible mechanism(s) involved. Investigation of receptor regulation, assessment of involved biomarkers such as hormones and biochemical factors through which crocin might affect brain functions might be suggested for the future study.

Acknowledgments

This work was supported by grants from Isfahan University of Medical Sciences, Isfahan, Iran.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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