

Original Article

# Effects of crocin on cognitive and spatial memories in rats under chronic isolation stress

Fatemeh Khani<sup>1</sup>, Maryam Radahmadi<sup>1\*</sup> , Hojjatallah Alaei<sup>1</sup>, Elahe Jafari<sup>2</sup>

1. Department of Physiology, School of Medicine, Isfahan University of Medical Sciences, Isfahan, Iran

2. Department of Psychology, Khorasgan Branch, Islamic Azad University, Isfahan, Iran

## Abstract

**Introduction:** Certain types of chronic mental stress impair memory. On the other hand, crocin is introduced in the medical literature as an effective component of saffron with remedial effects on memory impairment. This study investigated the effects of crocin on spatial and cognitive memories, locomotor activity, novel recognition conditions and serum corticosterone levels in rats under chronic isolation stress.

**Methods:** Male rats were randomly allocated to the five groups of control, sham, isolation stress (St.I), St.I-C30 and St.I-C60. The latter two groups were exposed to chronic isolation stress (6h/day) receiving two levels of crocin (30 and 60 mg/kg, respectively) over a period of 21 days. The object location and novel object recognition tests (OLT and NOR) were used to evaluate spatial and cognitive memories, respectively.

**Results:** The OLT results revealed that chronic isolation stress led to significantly decreased locomotor activity in all the stressed groups; the NOR test, however, yielded similar results only in the St.I group. Moreover, isolation stress was found to lead significant declines in spatial and cognitive memories. Finally, crocin administration led to improvements in impaired memory in St.I-C30 and St.I-C60 groups. There were significant enhancements in serum corticosterone levels in the St.I and St.I-C30 groups as compared with the control group.

**Conclusion:** Our findings indicate that spatial and cognitive memory impairments are strongly affected by isolation stress and crocin especially at its high dose of 60 mg/kg, exhibits better protective effects against cognitive memory deficit induced by chronic isolation stress.

## Keywords:

Crocin;  
Memory;  
Isolation stress;  
Rat

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## \*Correspondence to:

M. Radahmadi

**Tel:** +98-3137929176

**Fax:** +98-3136688597

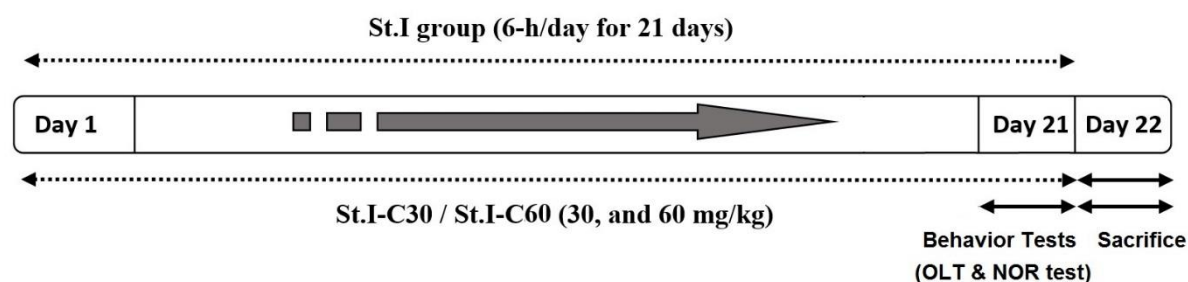
## Email:

m\_radahmadi@med.mui.ac.ir

## Introduction

Mental stress is defined as the situation in which the brain responds to internal and external stimuli with likely changes in the brain structure and functions (Radahmadi et al., 2014) accompanied by adverse

behavioral effects (Bandegi et al., 2014; Schreck et al., 2001; Zardooz et al., 2006) such as memory impairment (Eidelkhani et al., 2015; Ghadrdoost et al., 2011; McLaughlin et al., 2007; Radahmadi et al., 2015; Ranjbar et al., 2016). Isolation stress is one of a variety of mental stress types in rodent and human, organisms may be typically exposed to



**Fig.1.** Protocols for isolation stress treatments and crocin administration.

throughout their lives (Izadi et al., 2018a; Izadi et al., 2018b; Rudramma et al., 2003; Sampath et al., 2014; Sandstrom and Hart, 2005; Shao et al., 2015; Zimmerberg et al., 2003; Zlatković et al., 2014). It has been observed that isolation of rats affects their brain development, brain neurochemical responses and subsequent adult behavior (Arango et al., 2001; Caldji et al., 2000; Lehmann and Feldon, 2000; Weiss and Feldon, 2001). It is, therefore, hypothesized that stress impairs both the activity of the hypothalamic–pituitary–adrenal (HPA) axis and cognition, suggesting potential alterations in brain functions (Sandstrom and Hart, 2005). Certain studies have investigated possible ways of alleviating the deleterious effects of mental stress on memory as a risk factor of mood disorders associated with the Alzheimer's disease (Alkadhi, 2011).

Crocine, as a carotenoid pigment, is the effective compound of saffron (*Crocus sativus* L) that has long been used as a drug in medicine (Abdullaev, 2002; Alavizadeh and Hosseinzadeh, 2014; Mohajeri et al., 2010). Previous studies indicated the beneficial anti-oxidant, anti-lipidemic and anti-inflammatory effects of crocin (Abdullaev, 2002; Assimopoulou et al., 2005; He et al., 2005; Lee et al., 2005; Nam et al., 2010). The water maze test has shown crocin improves memory deficit induced by restraint stress (Ghadrdoost et al., 2011). Isolation stress is a kind of stress anyone is likely to be exposed to throughout their lives. Affected individuals may present memory deficits as a result of chronic isolation stress. However, few studies have been focused on the physiological aspects of isolation stress such as brain functioning. The present study was, therefore, designed and conducted to investigate the protective effects of crocin on serum corticosterone (CORT), locomotor activity and novel recognition. Moreover, the object

location and novel object recognition (OLT and NOR) tests were used to explore spatial and cognitive memories, respectively, only in rats affected by chronic stress isolation.

## Materials and methods

### Experimental design

The experiments were performed on forty adult male Wistar rats (Pasteur Institute, Tehran, Iran) weighing 250–300g. The animals were maintained under controlled temperature ( $22\pm 2^{\circ}\text{C}$ ) and humidity ( $50\pm 5\%$ ) conditions over 12-h light/dark cycles with *ad libitum* access to food and water. All the experiments were performed in accordance with the standards set by the Committee on Ethical Standards of Isfahan University of Medical Sciences (IR.mui.rec.1394.3.934) and the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publications No. 80–23, 1996 Rev.).

Rats were randomly assigned to the following five groups ( $n=8$  in each group): control (Co), sham (Sh; receiving saline as vehicle daily for 21 days), chronic isolation stress (St.I; stressed in individual housing 6 h/day for 21 days) and two groups receiving daily crocin doses (30 and 60 mg/kg) accompanied by a period of 21 days of isolation stress.

Finally, all rats were prepared on day 21 for the tests (Fig. 1). The animals were evaluated not only in terms of their locomotor activity and recognition of novel conditions but also for their spatial and cognitive memories as judged by the object location and novel object recognition tests, respectively. Serum corticosterone levels were also determined after decapitation.

### Drugs

Crocine (Sigma Aldrich Co., USA) was purchased in powder form and dissolved in a saline to be injected

intraperitoneally (IP) to the rats in the experimental groups at doses of 30 and 60 mg/kg/day for 21 consecutive days. The prevalently used and least effective doses of crocin administered to rodents in the experiments have been reported to be 30 and 60 mg/kg/day (Khalili and Hamzeh, 2010; Vakili et al., 2014). In addition, previous studies have shown no biochemical, hematological or histopathologic toxicity in rodents (mice and rats) due to chronic injection administration of 15-80 mg/kg, IP of crocin (Hosseinzadeh et al., 2010; Kianbakht and Hashem Dabaghian, 2015). However, higher doses administered over long periods (21 days) might be toxic.

### Chronic isolation stress

For the purposes of this study, isolation stress was induced 6h/day (from 8:00 to 14:00) for 21 consecutive days in the stressed groups. Briefly, each rat was left alone inside a cage (individual housing) before they were placed back in their communal home cage. Other protocols (e.g., lower or higher durations ranging from 3-6 h/day to 24 h/day for periods of 1 to 60 days) have also been reported for inducing isolation stress in rodent (Izadi et al., 2018a; Izadi et al., 2018b; Rudramma et al., 2003; Sampath et al., 2014; Sandstrom and Hart, 2005; Shao et al., 2015; Zimmerberg et al., 2003; Zlatković et al., 2014).

### Behavioral apparatus and method

Spatial memory is responsible for recording information on the surrounding environment and spatial orientation (León et al., 2016). Cognitive memory includes what has been called the 'executive memory' and/or 'memory of action' (e.g., skills and habits) (Fuster, 2006). The hippocampal-dependent spatial and cognitive memories were investigated using the object location and the novel object recognition tests, respectively, among others (Antunes and Biala, 2012; Broadbent et al., 2004; Ranjbar et al., 2016; Vann and Albasser, 2011). These tasks are widely used for assessing memory in both humans and rodents (Ainge et al., 2006; Brodziak et al., 2014; Clark et al., 2000; Gaskin et al., 2003; Hughes, 2007; Murai et al., 2007; Ranjbar et al., 2016; Sierksma et al., 2014). Other learning and memory tasks such as passive avoidance or Morris water maze are often associated with stress in the

animal (Ranjbar et al., 2016; Roozendaal et al., 2008; Roozendaal et al., 2003). However, the OLT and NOR behavioral tests used in the current study required no external motivation, reinforcement, punishment or stimulation (Silvers et al., 2007).

To perform these tests, each rat was initially habituated by being placed individually for 5min in the center of the apparatus (an open field box of 60×60×50 cm) on day 20 of the experimental period. The sample (acquisition) and test (retention) phases of the OLT and NOR tests were performed on the following day (i.e., day 21). In the sample phase trial, two identical objects (A1 and A2 cuboids with identical heights of 5cm) were placed on the adjacent corner of each area leaving a distance of 8cm from the walls. The rat was then placed between the two objects in order for it to start a 5min object exploration training. An interval of 7min was allowed between the sample and the test phase trials (Ranjbar et al., 2016).

In the test phase trial of the OLT, the cuboid objects were replaced with their identical copies; one placed in the same position as it was in the sample phase trial (representing familiar location, F) and the other moved to the opposite side (representing new location, N).

In the NOR test trial, one of the two cuboid objects was kept unchanged (representing familiar object, F) while the other was replaced with a new one (representing new object, N). N and F in the OLT represent the time spent to explore novel and familiar locations over a 5-min observation period while they represent the time spent to explore novel and familiar objects in the NOR test (Ranjbar et al., 2016). Animal performance was video-taped for later analysis; however, in order to avoid any bias in the analysis, a human coder totally alien to the treatment procedure was used for the behavioral test. Locomotor activity in the test phase (retention) trials of both tests was measured as the total time spent exploring each of the two objects (i.e.,  $T_2 = F + N$  in the test phase). Finally, two main and auxiliary discrimination indexes (D1 and D2) were considered as index measures of discrimination between new and familiar objects. Indeed, D1 represents the absolute difference between new and familiar objects, whereas D2 is the relative measure of discrimination that corrects D1 for the level of exploratory activity in the test-trial (Akkerman et al., 2012; Sik et al., 2003). Hence, D1

(the discrimination index) and D2 (the discrimination ratio) were calculated using the following formulas (Ranjbar et al., 2016; Waters et al., 2012):  $D1 = N - F$  (s);  $D2 = [N/(N+F)] \times 100$  in the test phase

In both the OLT and NOR tests, object exploration was defined as directing the nose toward the object at a distance of 2cm and touching it with the nose but excluding such irrelevant activities as turning around, climbing over and sitting on the object (Reagan et al., 2008). Between each two runs of the trial, the behavioral box and the objects were cleaned with 70% ethanol in order to prevent the build-up of olfactory cues (van Gaalen and Steckler, 2000).

### Assessment of serum corticosterone levels

At the end of the experiments, rats were anesthetized using an intraperitoneal injection of urethane (1.5 g/kg) before they were sacrificed at 14:00–16:00. Following decapitation on day 22, serum was separated from blood samples and stored at  $-80^{\circ}\text{C}$  until analysis. The commercial enzyme-linked immunosorbent assay (ELISA) kit (Zellbio Co., Germany) was used for serum corticosterone level measurement.

### Data analysis

All the data were reported as mean $\pm$ SEM. The behavioral data and serum corticosterone levels of the various groups (i.e., between-group comparisons) were compared using ANOVA followed by LSD post-hoc test for multiple comparisons. Comparisons of the novel and familiar explorations (within-group comparisons) were analyzed using the paired Student's *t*-tests. A *P*-value of less than 0.05 was declared statistically significant. Ultimately, the calculations were performed using SPSS 21 (SPSS Inc. Chicago, IL, USA).

## Results

None of the variables investigated exhibited any significant differences between the control and the sham groups, indicating no effect of intraperitoneal injection of normal saline.

### Total time of object exploration and discrimination indexes (D1 and D2) in the OLT

In the OLT test, the ANOVA assigned different significant levels to the different variables: in T2,  $F(4,$

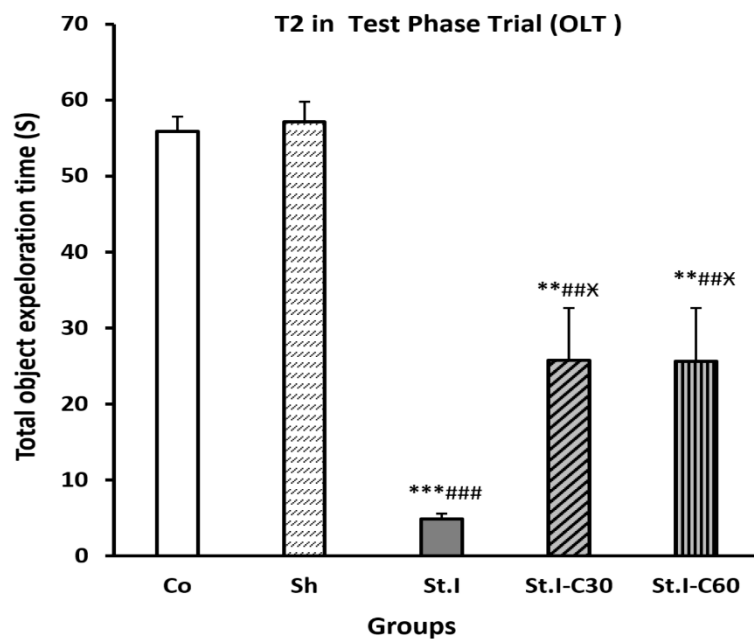
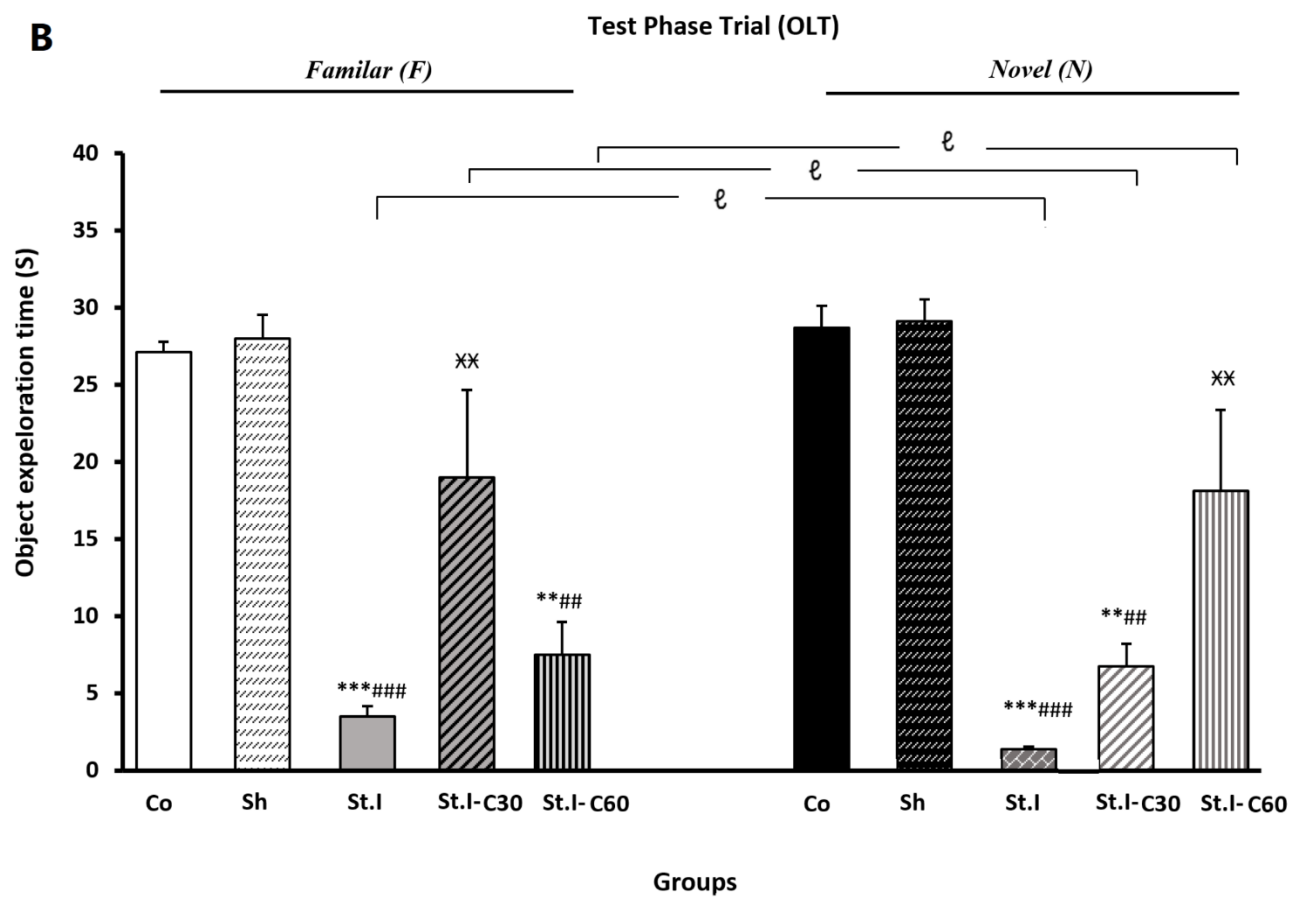
$33)=20.972$ ,  $P<0.001$ ; in new location (N) exploration times,  $F(4, 33)=21.363$ ,  $P<0.001$ ; in the main discrimination index (D2),  $F(4, 33)=20.635$ ,  $P<0.001$  and in the discrimination index (D1),  $F(4, 33)=8.145$ ,  $P<0.001$ .

The total times of object exploration in the test phase ( $T2=F+N$ ) of the OLT revealed significant decreases among the St.I, St.I-C30 and St.I-C60 groups compared with those measured for the Co group ( $P<0.001$  for the St.I group,  $P<0.01$  for the St.I-C30 and St.I-C60 groups), indicating reduced locomotor activity in these groups. Moreover, significant ( $P<0.05$ ) enhancements were observed in the T2 values of the OLT in the St.I-C30 and St.I-C60 groups relative to that recorded for the St.I group (Fig. 2A), suggesting the beneficial effects of crocin treatments (30 and 60 mg/kg) on induced memory deficit in rats under chronic isolation stress. However, crocin did not improve T2 in the stressed rats as much as it did in the control group.

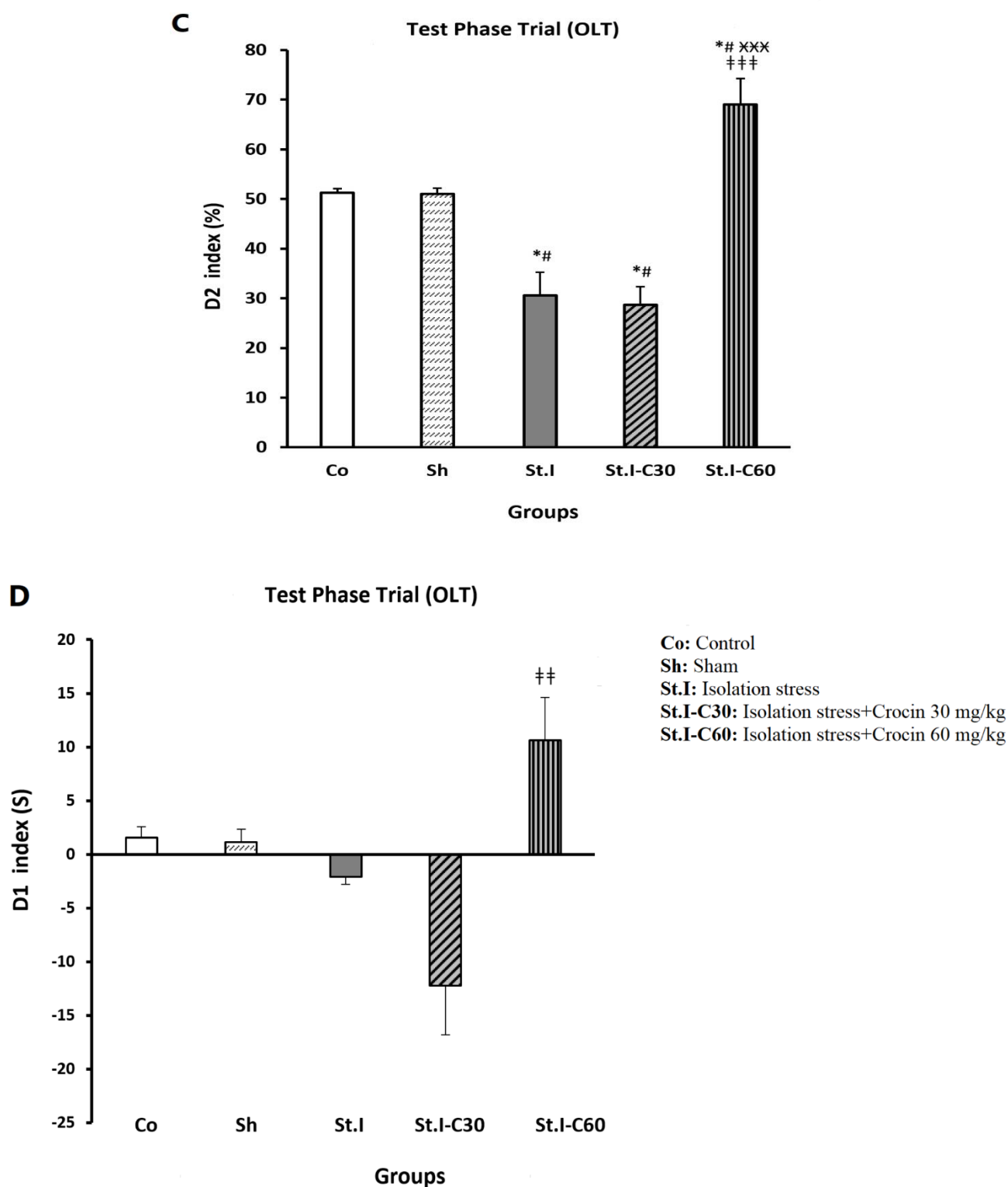
As shown in Figure 2B, new location (N) exploration times were significantly ( $P<0.05$ ) different from those of familiar location (F) in the St.I, St.I-C30 and St.I-C60 groups, suggesting that isolation stress led to deteriorating ability to recognize novel locations compared with recognizing familiar locations in the St.I and St.I-C30 groups, unlike in the St.I-C60 one. In other words, novel recognition was enhanced relative to familiar recognition only in the St.I-C60 group (Fig. 2B).

On the other hand, only in the St.I-C60 group, the exploration time of novel location (N) of the OLT showed a significant ( $P<0.01$ ) enhancement as compared with that in the St.I group (Fig. 2B). It, therefore, seems that a crocin dosage of 60mg/kg was more effective in improving novel location recognition.

In the OLT test, both St.I and St.I-C30 groups recorded significant ( $P<0.05$ ) decreases in their main discrimination indices (D2) as compared with the Co group (Fig. 2C), indicating the significantly decreased spatial memory in the St.I and St.I-C30 groups. This is while, compared with the Co group, St.I-C60 showed a significant ( $P<0.05$ ) enhancement in its D2. The significant ( $P<0.001$ ) enhancements observed in the values of D2 relative to those recorded by both St.I and St.I-C30 groups indicate that a crocin dosage of 60mg/kg improved spatial memory in the chronic isolation stress groups (Fig. 2C).

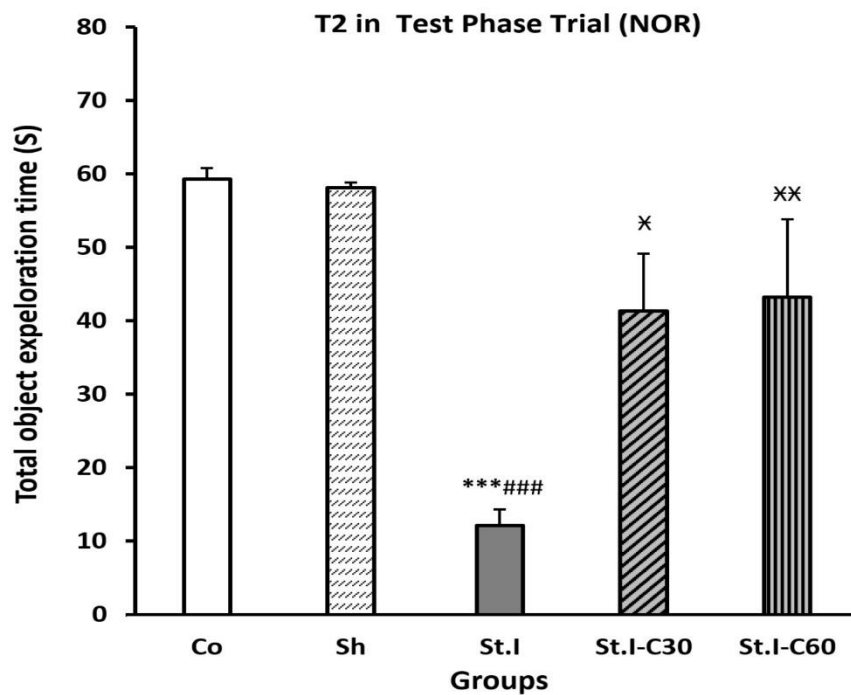
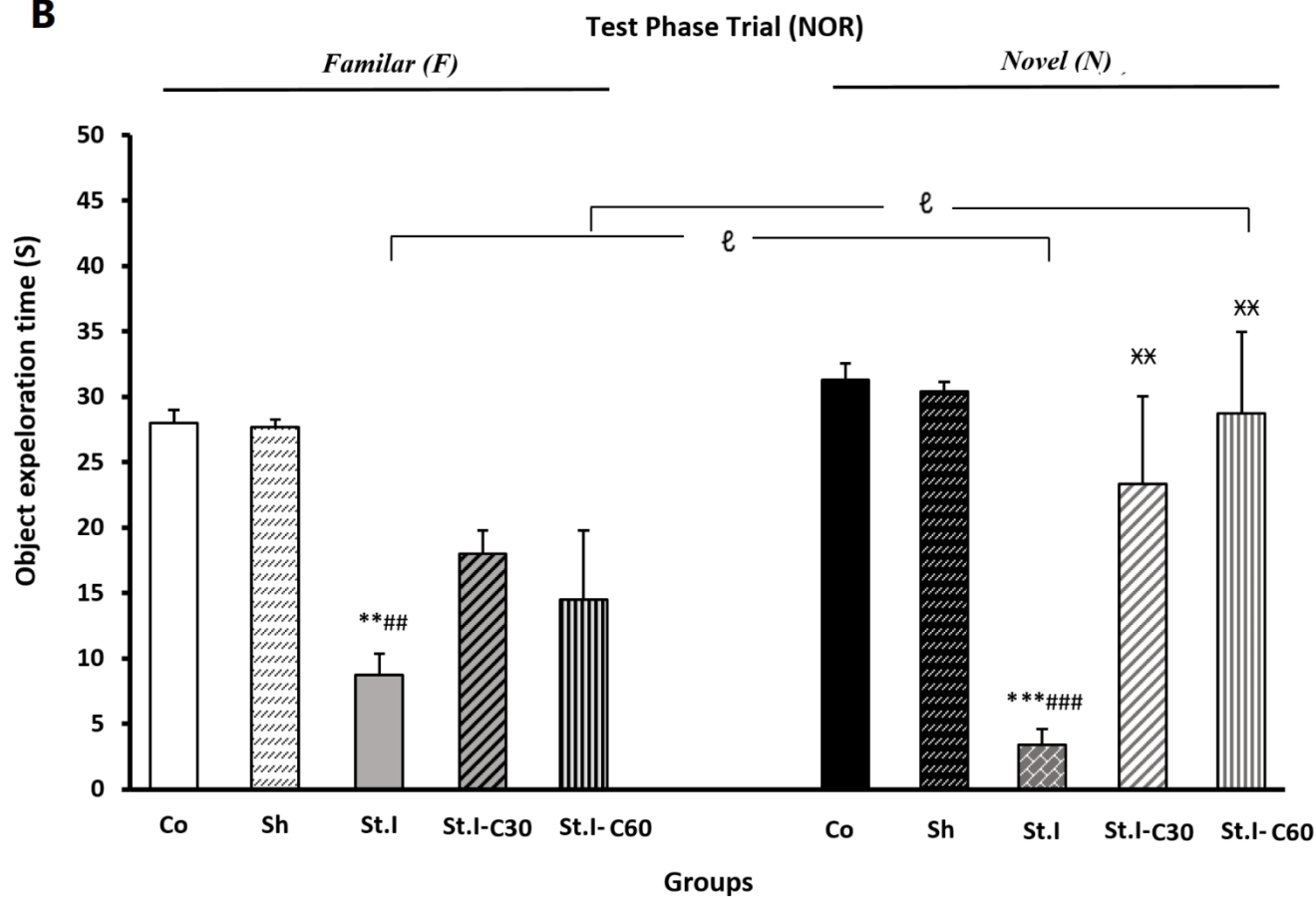
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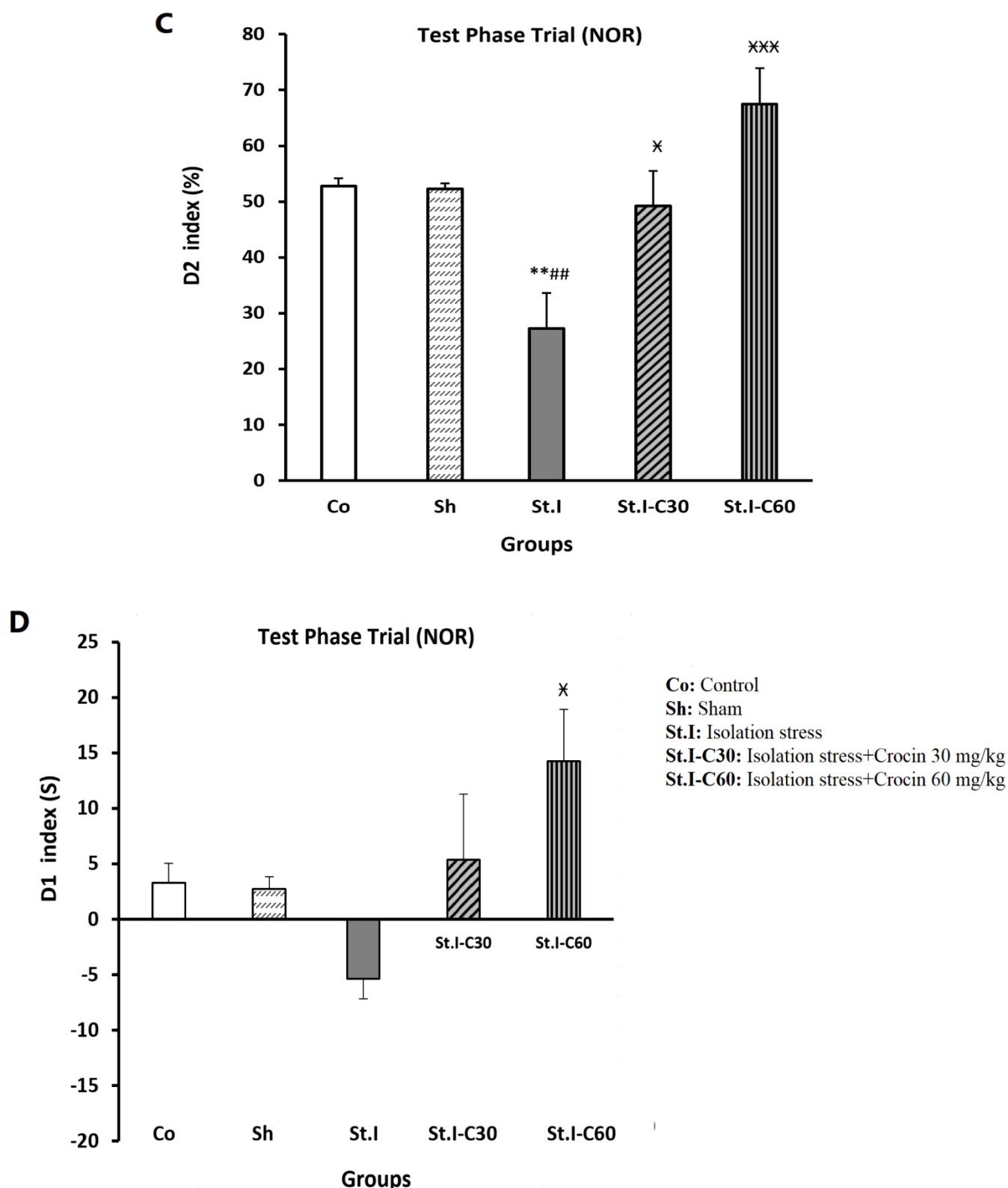




**Fig.2.** Effects of chronic isolation stress and different doses of crocin (30 and 60mg/kg) in the object location test (OLT): A) Total time of object exploration(s) for the test phase trial (T2) in the OLT; B) Exploration times of familiar and new locations (F and N, respectively) in the test phase trial of the OLT; C) Values of the main discrimination index (D2) for the test phase trials of the OLT and D) values of auxiliary discrimination index (D1) for the test phase trials of the OLT. Results are expressed as mean±SEM.

\*\*\* $P < 0.001$ , \*\* $P < 0.01$  and \* $P < 0.05$  compared to the control; ### $P < 0.001$ , ## $P < 0.01$  and # $P < 0.05$  compared to the Sham; XXX $P < 0.001$ , XX $P < 0.01$  and X $P < 0.05$  compared to the stress group; \*\*\* $P < 0.001$  and \*\* $P < 0.01$  compared to the stress-crocine 30 group.

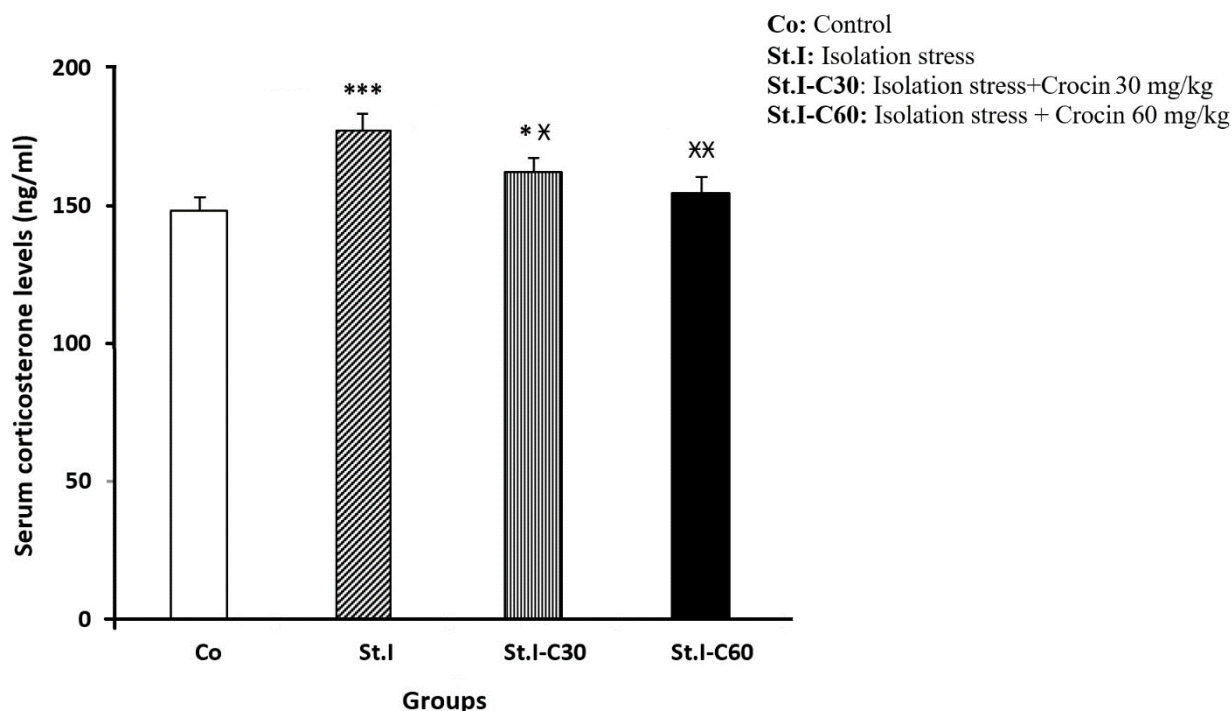
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**Fig.3.** Effects of chronic isolation stress and different doses of crocin (30 and 60mg/kg) in the novel object recognition tests (NOR): A) Total time of object exploration(s) for the test phase trial (T2) in the NOR; B) Object exploration times of familiar and new objects (F and N, respectively) in the test phase trial of the NOR; C) value for the main discrimination index (D2) for the test phase trials of the NOR and D) value of the auxiliary discrimination index (D1) for the test phase trials of the NOR. Results are expressed as mean±SEM.

\*\*\* $P < 0.001$  and \*\* $P < 0.01$  compared to the control; ### $P < 0.001$  and ## $P < 0.01$  compared to the sham; \*\*\* $P < 0.001$ , \*\* $P < 0.01$  and \* $P < 0.05$  compared to the stress group.





**Fig.4.** Effects of chronic isolation stress and different doses of crocin on serum corticosterone (CORT) levels (ng/ml) in the different treatments. Results are expressed as mean $\pm$ SEM.

\*\*\* $P$ <0.001 and \* $P$ <0.05 compared to the control; \*\* $P$ <0.01 and \* $P$ <0.05 compared to the stress group.

The auxiliary discrimination index (D1) of the OLT showed a significant ( $P$ <0.01) enhancement, in the St.I-C60 group compared with St.I-C30 (Fig. 2D). Clearly, a crocin dosage of 60mg/kg was effective in spending more time exploring the new rather than the familiar location, indicating the rats' improved performance in the OLT.

#### Total time of object exploration and discrimination indexes (D1 and D2) in the NOR

In the NOR test, an ANOVA assigned different significant difference levels to the different variables examined: in T2,  $F(4, 33)=9.133$ ,  $P$ <0.001; in new location (N) exploration times,  $F(4, 33)=7.217$ ,  $P$ <0.001; in the main discrimination index (D2),  $F(4, 33)=7.939$ ,  $P$ <0.001 and in the discrimination index (D1),  $F(4, 33)=3.652$ ,  $P$ <0.05.

Compared with the Co group, the values for T2 in the test phase of NOR decreased significantly ( $P$ <0.001) in the St.I group, indicating locomotor impairment in animals subjected to the chronic isolation stress in the test trial of NOR (Fig. 3A). Furthermore, significant enhancements ( $P$ <0.05 and  $P$ <0.01, respectively) were observed in the T2 values obtained for the St.I-C30 and St.I-C60 groups relative to that of St.I (Fig. 3A), suggesting enhanced

locomotor activity in both stressed groups as a result of daily injection of crocin (30 and 60 mg/kg) in the NOR test, albeit not as much as that in the Co group. As shown in Figure 3B, exploration times of new objects (N), as compared with those of familiar ones (F), showed a significant ( $P$ <0.05) decrease in St.I but a significant enhancement in St.I-C60. This suggested that a crocin dosage of 60mg/kg in the isolation stress groups improved their ability to recognize novel objects. Also, significant ( $P$ <0.01) enhancements were observed in the novel exploration times recorded by the St.I-C30 and St.I-C60 groups compared with those recorded for the St.I group (Fig. 3B). It, therefore, seems that both crocin doses had positive effects on novel object recognition in the NOR test.

The main discrimination index (D2) in the NOR test showed a significant ( $P$ <0.01) decrease in the St.I group compared with that in the control (Fig. 3C), indicating a significantly declining cognitive memory only in the St.I group. This is while significant enhancements ( $P$ <0.05 and  $P$ <0.001, respectively) were observed in the NOR test D2 results of the St.I-C30 and St.I-C60 groups as compared with that recorded by St.I (Fig. 3C). Clearly then, cognitive memory improved independently of the crocin

treatment although spatial memory under a crocin dosage of 60mg/kg experienced a greater enhancement in the St.I-C60 group than in the control.

The NOR auxiliary discrimination index (D1) showed a significant ( $P<0.05$ ) enhancement in St.I-C60 when compared with that in the St.I group (Fig. 3D). Thus, D1 values indicate that crocin (60mg/kg) led to more time being spent by the rats to explore the new rather than the familiar objects, indicating their improved performance in the NOR test.

#### Assessment of serum corticosterone levels

An ANOVA applied a significant difference level to serum corticosterone levels:  $F(4, 33)=6.306$ ,  $P<0.01$ . Compared to the control, the St.I and St.I-C30 groups exhibited significant ( $P<0.001$  and  $P<0.05$ , respectively) increases in their serum CORT levels (Fig. 4). As shown in Figure 4, CORT levels significantly ( $P<0.05$  and  $P<0.01$ , respectively) decreased in St.I-C30 and St.I-C60 groups when compared with that in the St.I group, indicating the protective effect of crocin on serum CORT levels as the main stress hormone in the stressed rats.

## Discussion

The effects of different doses of crocin were investigated on locomotor activity as well as exploration times of novel location and novel object. Spatial and cognitive memories were also examined in the rats subjected to chronic isolation stress using the OLT and NOR tests, respectively. The novelty of the present study lied in the stress type (chronic isolation stress) applied whose effects were evaluated using the two behavioral NOR and OLT tests in the absence and presence of crocin treatment (30 and 60 mg/kg) on changes in spatial and cognitive memories. Other types of stress, different crocin dosages and other behavioral tests such as Morris water maze had been employed in previous studies (Ghadrdoost et al., 2011). Present OLT findings showed that chronic isolation stress greatly reduced locomotor activity, novel location recognition and spatial memory (Fig. 2). These findings are in agreement with those of other studies that reported chronic stress impaired memory as revealed by a variety of spatial memory tasks such as the Morris water (Eidelkhani et al., 2015; Venero et al., 2002),

radial-arm (Atsak et al., 2016) and Y mazes (Hao et al., 2014). One study also reported that isolation stress was able to increase fear or anxiety in animals (Weiss et al., 2004). In contrast to the present findings, isolation stress was reportedly associated with behavioral deficits so that the affected rats were found to be less adaptive as evidenced by their more conservative movement patterns (Varty et al., 2000). The present NOR data indicated significant impairments in locomotor activity, novel object recognition and cognitive memory as a result of chronic isolation stress. Previous studies demonstrated that exposure to other types of chronic stress destroyed both cognitive memory and object recognition (Maeng and Shors, 2013; Oei et al., 2006; Ranjbar et al., 2016; Simoens et al., 2007). Only one study, however, showed that isolation stress gave rise to exploratory behavior (Varty et al., 2000). Reports have also indicated that stress might impair and/or enhance memory in both humans and rodents (Diamond et al., 2007; Howland and Wang, 2008; Wolf, 2009). The inconsistencies observed among the results reported in different studies are further augmented by those that demonstrated isolation stress either had no effects on locomotor activity (decreased resting time) or increased it (Levine et al., 2007; Ranjbar et al., 2017; Shao et al., 2015). It is interesting to note that the complex effects of stress on the results of behavioral tests depend on such varied factors as the behavioral task employed, stressor type, stress time and duration, sex, as well as age (Diamond et al., 2007; Ennaceur, 2010; Joëls et al., 2006; Sandi and Pinelo-Nava, 2007; Wolf, 2008). Moreover, the present data indicate greatly increased CORT levels in the chronic isolation stress groups. It may, thus, be claimed that memory deficits are mediated mainly via elevated CORT levels and neurochemical factors (Radahmadi et al., 2015; Sato et al., 2010). This is further supported by previous studies that showed stress hormones activated memory impairment mechanisms (Aziriova et al., 2014; Li et al., 2008).

Based on the current results, crocin improved locomotor activity and stress-induced memory impairments as evidenced by the increasing total object exploration time and the values obtained for the discrimination indexes in the OLT and NOR tests, respectively. Moreover, both crocin dosages (30 and 60 mg/kg) equally improved locomotor activity in the

OLT test (Fig. 2A) whereas the higher crocin dosage of 60mg/kg yielded better NOR test results than did the 30mg/kg dosage (Fig. 3A). It seems that although the improvements were observed in locomotor activity in both the OLT and NOR tests in the stressed groups, the control group recorded a greater increase in this parameter, which might have been due to the relaxation due to the decreased movement as a result of crocin treatment (Hosseinzadeh and Noraei, 2009). On the other hand, previous studies reported inconsistent results in that some reported saffron extract and its components such as crocin to increase and others observed them to decrease locomotor activity while still others reported no effect (Amin et al., 2015; Karami et al., 2013). It seems that saffron extract changed locomotor activity in normal animals in a dose-dependent manner (Hosseinzadeh and Noraei, 2009). High doses of saffron and crocin (>400mg/kg of crocin) have been found to have reduced effects on locomotor activity (Karami et al., 2013). This is while locomotor activity has been observed to improve at low doses of crocin (about 100-200mg/kg) (Karami et al., 2013). In the present study, locomotor activity improved at a crocin dosage of 30mg/kg and particularly was enhanced with a crocin dosage of 60mg/kg, similar to what has been achieved under 100-200mg/kg. It, therefore, seems that crocin serves as a stimulator (at its low dosages) and/or an inhibitor (at very high doses) on locomotor activity. Hence, different neurotransmitters might be involved at different crocin doses (Anwar et al., 2017; Hosseinzadeh and Sadeghnia, 2007). However, some specific mechanisms might be involved in the observed inhibitory stress response of crocin. Previous study has shown that some saffron constituents such as crocin might inhibit HPA activity to reduce adrenocorticotropin hormone and corticosterone secretion in stressed animals by inhibiting NMDA glutamatergic and/or sigma opioid receptors located on the adrenal cortex (Halataei et al., 2011; Iyengar et al., 1990; Lechtenberg et al., 2008). Also, crocin might influence the release of such brain neurotransmitters as dopamine, norepinephrine, serotonin, acetyl choline and GABA (Anwar et al., 2017; Hosseinzadeh and Sadeghnia, 2007).

A crocin dosage of 60mg/kg was observed in the current study to improve both spatial and cognitive memories. A dosage of 30mg/kg, however, enhanced only the cognitive memory in isolation stressed rats.

Hence, it seems that administration of 60mg/kg of crocin might well abolish the deleterious effects of chronic isolation stress. Similar effects of crocin on cognitive memory under stress conditions have been reported elsewhere (Ghadrdoost et al., 2011). In general, study has shown that crocin acts on different memories under different conditions. Despite the fact that various mechanisms seem to be involved in memory improvement, the significantly decreased CORT levels observed in the crocin treatment (especially the one with 60mg/kg of crocin) of stressed rats in the present study suggest crocin to be involved in the mechanisms responsible for improving spatial and cognitive memories. The interaction of the HPA axis and cognitive memory has been reported to be a possible mechanism mediating the protective effects of crocin (Ghadrdoost et al., 2011).

Previous study has also shown, crocin affect memory functions particularly in the hippocampus (as the main memory region) in stressed rats (Ghadrdoost et al., 2011) largely due to the anti-oxidant activities of crocin (Ahmad et al., 2005; Ochiai et al., 2007; Zheng et al., 2007). Evidence has been indicated the neuroprotective activity of crocin in various experimental models of brain disorders (Ahmad et al., 2005; Zheng et al., 2007), through which cells are protected against oxidative stress (Abe and Saito, 2000). Thus, different mechanisms may be proposed for the beneficial effects of crocin such as its free radical scavenging (Abe and Saito, 2000), enhancement of anti-oxidants (Abe and Saito, 2000; Asdaq and Inamdar, 2010; Bickford et al., 2000; Ochiai et al., 2004; Papandreou et al., 2006), modulation of NMDA receptor functions (Abe and Saito, 2000), enhancement of anti-oxidants (Abe and Saito, 2000; Bickford et al., 2000), hormonal changes and changes in neurotransmitters such as dopamine and glutamate (Ettehad et al., 2013).

## Conclusion

The present study demonstrated the severe impairment of spatial and cognitive memories as a result of chronic isolation stress. Crocin, especially at high dosage, was found to have better protective effects on memory deficits induced by chronic isolation stress. Finally, further investigation are required to determine the mechanism(s) contributing

to the effects of crocin on spatial and cognitive memories under chronic isolation stress.

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## Conflict of interest

There are no conflicts of interest that have been reported by the authors or by any individuals in control of the content of this article.

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