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Original Article

Saffron (Crocus sativus) aqueous extract reverses the hypothalamus-pituitary-adrenal axis activity in rat model of post-traumatic stress disorder

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ABSTRACT

Crocus sativus L., Iridaceae, has been used worldwide in traditional medicinefor treatment ofsome neurological disorderssuch as depression. Post-traumatic stress disorder is a mental disorder developed in peoplewho experience stressful events. Since stress has been proposed tocause thehypothalamic-pituitary-adrenal axis malfunction in post-traumatic stress disorder patients, this study aimed at investigating the effect of saffron aqueous extract on hypothalamic-pituitary-adrenal axis activity in rats of post-traumatic stress disorder model. Here, Post-traumatic stress disorder animals received an acute electro foot shock; however, 5 min before the stress session, these animals received an intra-cerebral-ventricular (10 μg/rat) infusion of either saffron aqueous extract or saline. Twenty one days later, they were re-exposedto the stress box withoutinducing stress, andthen were examined for their freezing behavior. The impact of stress and saffron aqueous extract on serum corticosterone, corticotrophin releasing hormone gene expression in hypothalamus and glucocorticoid receptor gene expression in pituitary gland werethen evaluated on day 28. Intra-cerebral-ventricular injection of saffron aqueous extract resulted in an increase in serum corticosterone level and reduced symptoms of freezing behavior, and corticotrophin releasing hormone and glucocorticoid receptor gene expression in post-traumatic stress disorder groups.Saffron administration could improve the symptoms of stress-induced post-traumatic stress disorder, possiblythrough the adjustment ofhypothalamic-pituitary-adrenal axis function.

Introduction

Post-Traumatic Stress Disorder (PTSD) is a chronic psychiatric disorder triggered by a traumatic and/orlife-threatening event. If most people experience at least one traumatic event in their life span, they can easily develop PTSD in the community (Breslau, 2009). According to Diagnostic and Statistical Manual of mental Disorders-Text Revision (DSM-V), PTSD is a kind of weakening stress associated with neurological disorders (Breslau et al., 1998). Patients with PTSD consistently respond to their traumatic experiences and injuries with fear, panic and frustration Besides that,they might show some psychological symptoms such as flashback of memories, nightmares, changes in the mood andbehavior, changes in the stimulation threshold, or avoidance of theplaces or people reminding them of those inconvenient events (Kupfer et al., 2013). Notably, some different reports revealed that 8% of the population in the future will develop PTSD, because of social problems and violence (Kessler, 2000), as PTSD is usually associated with a major challenge in the society. Many studies have focused on the use of some drugs such as antidepressants, anti-anxiety, β-adrenergic antagonist, opium, and cortisol for individual and group treatments of this disorder (Kar, 2011). The hypothalamic-pituitary-adrenal (HPA) axis, including stress hormones such as corticotrophin releasing hormone (CRH), ACTH and primarily cortisol,isthe most important hormonal response system to stress.Theactivation of the HPA axis leads tothe release of CRH from the hypothalamic periventricular nucleus to the pituitary portal circulation along withthe stimulation of ACTH secretion, and glucocorticoids from the adrenal cortex, which are essential for homeostasis and stress adaptation (Jacobson, 2005). Dysregulation of the HPA axis andincrease in

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the noradrenergic activity, in the central andperipheral areare-spectively, are commonly observed in patients with PTSD. PTSD is also determined by the impairment of neuroendocrine system that results in thediminished concentration of blood corticosteroid level and increased negative feedback of HPA axis (Yehuda, 2002). Moreover, neuroendocrinologic studies have reported the increased inhibition of the hypophysis-adrenal system (HAS), which leads to the reduction in the cortisol levels by enhancing negative feedback in PTSD patients (Carvalho et al., 2008). In addition to HPA, CRH is also an important hormone in the brainneuroendocrine system for responding to stress, which modifies behaviors and the automated body position. In fact, CRH initiates and coordinates the components of stress response (Vale et al., 2013). Hence, the main part of the change in the HPA axis is probably associated with high CRH production.Moreover,studies showed that glucocorticoid receptor (GR) plays a major role in modulating the effects of glucocorticoid negative feedback responses following stress.In fact, itplaysan intermediary role in HPA axis regulation under stress (De Kloet et al., 1998).

Crocus sativus L., Iridaceae, stigma is commonly known as saffron. This perennial stemless herb of the Iridaceae family is widely cultivated and harvested in different countries such as Iran, India, China, Spain, Italy and Greece.Commercial saffron contains red stigma with a small portion of the yellowish style that is added to the food because of its color and taste.The cultivation of saffron has been also common in Iran since ancient times, when saffron was used to prepare different nourishing and energizing drinks, and served with cinnamon and cardamom (Asalgoo et al., 2015, Gohari et al., 2013). Saffron has been also extensively used as a healthy additive to give flavor, color, scent and aroma to Iranian foodincluding many traditional foods served with rice, *e.g.* chlokabab. It is also used as the main foodstuff in preparing some other traditional desserts and confections called Sholezard, Zoolbia and Halva (Moshiri et al., 2006).

According to the photochemical research, its color is mainly because of the carotenoid substances such as crocin and crocetin. Moreover, its flavor comes from carotenoid oxidation products especially safranal (Gohari et al., 2013). Saffron is also used in traditional medicine to deal with a wide range of indicators including cramps, asthma and depression (Berger et al., 2011). Moreover, in modern pharmacological studies, saffron or its active constituents are used as anti-convulsant, anti-depressantand anti-tumor drug (Gohari et al., 2013) and improves some disorders related to memory and learning (Hadipour et al., 2018).

The aim of this study was to evaluate the effects of saffron aqueous extract on the function of HPA axis in the rat model of PTSD. We used an electric foot shock system to create a model of PTSD in rat, which is used in other similar studies as well (Asalgoo et al., 2017, Hashtjini et al., 2018). To follow the aim of the study, the effects of ICV injection of saffron aqueous extract was evaluated through the measurement of the quantitative expression of CRH and GR genes, which have a fundamental role in the HPA pathway, and the serum levels of corticosterone as well asthe assessment of the behavioral symptoms' improvement in rat model of PTSD.

Material and methods

Plant material and extract preparation

Saffron (stigmas of *Crocus sativus* L., Iridaceae) was provided by Talakaran-E- Mazraeh Company (Torbat Heydarieh, Khorasan, Iran) and recognized by Mr. Mohammad Kamalinejad, from the Department of Pharmacology, Shahid Beheshti University of Medical Sciences, Tehran, Iran. It was registered as a voucher specimen (code: P-408) in the Department of Pharmacognosy, the Faculty of Pharmacy, Shahid Beheshti University of Medical Sciences. The extract was obtained as follows: 100 g of saffron stigmas were dried, milled, and, then mixed with 1000 ml of distilled water. After that, it was kept for 48 h at 30-33 °C in order to get dried. The yield of extraction was 24 g of freeze-dried powder for 100 g of the dry stigma. Extract was then dissolved in saline, and immediately injected into the left lateral ventricle of the rats' brain. Considering the previous studies, different doses of aqueous extract of saffron showed the same effect, so 10 μg/rat of the extract was chosen for ICV injection in this study (Sahraei et al., 2012a, 2012b). However, median lethal doses (LD50) of *C. sativus* have been reported 200 mg/ml and 20.7 g/kg *in vitro* and animal studies respectively (Khazdair et al., 2015).

Measurement of crocin in saffron extract

The measurement of crocin in aqueous extract of saffron is carried out according to the method which is described previously (Hosseinzadeh and Noraei, 2009, Mokhtari Hashtjini et al., 2018). Briefly, saffron extract was filtered through a 0.2 μm Millipore filter (Millipore, Bedford, MA, USA) and eluted with methanol. This quantification was done bya Shimadzu HPLC LC-10ADvp system integrated witha Shimadzu SCL-10Avp system controller and a SPD-10Avp UV-visible spectrophotometric on a reversed phase Shim-pak C18, VP-ODS analytical column (25 cm × 4.6 mm I.D. with a 12.0 ± 1.0 nm pore size and 4.6 ± 0.3 μm particle size), using an isocratic mobile phase of acetonitrile:water (76:24%) at a flow rate of 1.2 ml/min. By Rheodyne Shimadzu Model 7725i injector, 25 μl of the sample was injected onto the column. All data were recorded and analyzed on a chromatography workstation Shimadzu Class-VP™ 6.10 software (Hosseinzadeh & Noraei, 2009).

Animals

The present study was approved by the ethics committee of Baqiyatallah University of Medical Sciences, Tehran, Iran (ID: IR.BMSU.REC.1395.380). In this study, male Wistar rats (weighing from 220 to 250 g) were used from the Pasteur Institute, Tehran, Iran. The animals were housed in the cages (n = 4/cage) under the standard conditions (22 ± 2 °C temperature and alternate 12/12 h dark/light cycle) for adaptation to the laboratory conditions. They were kept for 28 days in the laboratory and tests were carried out during daylight under the standard conditions.The animals were freely provided with food and water except for the time of testing. We randomly divided them into two categories namely receiving stress (PTSD) and without stress (non-PTSD). The non-PTSD group included three groups as without manipulation group (negative control ($Ctrl^-$)), surgery group without the insertion of cannula (Sham) and drug control with receiving saffron extract. The PTSD group included three groups as with Stress exposure only (Positive control ($Ctrl^+$)), Stress exposure, cannulation surgery and, then, treated with saffron (Treat-Saffron), and finally Stress exposure, cannulation surgery and treated with normal saline injection (PTSD-saline). Rats were evaluated after giving them 7 days for surgical recovery (Fig. 1).

Surgery and creation of shock

Surgery method

During surgery,the rats were anesthetized with ketamine hydrochloride (70 mg kg^{-1}, *i.p.*) and xylazine (10 mg kg^{-1}, *i.p.*). Then, a stainless steel cannula (23-gauge) was attached to stereotaxic apparatus (Stolting Instruments, USA). The exact location of the cannula was determined according to Paxinosand Watson's Atlas (Paxinos and Watson, 2006). Unilateral cannulation was implanted in the left lateral ventricle of the brain according to the follow-

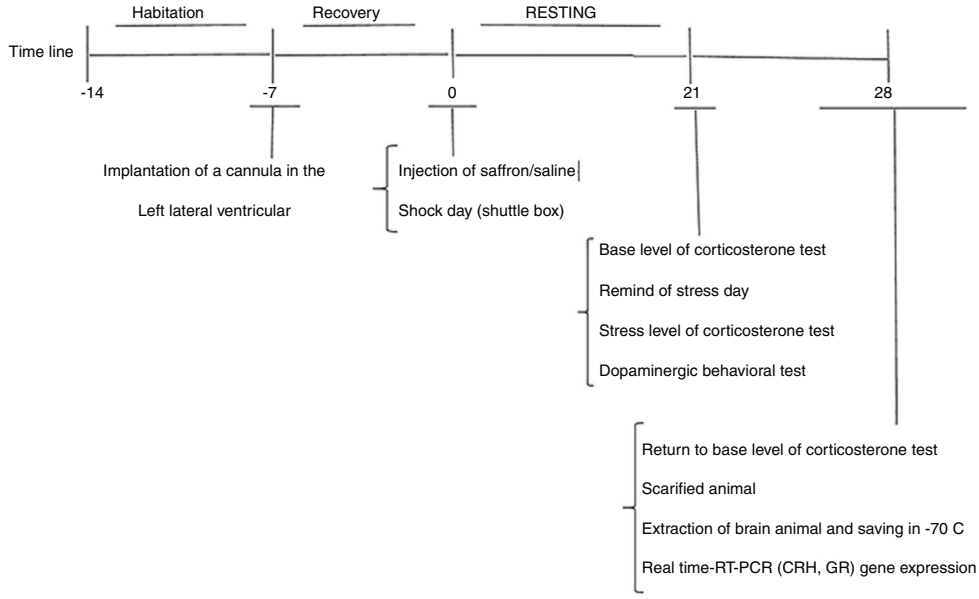

Figure 1. Protocol time line.Evaluation of rats was performed after post-surgery recovery (7 days) of the electrode implantation in the left lateral ventricle of the brain. In the first day (day 0 cited in Figure 1) after recovery, 5 min before giving the electric foot shocks, saffron extract (10 μg/rat) was gently injected during 60 s. On day 21 as cited in Figure 1 all of groups undertook the freezing behavior test in the re-exposure time and blood sampling from orbital sinus as will be mentioned later was collected. Next day (day 28 cited in Figure 1), ultimately, blood sampling from orbital sinus was collected and all animals were sacrificed fast enough for avoiding any problem in brain sampling.

ing stereotaxic adjustments: –0.9 mm posterior to the bregma, + 1. 5 mm lateral to the sagittal suture, -3.5 mmfrom dura matter of upper cranium and –3.3 mm from incisor bar location.The injection cannula was fixedon the animal's skull using dental acrylic.A 7-day period was considered for full recovery from the surgery and anesthesia. For injection, animals were gently restrained by hand and Hamilton needle was then inserted into the cannula through apolyethylene connector. Five minutes before giving the electric foot shocks, saffron extract (10 μg/rat) was gently injected during 60 s. Trepan blue was used to confirm the location of the cannulation.

Creation of a rat model of PTSD by electric foot shock

Electric foot shock was applied once during one day. All animals were placed in the stress box and randomly divided into two groups of non-PTSD without receiving any shock and PTSD with shock exposure. They were then transported from the laboratory to the experiment room 1 h prior tothe exam for the environment adaptation. We used a similar inescapable shocks paradigm as described by Mikics et al. (2008). The electric shock box was made of the transparent Plexiglas (16 × 16 × 54 cm) manufactured by Borj-e-Sanat Co., Tehran, Iran, which contains nine separate partitions. Chamber floor wascovered with the stainless steel rods (4 mm in diameter) and connected to a power generator. The rats received electric shocks (1 mA, 1s duration) applied through the cage floor at intervals of 30 s; the entire session lasted for 5 min. After removing all animals, including those that received shock (PTSD) and those that were only placed in the box without any shock exposure (non-PTSD), from the electric shock box, they were returned and kept in their cagesfor 21 days without any shock re-exposure.

Freezing behavior

After a 21-day intervalwithout shock exposure, all animals were again placed in the stress box for 5 min without receiving any shocksin order to remind them of the previous stresscondition. Animals' locomotors activity was recorded by a digital camera in 5 min.

Evaluation of corticosteroneconcentration in three Levels

The serum corticosterone concentration was measured by ELISA kit at basal, stress and return toward basal level in all groups. Blood samples were obtained from retro-orbital sinus (2 ml)between midday and two o' clock before stress reminder (basal level), after stress reminder (stress level) and, finally, on day 28 which was considered as return toward basal level. Blood samples were used to separate the supernatant serum by centrifugation at 805 × *g*, 4° C for 8 min. Then, the concentration of corticosterone was measured by CorticosteroneELISA kit (DRG) (Cat. Nr EIA 4164) and was read by Eliza reader system at 450 nm absorption.

Real-time RT-PCR method

All rats were sacrificed at the end of the experiments and the whole brain tissue was immediately removed. Hypothalamus and pituitary were carefully dissected according to anatomical atlases and Paxinos and Watson Atlas (Paxinos and Watson, 2006) and transferred to nitrogen tank. After that, they were placed in an ultra-cold freezer (-80° C). Total RNA was extracted from hypothalamus and pituitary glands of the rats using RNA purification kit (Gene All® Hybrid-R™). Purified RNA concentration was measured using a NanoDrop spectrophotometer at 260/280 ratio which showed 2.2. Primers were designed as follows using three software including CLC Sequence Viewer 6, Oligo 7, and GENRUNR:β-actin (NM_007393); CRH (NM_205769.3); GR (NM_DQ504162) (Table 1). Hyper Script™ reverse transcriptase kit (GeneAll®) was used for cDNA synthesis. The expressions of CRH inhypothalamus and GR in pituitary were evaluated using ABI7500 Real Time System (Applied Biosystem), and composition of Real Q plus 2x Master Mix Green, Low Rox™ (Ampliqon) kit (Cat No: A324402). Terms of timing cycles are as follows: 15 min at 95 °C, followed by 20 s of 40 cycles at 95 °Cand 60 s at 60 °C. The analysis of real time RT- PCR products in ABI7500 system was done at theend of each 60 °C cycle (the amplification of each gene was assessed by melting curve and viewing ongel electrophoresis). The $2^{-\Delta\Delta CT}$ was used for the quantitative analysis of gene expression (Winer et al., 1999) (Table 1).

Table 1
The forward and reverse sequences of primers designed for β-actin, CRH, GR.

Reference	Primer	Length of sequence (bp)	Target gene
The current study	R: GAACCGCTCGTTGCCAATAG F: GGGAAATCGTGCGTGACATC	β-actin	149bp
(Chen et al., 2012)	F:CTCTCTGGATCTCACCTTCCAC R:CTAAATGCAGAATCGTTTTGGC	CRH	150bp
The current study	F1: GTGATGGGGAATGACTTGG R1:GGAAGAAAGCATTGCAAACC	GR	98bp

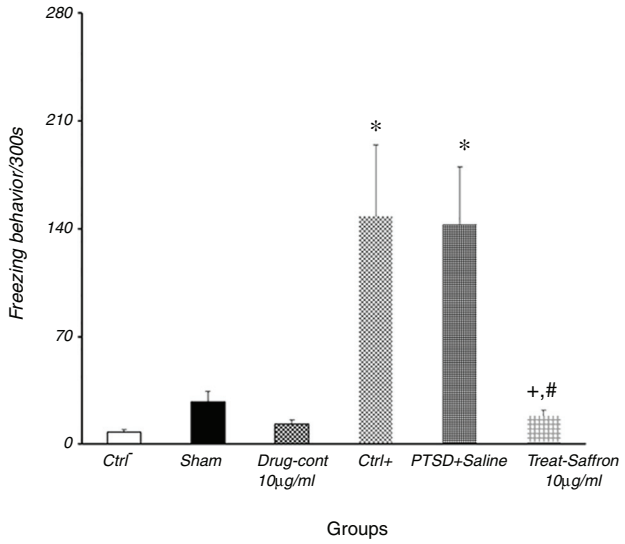

Figure 2. Changes in the freezing time; the exploratory behavior was measured on day 21, post-shock, in six groups at the time of re-exposure. *p< 0.05 was significantly different from the Ctrl⁻ group; ?p<0.05, #p< 0.05 was significantly different from the Ctrl⁺ and PTSD-saline groups.

2.7.Statistical analysis

The Statistical Package for the Social Sciences (SPSS, version 19.0) was used for data analysis. Quantitative findings were expressed as mean ± standard error of mean (SEM). The non-parametric analysis (Kruskal-Wallis and Mann Whitney test) was also used to evaluate corticosterone serum, freezing behavior and CRH and GR gene expression. p< 0.05 was considered as significant.

Results

The measurement of crocin in saffron extract

Our results showed that the aqueous extract of saffron contains 22.5% of crocin. Extract doses used in these tests are standardized based on their crocin content.

The effect of saffron aqueous extract on freezing behavior

The results indicated that freezing behavior significantly increased in the Ctrl⁺ group (147.71 ± 46.44) and PTSD-saline group (142.85 ± 37.41) compared to the Ctrl⁻ group (7.66 ± 1.85) (p <0.01 and p <0.005, respectively) (Fig. 2). After the administration of saffron aqueous extract by ICV, 5 min prior to the shock, freezing behavior significantly decreased in the Treat-Saffron group (18. 42 ± 3.8) comparing with the Ctrl⁺ (p < 0.05) and PTSD-saline (p < 0.005) groups. Although the freezing was decreased in the treat-saffron group, there existed a significant difference with the Ctrl⁻ group yet (p < 0.05).

The effect of saffron aqueous extract on serum corticosterone

PTSD caused a sharp decline in serum concentration of corticosterone at three levels (basal, stress and return toward basal). The serum concentration of corticosterone at the basal level in the Ctrl⁺ group (28.20 ± 1.90) and PTSD-saline group (27.25 ± 2.13) significantly decreased compared to the Ctrl⁻ group (82.33 ± 6.93, p <0.05). In addition, the serum concentration of corticosterone at the stress level in the Ctrl⁺ group (36.20 ± 3.26) and PTSD-saline group (34.50 ± 1.75) significantly decreased compared to the Ctrl⁻ group (85.33 ± 9.24, p <0.05). Finally, at the return toward basal level in the Ctrl⁺ group (41.20 ± 3.15) and PTSD-saline group (38.25 ± 3.90), a significant decrease in serum concentration of corticosterone was observed compared to the Ctrl⁻ group (78.00 ± 6.11) (p <0.05, p <0.05, respectively) (Fig. 3). Notably, the concentrations of corticosterone at three levels increased in the treatment group, while the comparison with the Ctrl⁻ group showed no significant changes. Instead, a significant increase was observed in the concentration of corticosterone in the treatment group at basal level (98.25 ± 7.02) compared to the Ctrl⁺ and PTSD + saline groups (p <0.05, p <0.05, respectively), at stress level (111 ± 12.35) compared to the Ctrl⁺ and PTSD + saline groups (p <0.05, p <0.05, respectively), and at return toward basal level (96.75 ± 3.59) compared to the Ctrl⁺ and PTSD + saline groups (p <0.05, p <0.05 respectively).

The effect of saffron aqueous extract onCRH gene expression

CRH gene expression level was examined 28 days after the electric shock by Real-Time RT-PCR. According to the results, the acute stress exposure increased CRH gene expression in the Ctrl⁺ group (6.06 ± 1.61) and PTSD-saline group (5.73 ± .93) compared to the Ctrl⁻ group (1.09 ± 0.36) (p <0.05), while no significant changes in CRH gene expression was found in the treat-saffron group (0.87 ± 0.32) compared to the Ctrl⁻ group. On the other hand, results indicated a significant decrease in the CRH gene expression in the treat-saffron group (0.87 ± 0.32) compared to the Ctrl⁺ and PTSD + saline groups (p <0.05) (Fig. 4).

The effect of saffron aqueous extract onGR gene expression

Twenty eight days after receiving shock, GR gene expression in pituitary was evaluated using real-time techniques. Results of the current study showed that the acute stress exposure increased GR gene expression in the Ctrl⁺ group (4.69 ± 1.07) and PTSD + saline group (4.58 ± 0.65) compared to the Ctrl⁻ group (0.88 ± 0.13) (p <0.05), while no significant changes in GR gene expression was found in the Treat-Saffron group (0.89 ± 0.06) compared to the Ctrl⁻ group. Instead, a significant decrease in the GR gene expression was observed in thetreat-saffron group (0.89 ± 0.06) compared to the Ctrl⁺ and PTSD + saline groups (p <0.05) (Fig. 4).

Discussion

In the present study, we used electric foot shock in order to create an animal model of PTSD, assimilar studies (Mokhtari Hashtjini et al., 2018). Twenty one days after receiving shock, the

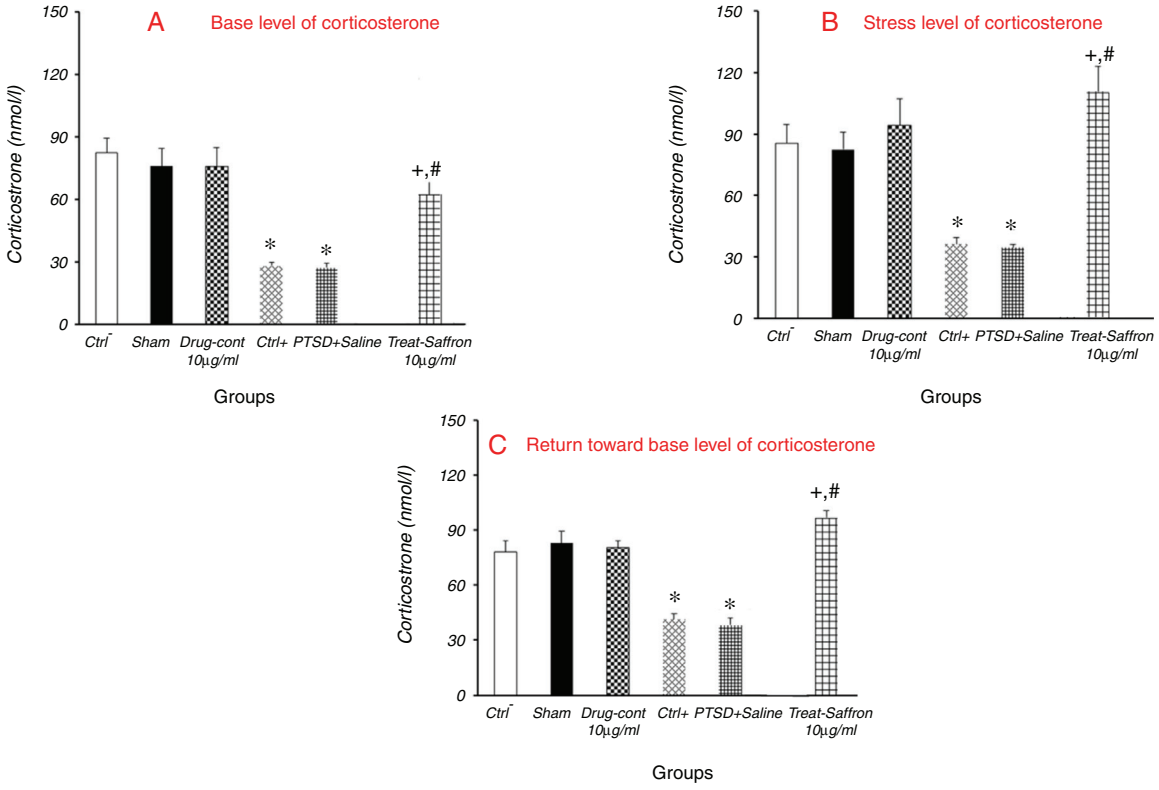

Figure 3. Evaluation of the corticosterone serum levelatthree stages (basal, stress, return toward basal level) A. Evaluating the basal level of serum corticosterone, before re-exposure time in the groups; a significant increase in the treat-saffron group compared with Ctrl$^+$ and PTSD-saline groups (^+p <0.05, $^\#p$ < 0.05); a significant decrease in the Ctrl$^+$ and PTSD-saline groups, compared with Ctrl$^-$ group ($^*p$ < 0.05);B. Evaluating post-shock, stress-induced corticosterone level in the groups; a significant increase in the Treat-saffron group, compared with the Ctrl$^+$ and PTSD-saline groups (^+p < 0.05, $^\#p$ < 0.05); a significant decrease in the Ctrl$^+$ and PTSD-saline groups compared with the Ctrl$^-$ group ($^*p$ < 0.05); C.Evaluating post-shock, return toward basal level of corticosterone in the groups; a significant increase in thetreat-saffron group compared with the Ctrl$^+$ (^+p < 0.05) and PTSD-saline ($^\#p$ < 0.05) groups; a significant decrease in the Ctrl$^+$ and PTSD-salinegroups compared with the Ctrl$^-$ group ($^*p$ < 0.05).

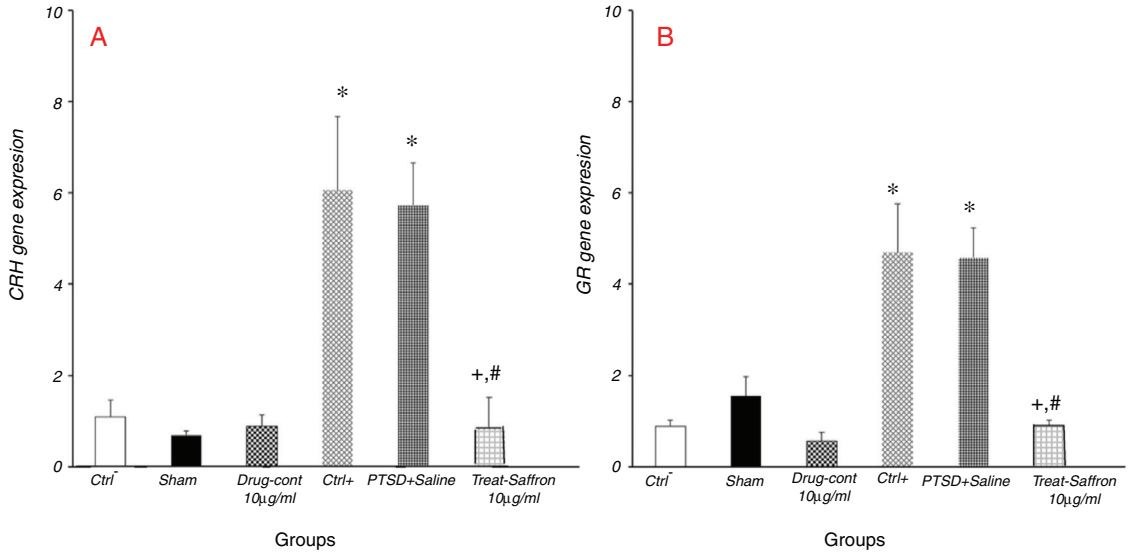

Figure 4. The level of genes expression was measured by Real-Time RT-PCR, on day 28, post-shock; A. The post-shock hypothalamic CRH expression in the groups, $^*p$ < 0.05shows a significant increasecompared tothe Ctrl$^-$ group, ^+p < 0.05,$^\#p$ < 0.05shows a significant decrease compared to the Ctrl$^+$ and PTSD-saline groups respectively;B. The expression of GR gene in pituitary after the re-exposure time in the groups, $^*p$ < 0.05 shows a significant different from the Ctrl$^-$ group, ^+p < 0.05, $^\#p$ < 0.05 shows a significant decrease compared to theCtrl$^+$ and PTSD-saline groups, respectively.

decreased level of serum corticosterone, increased freezing symptoms, increased CRH gene expression in hypothalamus and GR gene expression in pituitary gland were detected in the PTSD rats. Our results suggest that ICV injection of saffron aqueous extract reduces gene expression of CRH and GR, while increases serum corticosterone. These results could be considered as the cause of PTSD symptoms' improvement *e.g.* freezing behavior. In line with our results, it has been already shown thatthe intraperitoneal administration of ethanol extract of saffron could inhibit PTSD in rats (Sahraei et al., 2012b); however, the cellular and molecular

mechanisms of saffron extract have not yet been investigated in the previous studies. Here, we observed an increase in the expression of CRH gene in animal models of PTSD, while a significant reduction of CRH gene expression was observed in the group treated with saffron aqueous extract. These findings indicated that HPA axis malfunction occurred in PTSD may be at least due to the over expression of the CRH and GR genes in the hypothalamus and pituitary gland respectively. In addition, our findings indicated that the therapeutic effect of saffron aqueous extract on PTSD might be made through the alteration in HPA axis activity in order to reduce CRH and GR gene expression in hypothalamus and pituitary gland.

The exact mechanism(s) by which the saffron extract inhibits-the PTSD initiation is not clear. However, the HPA axis is shown to be responsible for increasing the secretion of CRH from PVN of hypothalamus, following by the increased secretion of ACTH and corticosterone from anterior pituitary and adrenal cortex in stressful events respectively (Dunn and Swiergiel, 2008). It is demonstrated that CRH is secreted in other regions of brain such as the hippocampus and amygdale in which acts as a neurotransmitter in response to tensional behaviors and autonomic stress (Chen et al., 2012).

According to the previous studies, it has been shown that the glutamate system plays an important role in the regulation of CRH excretion from hypothalamus neurons (Yuen et al., 2012). It is also demonstrated that glutamate innervations of PVN can be affected by neuroplasticity changes in chronic stress conditions which results in the desensitization of HPA axis response (Popoli et al., 2012). Other studies have reported the inhibitory effects of saffron components on NMDA glutamate receptor function (Lechtenberg et al., 2008a). Given the results of the previous studies,it can be concluded that saffron aqueous extract probably regulates the secretion of the hypothalamic CRH through a glutamate-dependent mechanism (Ettehadi et al., 2013, Lechtenberg et al., 2008a), so that one can come to the conclusion that saffron aqueous extract decreases the expression of CRH gene in the HPA axis by inhibiting the glutamate receptors in the treated group. Our results showed an increased expression of GR gene in the animal models of PTSD, while PTSD group treated with saffron aqueous extract showed a significant reduction of GR gene expression in the pituitary gland. Investigations revealed that the negative feedback responsible for the secretion of glucocorticoid, which inhibits GR activity in pituitary, hypothalamus, hippocampus, and amygdale, may not happen in some degrees based on the severity and type of the perceived stress; and, consequently, serum glucocorticoid hormones levels remain high (McEwen et al., 1992). It seems that the negative feedback mechanism in the HPA axis is not available in patients with PTSD. Therefore, the patients demonstrate a decreased level of cortisol in serum and urine (Yehuda et al., 2005). Similarly, our results showed a significant decrease in serum corticosterone in PTSD rats. Interestingly, it seems that, following the saffron extract administration and possible glutamate receptor antagonism (Lechtenberg et al., 2008b), this HPA dysregulationis inhibited, and, as a result,the decreased amount of CRH gene expression and increased levels of corticosterone secretion are then observed.

The current study also investigated the dopamine-related behavior in the animals of PTSD model. According to the results, increased freezing behavior is evident in PTSD group whereas it decreases in saffron treated group. As demonstrated by Howes and his colleagues, freezing behavior control is performed by dorsal striatum dopamine system (Howes et al., 2017). On the other hand, some studies showed that the freezing behavior is caused by stress-induced fear in the animal and the amygdala is involved in mediating fear in animals (McEwen, 2009). Besides that, it has been revealed that one of the most important signs of fear in rodents is freezing, which is due to the strong inhibitory effect of the amygdala on the dorsal striatum (Hooshmandi et al., 2011). It seems that, in our experiment, aqueous extract of saffron had an effect on the amygdala modulation performance as well. From the cellar and molecular view, it is shown that glutamate receptors in the meso-limbic system plays an important role in inducing dopamine-dependent behaviors (Howes et al., 2017). On the other hand, it is demonstrated that at least some constituents of the saffron aqueous extract can occupy the NMDA glutamate receptors in the central nervous system (CNS) (Lechtenberg et al., 2008a). In addition, Mojabi and collegues (Mojabi et al., 2017) have also shown that interaperitoneal injection of saffron water extract in the rats can increase glutamate concentration in the brain as well. These results may suggest the possible involvement of the brain glutamate system and its NMDA receptors in the mediation of saffron water extract. Also, studies have found that suppressing dopamine receptors by the D_2 dopamine receptor antagonist-sulpiride- can suppress dopamine-related behaviors, suggesting a close relationship between both glutamatergic and dopaminergic pathways in this regard (Tzschentke, 2007). Given the mentioned arguments about the inhibitory effect of saffron extract on NMDA receptor glutamate, our study suggests that a mixed mechanism of glutamate system, possibly via its NMDA receptors and glucocorticoids, may exist which results in reducing the complications of PTSD with saffron aqueous extract. In order to support such hypothesis, the effect of saffron extract on NMDA glutamate receptors could be also investigated using more precise methods such as HPLC. In addition, regarding this section, further studies needto be done on these types of communication in different parts of brain,especially the amygdala and hippocampus,in more advanced models of PTSD disease.

Conclusion

The current study showed that saffron aqueous extract can exert beneficial effects on behavioral and molecular consequences of PTSD in the rat model of this disorder. According to the results, saffron aqueous extract can modify thestress-induced disrupted HPA axispossibly via relatedglutamatergic system.However, further investigates are needed to explain the exact mechanism of this amelioration.

Ethical Disclosures

Protection of human and animal subjects. The authors declare that the procedures followed were in accordance with the regulations of the relevant clinical research ethics committee and with those of the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

Author contributions

SA: experimental work; GPJ: design of the study, experimental work, drafting of the paper, major revisions and final approval for publication; BH: statistical analysis, scientific writing and final approval for publication; HS: design of the study and extract preparation; JRS: experimental work; SSS: design of the study; GHM: scientific writing and revision.

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Declaration of interest

Nothing to disclose.

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