Protective effects of saffron extract and its active constituent crocin against oxidative stress and spatial learning and memory deficits induced by chronic stress in rats

Behshid GhardLeod a, Abbas Ali Vafaei a,1, Ali Rashidy-Pour a,⁎, Razieh Hajisoltan a, Ahmad Reza Bandegi b,1, Fareshteh Motamed c, Saeed Haghighi d, Hamid Reza Sameni d, Sharzad Pahlvan d

a Laboratory of Learning and Memory, Research Center and Department of Physiology, School of Medicine, Semnan University of Medical Sciences, Semnan, Iran
b Laboratory of Endocrine Research, Department of Biochemistry, School of Medicine, Semnan University of Medical Sciences, Semnan, Iran
c Neuroscience Research Center, Shaheed Beheshti University of Medical Sciences, Tehran, Iran
d Department of Histology, School of Medicine, Semnan University of Medical Sciences, Semnan, Iran

ARTICLE INFO

Article history:
Received 11 December 2010
Received in revised form 21 April 2011
Accepted 3 May 2011
Available online 18 May 2011

Keywords:
Chronic stress
Spatial learning
Spatial memory
Saffron
Crocin
Oxidative stress

ABSTRACT

Although it is well established that chronic stress impairs spatial learning and memory, few studies have investigated possible ways to prevent its deleterious effects. Here, we investigated the effects of Crocus sativus L., commonly known as saffron, and its active constituent crocin on learning and memory loss and the induction of oxidative stress in the hippocampus by chronic stress. Rats were injected with saffron extract, crocin or vehicle over a period of 21 days while being exposed to chronic restraint stress (6 h/day). After this, they were trained and tested on a water-maze spatial memory task. They performed four trials per day for 5 consecutive days, and this was followed by a probe trial two days later. At the end of the behavioral testing, several parameters of oxidative stress in the hippocampus were measured. Treatment with saffron extract or crocin blocked the ability of chronic stress to impair spatial learning and memory retention. Relative to controls that received vehicle, stressed animals that received saffron extract or crocin had significantly higher levels of lipid peroxidation products, significantly higher activities of antioxidant enzymes including glutathione peroxidase, glutathione reductase and superoxide dismutase and significantly lower total antioxidant reactivity capacity. Finally, crocin significantly decreased plasma levels of corticosterone, as measured after the end of stress. These observations indicate that saffron and its active constituent crocin can prevent the impairment of learning and memory as well as the oxidative stress damage to the hippocampus induced by chronic stress. Thus, using these substances may be useful in pharmacological alleviation of cognitive deficits.

© 2011 Elsevier B.V. All rights reserved.

1. Introduction

Chronic stress has been reported to impair spatial learning and memory in a variety of spatial tasks. This effect is mediated mainly via the elevation of glucocorticoid levels (Conrad, 2010). It is well known that stress triggers the activation of the hypothalamus–pituitary–adrenal (HPA) axis, culminating in the production of glucocorticoids by the adrenals glands. Receptors for these steroids are expressed throughout the brain, and expression is particularly strong in brain structures involved in cognition and mental health, including the hippocampus (De Kloet et al., 1987; Reul and De Kloet, 1985). Glucocorticoids can have short and long-lasting effects on behavior and cognitive functions via genomic and non-genomic mechanisms (Haller et al., 2008; Lupien et al., 2009). In fact, chronic stress induces a series of morphological changes in the hippocampi of rats and primates. These alterations include retraction of the apical dendrites in the CA3 region of the hippocampus, modification of hippocampal dendritic spine number and shape, and cell death (Conrad et al., 2007; Kleen et al., 2006). These structural and functional changes of the hippocampus following chronic stress may contribute to the impairment of cognitive functions (McLaughlin et al., 2007). While stress-induced memory impairments have been extensively studied (Conrad, 2010), very few studies have examined possible ways of preventing the deleterious effects of stress.

Oxidative stress is caused by an imbalance between the production of reactive oxygen species and a biological system’s ability to detoxify the reactive intermediates or easily repair the resulting damage (Sies, 1997; Storz and Imlay, 1999). Oxidative stress is an important mechanism that may contribute to the cytotoxicity and impairment of learning and memory induced by chronic stress (Muriach et al., 2010; Palumbo et al., 2007; Sharma et al., 2009). Oxidative stress has been implicated in the pathophysiology of several neurodegenerative...
disorders characterized by progressive cognitive deficits (Coyle and Puttfarcken, 1993; Olanow, 1993); for example, chronic stress can induce dysfunction of the HPA axis in Alzheimer’s disease, resulting in increased glucocorticoid levels in serum (Swannick et al., 1998). Moreover, exposure to high levels of glucocorticoids or chronic stress may lead to oxidative injury of various tissues including the hippocampus, which may impair learning and memory functions (Behl et al., 1997; McIntosh et al., 1998a, 1998b; Sato et al., 2010; You et al., 2009). Also, glucocorticoids have been shown to impair antioxidant defenses in several brain regions including the hippocampus, cortex, and cerebellum, and these changes have been reported as possible components of glucocorticoid-mediated neuronal damage (Abraham et al., 2001; McIntosh and Sapolsky, 1996a, 1996b; Patel et al., 2002). Furthermore, recent research has found that oxidative stress induces oxidative damage of the hippocampus and pyramidal cell apoptosis. These changes are accompanied by a marked decline in glucocorticoid receptors in the CA1 region of the hippocampus, which results in an increase in glucocorticoid production (Kobayashi et al., 2009). Thus, it is likely that chronic oxidative stress induces oxidative damage in the HPA axis, leading to an increase in serum glucocorticoids, which impairs cognitive functions. The resulting cognitive deficits arise due to toxicity from either abnormally high glucocorticoid levels or oxidative stress.

*Crocus sativus L., commonly known as saffron, is a plant cultivated in various parts of the world, particularly in Iran. The major biologically active ingredients of saffron are crocin, picrocrocin and safranal (Tarantilis et al., 1995). Prior studies have shown that saffron extract and its two major components, crocin and safranol, have antitumor, anticonvulsant, antidepressant, anti-inflammatory, anti-hyperlipidemic, free radical scavenging and antioxidant effects (Abdullaev, 1993; Aasdaq and Inamdar, 2010; Rios et al., 1996). These components also have chemopreventive and geno-protective effects and protect from genotoxin-induced oxidative stress and methyl methanesulfonate-induced DNA damage in mice (Abdullaev, 1993; Hosseinzadeh et al., 2008; Nair et al., 1995). Additionally, recent work has shown that saffron extract and its active constituent crocin improved scopolamine or ethanol-induced impairments of learning and memory (Abe and Saito, 2000; Pitsikas and Sakellaridis, 2006; Zhang et al., 1994), antagonized the extinction of recognition memory in the object recognition test (Pitsikas et al., 2007), and prevented ethanol-induced inhibition of hippocampal long-term potentiation, a cellular model of long-term memory storage (Sugiuara et al., 1994). However, previous experiments have not investigated the possibilities that saffron extract and crocin may prevent chronic stress-induced learning and memory deficits or protect against oxidative stress.

Here, we investigated the effects of systemic administration of saffron extract and its active constituent crocin on chronic stress-induced spatial learning and memory impairments and oxidative stress in the hippocampus. Because previous studies have shown that both saffron extract containing crocin and crocin alone can improve learning and memory (Abe and Saito, 2000; Pitsikas et al., 2007; Pitsikas and Sakellaridis, 2006; Zhang et al., 1994), we examined whether these products are able to protect against the cognitive deficits induced by chronic stress. Rats were subjected to stress for 21 days and received systemic daily injections of saffron extract or crocin. Following the end of stress, their spatial learning and memory functions and hippocampal oxidative markers were evaluated.

2. Materials and methods

2.1. Animals

Adult male Wistar rats (220 ± 10 g) were individually housed in cages (50 x 26 x 25 cm) on a 12-h light/dark cycle at 22–24 °C, with food and water ad libitum. All of the experimental procedures were conducted in accordance with the National Institutes of Health’s Guide for the care and use of laboratory animals. Additionally, care was taken to use the minimum number of animals possible in each experiment.

2.2. Drugs

Pure red saffron powder was kindly supplied by the Pharmaceutical Research Center, Mashhad University of Medical Sciences, Mashhad, Iran. Crocin was purchased from Sigma Aldrich. Both substances were dissolved in a physiological saline and injected subcutaneously in a volume of 1 ml/kg. Saffron extract at a dose of 30 mg/kg and crocin at doses of 15 and 30 mg/kg were injected for 21 days in stressed and control groups. These doses were chosen on the basis of our pilot studies and previous reports (Pitsikas et al., 2007; Pitsikas and Sakellaridis, 2006).

2.3. Chronic restraint stress

Animals in the stress group were retrained daily for 6 h (from 10:00 to 16:00) for a total of 21 days in well-ventilated Plexiglass tubes (20 cm length, 6.5 cm diameter) without access to food and water. A 1-cm hole in one end was provided for breathing. The animals were not physically compressed and did not experience pain. During restraint, control animals stayed in their home cage without access to food and water to match access with the stressed group. After the restraint procedure, the stressed rats were placed back in their home cage. Body weights were recorded daily prior to the onset, and during the entire period, of daily restraint.

2.4. Experimental groups

Rats were randomly divided into eight experimental groups as follows: saline (SAL) + no-stress (NS) (control; SAL-NS); saffron extract (30 mg/kg) + no-stress (SE-NS), crocin (15 mg/kg) + no-stress (C15-NS); crocin (30 mg/kg) + no-stress (C30-NS); saline + stress (Sal-S); saffron extract (30 mg/kg) + stress (SE-S), crocin (15 mg/kg) + stress (C15-S), and crocin (30 mg/kg) + stress (C30-S). With the exception of the SE-treated groups (n = 10 in each group), all groups consisted of 20 rats. The first four groups received systemic administrations of physiological saline, saffron extract or crocin daily for 21 days. The last four groups were stressed in Plexiglass tubes 6 h/day for 21 days and received systemic injections of saline, saffron extract or crocin 1 h before the application of stress. Immediately after the last day of stress, half of the animals in each group (with the exception of the SE-treated groups) were decapitated, and trunk blood was collected for corticosterone assay (see below for details). The rest of the animals were subjected to water maze tests of learning and memory (described below). On the day after the end of behavioral testing, animals were decapitated, their brains removed, and the bilateral hippocampi dissected and used for measurement of oxidative stress markers (see below for details).

2.5. Testing learning and memory using the water maze

The water maze apparatus and tracking system have been described in detail in our earlier works (Akavan et al., 2008; Ebrahimi et al., 2010). In brief, the water maze was a black circular pool (140 cm in diameter and 60 cm high) that was filled to a depth of 25 cm with 20 °C water.

The water maze protocol was a stringent protocol consisting of four trials per day for 5 days. At the start of each trial, rats were placed into the water at one of the four cardinal points of the compass (N, E, S, and W, varied from trial to trial in a pseudo-random order). Rats had to swim until they climbed onto the escape platform. Rats were guided by hand to the platform if they failed to locate it within 60 s. Rats were allowed to stay on the platform for 20 s during the intertrial interval. After the last trial, each animal was towel dried and returned to its home cage with no access to a running wheel. The
parameters escape latency (i.e., time to reach the platform, in seconds), path length, and thigmotaxis (i.e., the portion of the total distance that the rats swam in the outer 10 cm of the pool) were analyzed for all 5 days.

A spatial probe test in which the platform was removed was performed 2 days after the last acquisition trial. The rats were allowed to swim for 60 s while the latency to reach the former location of the platform and times spent swimming in the target zone (defined by a 20 cm radius circle centered on the former location of the platform) or the opposite zone (equivalent to the target zone, but located in the opposite quadrant) were recorded. The velocity of each animal was also calculated. Time spent within a specified zone is a sensitive measure for detecting group differences in water maze probe test performance (Gallagher et al., 1993; Maet al., 2009).

Immediately after the probe test, the rats performed a visible platform task to assess motor ability. The platform extended 2 cm above the surface of the water and was moved to a novel quadrant in the pool in each trial. Four trials were conducted with the visible platform following the same procedure described above for the learning and memory tests.

2.6. Measurements of oxidative stress markers in the hippocampus

2.6.1. Preparation of tissue homogenates

A fraction of the hippocampus was washed in cold 0.9% saline and kept at −70 °C until used for preparation of homogenates with a homogenizer (Polytron PT 2100, KINEMATICA AG, Switzerland). For ferric reducing antioxidant power (FRAP) and malondialdehyde (MDA) assays, a fraction of tissue was homogenized (1:10 w/v) in cold 1.15% KCl. Homogenates for superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GR) measurements were prepared in a ratio of 100 mg tissue to 1 ml phosphate buffer (50 mmol/l; pH 7.5) containing 1 mM EDTA. The supernatants was determined by the Bradford method using bovine serum albumin as a standard (Bradford, 1976).

2.6.2. Total antioxidant activity assay

Total antioxidant activity was measured by FRAP according to the method of Benzie and Strain (1999). Briefly, 1.5 ml of working FRAP reagent (25 ml 0.3 M sodium acetate buffer, pH 3.6; 2.5 ml 0.01 M TPTZ, 2, 4, 6-tri (2-pyridyl)-1, 3, 5-triazine) in 0.04 M HCl; 2.5 ml 0.02 M FeCl3·6H2O, preheated to 37 °C) was mixed with 50 μl of supernatant. The mixture was incubated at 37 °C for 5 min, and the absorbance was determined at 593 nm. FeSO4 solutions from 0.2 to 1.2 mM in 1.15% KCl were used for calibration. FRAP values are expressed as μmol/mg of protein.

2.6.3. Lipid peroxidation assay

MDA results from degradation of polyunsaturated fatty acids. The production of this substance is used as a biomarker to measure the level of lipid peroxidation. MDA reacts with thiobarbituric acid (TBA) as a thiobarbituric acid reactive substance (TBARS) to form a 1:2 MDA–TBA adduct, which absorbs at 535 nm. Thus, the quantity of TBARS is proportionate to the amount of MDA. Concentration of TBARS is determined according to the method of Mihara and Uchiyama (1978). Briefly, 3 ml of 1% phosphoric acid and 1 ml of 0.6% w/v TBA aqueous solution were added to 0.5 ml of homogenate supernatant and heated for 45 min in a boiling water bath. After cooling, 4 ml n-butanol was added, the mixture was shaken and then centrifuged at 3000 × g for 10 min. Then the absorbance of the samples at 535 nm was measured using a Milton–Roy spectrophotometer 1201. The concentration of TBARS was calculated using the MDA standard curve and is expressed as nmol/mg of protein.

2.6.4. Enzymes activity assay

2.6.4.1. SOD assay. SOD activity in the hippocampus homogenates was assayed using the Ransod kit (Randox, UK). This method employs xanthine oxidase to generate superoxide radicals which react with 2-(4-iodophenyl)-3-(4-nitrophenol)-5-phenyltetrazolium chloride to form a red formazan dye. The SOD activity was assayed spectrophotometrically (STAT FAX 3300, Awareness Technologies) at 505 nm. The inhibition of the produced chromogen is proportional to the activity of the SOD present in the sample. A 50% inhibition is defined as one unit of SOD.

2.6.4.2. GPx assay. GPx activity was measured in homogenates by the method of Paglia and Valentine (1967). GPx catalyzed the oxidation of glutathione by cumene hydroperoxide. In the presence of GR and NADPH, the oxidized glutathione was immediately converted to the reduced form with a concomitant oxidation of NADPH to NADP+. The decrease in absorbance of NADPH was measured at 340 nm (Ransel kit, Randox, UK).

2.6.4.3. GR assay. GR activity in homogenates was determined by the method of Goldberg and Spooner (1983), using the Randox kit, UK. GR catalyses the reduction of glutathione in the presence of NADPH, which is oxidized to NADP+. The decrease in absorbance at 340 nm was measured.

2.7. Radioimmunoassay for corticosterone

At the end of 21 days’ stress, the rats were decapitated, trunk blood was collected in tubes with EDTA and centrifuged (3000 × g, 20 min) and the plasma was stored at −70 °C until used for the corticosterone assay. Corticosterone levels were determined by a radioimmunoassay kit (DRG diagnostics, DRG Instruments GmbH, Marburg, Germany). The sensitivity of the assay was 0.39 ng/ml.

2.8. Statistical analysis

The data express as the mean ± standard error of the mean (S.E.M.). Data analysis consisted of mixed- and between factor analyses of variance (ANOVARs). Post-hoc analyses consisted of Turkey’s test. Student’s t-tests were used to compare two independent groups. Statistical differences were considered significant when P<0.05.

3. Results

3.1. Corticosterone levels

Fig. 1 shows the plasma corticosterone levels in multiple experimental groups. One-way ANOVA indicated significant differences among groups (F5,30 = 88.79, P < 0.0001). Post-hoc comparison indicated that chronic stress significantly increased the plasma corticosterone concentrations in the SAL-S group as compared with the SAL-NS group (P < 0.01). Interestingly, corticosterone levels in the C15-S and C30-S groups were significantly lower than that of the SAL-S group (P < 0.01). A significant difference was found between the C30-NS group and the SAL-NS or C15-NS groups. These findings indicate that crocin did significantly prevent chronic-stress induced elevations in corticosterone levels.

3.2. Effects of saffron extract and crocin on chronic stress induced impairments of learning and memory

An ANOVA carried out on swim speed revealed no differences between groups. Moreover, data related to the distance swam to reach the platform and latency to reach the platform followed similar patterns; thus, we only present latency data. Latency data of the
Effects of saffron or crocin pretreatment on chronic stress induced impairment of spatial memory. (A) The platform location latency. (B) Mean time (%) spent in target and opposite quadrants. Chronic stress impaired memory retention as the stressed animals spent significantly less time in the target quadrant and had longer platform location latencies than their non-stressed control counterparts. Saffron or crocin pretreatment inhibited the stress-induced impairment of memory retention. Data are expressed as the mean ± S.E.M. In (A): **P < 0.01 as compared with the SAL-NS group; # P < 0.05 and ## P < 0.01 as compared with the SAL-S group. In (B): * P < 0.01 as compared with the SAL-NS; # P < 0.05 as compared with the SAL-S in the same zone.
3.3. Effects of saffron extract and crocin on chronic-stress induced changes of oxidative stress markers in the hippocampus

3.3.1. FRAP levels
Two-way ANOVA on FRAP level data (Fig. 4) revealed significant effects of stress ($F_{3,56} = 152$, $P < 0.0001$), treatment ($F_{3,56} = 25.66$, $P < 0.0001$) and a significant interaction ($F_{3,56} = 2.96$, $P < 0.05$). Between-group comparisons indicated that the FRAP levels of the SAL-S group were significantly lower than those of all other groups (all, $P < 0.01$). FRAP levels in the C15-NS, C30-NS, and SE-NS groups were also significantly higher than those of the SAL-NS (all, $P < 0.01$).

3.3.2. TBARS levels
Two-way ANOVA on TBARS levels (Fig. 5) revealed significant effects of stress ($F_{3,56} = 134.14$, $P < 0.0001$), treatment ($F_{3,56} = 30.25$, $P < 0.0001$) and a significant interaction between factors ($F_{3,56} = 6.04$, $P < 0.01$). Between-group comparisons indicated that the TBARS levels of the SAL-S group were significantly higher than those of all other groups (Ps ranging between $0.01$ and $0.05$). The TBARS levels in the C15-NS ($P < 0.05$), the C30-NS ($P < 0.01$), and the SE-NS ($P < 0.05$) were also significantly lower than those of the SAL-NS.

3.3.3. Activity of antioxidant enzymes

3.3.3.1. SOD activity. Two-way ANOVA on SOD activity (Fig. 6A) revealed significant effects of stress ($F_{3,56} = 253.27$, $P < 0.0001$), treatment ($F_{3,56} = 30.25$, $P < 0.0001$) and a significant interaction ($F_{3,56} = 13.73$, $P < 0.01$). Between-group comparisons indicated that SOD activity in the SAL-S group was significantly higher than that of all other groups except the C15-NS group (all, $P < 0.01$). SOD activity in the C30-NS and SE-NS groups was also significantly lower than that of the SAL-NS group (both, $P < 0.01$).

3.3.3.2. GPx activity. Two-way ANOVA on GPx activity (Fig. 6B) revealed significant effects of stress ($F_{3,56} = 304.6$, $P < 0.0001$), treatment ($F_{3,56} = 45.84$, $P < 0.0001$) and a significant interaction between factors ($F_{3,56} = 18.42$, $P < 0.001$). Between-group comparisons indicated that GPx activity in the SAL-S group was significantly higher than that of all other groups (all, $P < 0.01$). GPx activity in the C15-NS ($P < 0.05$), the C30-NS ($P < 0.01$), and the SE-NS ($P < 0.01$) was also significantly lower than that of the SAL-NS.

3.3.3.3. GR activity. Two-way ANOVA on GR activity (Fig. 6C) revealed significant effects of stress ($F_{3,56} = 80.89$, $P < 0.0001$), treatment ($F_{3,56} = 16.35$, $P < 0.0001$) and a significant interaction ($F_{3,56} = 3.72$, $P < 0.01$). Between-group comparisons indicated that GR activity in the SAL-S group was significantly higher than that of all other groups (Ps ranging between $0.01$ and $0.05$). GR activity in the CR30-NS ($P < 0.01$)

4. Discussion
The main findings of the present study are that chronic restraint stress impairs spatial learning and memory and induces oxidative stress. These harmful effects of chronic stress can be prevented by saffron and crocin pretreatment, suggesting that these substances...
have potential therapeutic applications protecting against the detrimental effects of chronic stress on cognitive functions.

4.1. Chronic restraint stress impairs spatial learning and memory

We have found that the chronic stress produced by 6 h/d/21 d restraint impairs hippocampal-dependent spatial learning and memory performance. The finding that chronic stress impairs spatial learning and memory is in agreement with other studies showing similar deficits in spatial learning and memory following chronic restraint stress (Abidin et al., 2004; Kitraki et al., 2004; Kleen et al., 2006; Ma et al., 2007; Moosavi et al., 2007; Radecki et al., 2005; Song et al., 2006; Touyarot et al., 2004). Although we found that chronic stress impairs spatial learning and memory, it is necessary to deduce whether altered acquisition reflects impairment of learning or memory. Analyses of swimming velocity and latencies to reach the visible platform revealed no differences between stressed and non-stressed animals, ruling out any non-specific effects of chronic stress on spatial acquisition and memory. Moreover, the lack of significant differences in swimming velocity between the stressed and the non-stressed animals in the probe trial further rule out any non-specific effects. These findings demonstrate that the impairing effects of chronic stress on spatial learning and memory are not due to any non-specific changes in gross motor activity or motivational state.

The impairing effects of chronic stress on learning and memory are mainly mediated via activation of the HPA axis, which culminates in the production of glucocorticoids by the adrenal glands. Chronically elevated levels of glucocorticoids in rodents cause dendritic atrophy in hippocampal CA3 pyramidal neurons (Conrad et al., 2007; Magarinos and McEwen, 1995), inhibit neurogenesis (Gould et al., 1997) and cause hippocampal volume loss (McEwen, 2000). These changes in the hippocampus after chronic stress or elevation of glucocorticoids have been related to changes in spatial learning and memory (McEwen, 2001). Oxidative stress could be one of the mechanisms by which chronic stress or glucocorticoids negatively affect learning and memory (Abidin et al., 2004) and induce neuronal damage (Abraham et al., 2001; McIntosh and Sapolsky, 1996a, 1996b; Patel et al., 2002). As explained in the Introduction, oxidative stress is an imbalance between oxidants and antioxidants with the former prevailing. Oxidative stress can be increased by an increased production of reactive oxygen species or by a decrease in antioxidant enzymes (Storz and Imlay, 1999). Brain cells are at particular risk of being damaged by free radicals because the brain has a high oxygen turnover, and central nervous system neuronal membranes are rich in polyunsaturated fatty acids that are potential targets for lipid peroxidation (Anderson et al., 1985; Metodiewa and Koska, 2000). Our findings demonstrate that 21 days of chronic restraint stress increased the plasma levels of corticosterone, induced oxidative stress in the hippocampus, and impaired spatial learning and memory. These findings corroborate the findings of others (Abidin et al., 2004) and further support the idea that chronic stress affects spatial learning and memory through oxidative stress.

4.2. Saffron extract and crocin prevent chronic-stress induced deficits in learning and memory

Our findings show that pretreatment with both saffron and crocin abolishes the deleterious effects of chronic stress on learning and memory. These findings are in agreement with previous studies showing saffron and crocin ameliorate impairments of learning and memory in a variety of tasks and conditions (Pitsikas et al., 2007; Pitsikas and Sakellaridis, 2006; Zhang et al., 1994). The present data allow for two interpretations of the effects of saffron and crocin on spatial ability in chronically stressed rats. First, these substances may have prevented cognitive deficits by interacting with the mechanism(s) that cause spatial learning and memory impairments in chronically stressed rats. This hypothesis predicts that chronic stress and saffron and crocin act on similar neurobiological substrates. Alternatively, saffron- or crocin-treated rats may have enhanced spatial learning and memory compared with control (vehicle-treated) rats in the absence of chronic stress. This hypothesis predicts that chronic stress and saffron and crocin work in parallel to influence spatial ability. However, our findings do not support the latter hypothesis, because we did not see any improvement in learning and memory due to saffron or crocin in non-stressed animals. Regardless of the interpretation, to our knowledge, these data are the first to show that saffron and crocin can prevent chronic stress-induced spatial learning and memory deficits in adulthood. We found no differences in thigmotaxis, an indicator of anxiety or fear (Treit and Fundytus, 1989), among groups. This indicates that the observed effects are not simply due to any anxiolytic effects of the compounds but result from their specific effects on learning and memory.

The anti-oxidative stress properties of saffron and crocin provide one possible mechanism by which these compounds block stress-induced impairment of learning and memory. Saffron contains many carotenoids with powerful antioxidant effects and may protect CNS neurons from oxidative damage (Ochiai et al., 2007; Zheng et al., 2007). Our data show that saffron and crocin significantly modulate the levels of oxidative markers in the hippocampus. As mentioned above, the brain tissue is highly vulnerable to oxidative stress because of its oxidative damage potential (Anderson et al., 1985; Metodiewa and Koska, 2000). Reactive oxygen species can react with polyunsaturated fatty acids to form lipid peroxides, and the accumulation of the end products of lipid peroxidation due to chronic stress may contribute to cognitive deficits (Cantuti-Castelvetri et al., 2000; Launer and Kalmin, 1998). We found that chronic stress increased lipid oxidation in the hippocampus, and this effect was blocked by both saffron and crocin treatment. This finding is in line with a recent work showing a decrement of lipid peroxidation products by saffron and crocin in some pathological conditions (Joukar et al., 2010). We also found a significant reduction in total antioxidant power in the stressed animals, which was prevented by either saffron or crocin pretreatment. This finding shows the antioxidant effects of these substances in chronic stress.

Organisms have some antioxidant defense enzymes to protect against oxidative damage. These antioxidant defense enzymes include SOD and GPx. In line with earlier studies (John et al., 2001; Kakkar et al., 1992; Limaye et al., 2003; Shull et al., 1991), we demonstrated a significant increase in the activities of these enzymes in the hippocampus following chronic stress. The results of the present study can be interpreted in terms of two mechanisms: an elevation of glucocorticoid levels following chronic stress and a compensatory response to chronic stress. The existing data in the literature are not in the favor of the first mechanism. In fact, previous studies have observed different effects on antioxidant enzymes following glucocorticoid administration. Rats treated with glucocorticoids showed decreased activities of SOD and GPx in the brain (Mcintosh et al., 1998b), and glucocorticoids prevented induction of antioxidant enzymes after kainic acid administration in the hippocampus (Mcintosh et al., 1998a). Corticosterone has been shown to decrease GPx activity and reduce GSH levels in hippocampal cell cultures (Patel et al., 2002). Therefore, the increased GPx, SOD and GR activities observed in the present study are possibly adaptations to chronic stress. In fact, elevations of hippocampal GPx, SOD and GR activities after chronic stress suggest a possible chronic stress-induced increase in reactive oxygen species production and an accompanying compensatory adaption of the tested free-radical scavenging enzymes. More importantly, the increases in the activities were parallel and balanced. These parallel changes, especially in SOD and GPx activities, are very important because SOD metabolizes excess O2− and produces H2O2, which is converted to H2O by GPx. We have shown that both saffron and crocin, alone and in the presence of chronic stress, decrease the activities of these enzymes. This could be due to the fact
that saffron and crocin lead to reductions of reactive oxygen species, which in turn decreases the need for antioxidant enzymes. Another possibility is that saffron or crocin directly modulate synthesis of these enzymes (Soeda et al., 2007).

Finally, an interaction with the HPA axis might be another mechanism that mediates the protective effects of saffron and crocin against chronic stress-induced impairment of learning and memory. Interestingly, we have found that crocin can decrease the corticosterone response to chronic restraint stress. Presently, the underlying mechanisms and anatomical sites of the inhibitory action of crocin on corticosterone levels are not known and need further research.

Repeated restraint stress has long been used as an interesting animal model of chronic stress and stress-related mental illnesses such as depression and cognitive disorders. This model has a good degree of construct, face, and predictive validity in terms of physiological and behavioral changes (Mitchell and Redfern, 2005; Siegmund and Wotjak, 2006). Our findings that saffron extract and crocin can ameliorate the oxidative stress and cognitive deficits induced by chronic stress provide evidence of the possible therapeutic effects of saffron extract and crocin in chronic stress-related disorders, particularly cognitive dysfunction.

One limitation of the present study is the narrow range of saffron extract and crocin doses used. Another limitation is the lack of measurement of the amount of crocin present in the saffron samples. These issues should be taken into account in future studies on the effects of saffron or crocin on learning and memory in different conditions.

In conclusion, the present work demonstrates that saffron can prevent chronic-stress induced deficits in spatial learning and memory and oxidative stress in the hippocampus. Thus, these substances should be useful as new pharmacological tools for studying the mechanism of chronic stress-induced cognitive impairment and for alleviating cognitive deficits.

Acknowledgment

This work was supported by grants from Semnan University of Medical Sciences and Iranian Neuroscience Research Network. We would like to thank Dr. H. Hossienzadeh for providing us the saffron powder.

References


