ORIGINAL ARTICLE

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Pretreatment with crocin along with treadmill exercise ameliorates motor and memory deficits in hemiparkinsonian rats by anti-inflammatory and antioxidant mechanisms

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Abstract

The motor symptoms of Parkinson's disease (PD) are preceded by non-motorized symptoms including memory deficits. Treatment with dopamine replacement medications, such as L-DOPA only control motor symptoms and does not meet the clinical challenges of the disease, such as dyskinesia, non-motor symptoms, and neuroprotection. The purpose of the current study was to examine the neuroprotective potential of crocin and physical exercise in an animal model of PD. Male Wistar rats ran on a horizontal treadmill and/or pretreated with crocin at a dose of 100 mg/kg. Then, 16 μg of the neurotoxin 6-hydroxydopamine (6-OHDA) was microinjected into left medial forebrain bundle. Crocin treatment and/or exercise continued for 6 more weeks. Spatial and aversive memories, rotational behaviour, inflammatory and oxidative stress parameters were assessed at the end of week 6 post surgery. The results showed that pretreatment with crocin alone and in combination with exercise decreased the total number of rotaions as compared with 6-OHDA-lesioned group. Furthermore, treatment of parkinsonian rats with crocin along with exercise training improved aversive and spatial memories. Biochemical analysis showed that crocin and exercise (alone and in combination) reduced tumor necrosis factor- (TNF) α levels in the striatum. Moreover, treatment with crocin at a dose of 100 mg/kg decreased the lipid peroxidation levels in the hippocampus, while exercise training increased the total thiol concentration. In conclusion, our findings indicated that pretreatment with crocin along with treadmill exercise ameliorated motor and memory deficits induced by 6-OHDA, which is considered to be due to their antioxidant and anti-inflammatory activities. The results suggest that combined therapy with crocin and exercise may be protective for motor and memory deficits in PD patients.

Keywords Crocin · Memory · Motor activity · Treadmill exercise · Cytokine · 6-OHDA · Inflammatory biomarkers · Oxidative stress · Parkinson's disease

Introduction

Parkinson's disease (PD) is a chronic and progressive neurodegenerative disease with a prevalence of 1–3% in the population over 55 years old (De Lau and Breteler 2006). The motor symptoms of the disease including; bradykinesia, tremor, muscular stiffness and imbalance, are due to death of dopaminergic neurons in the substantia nigra and dysfunction of the basal ganglia (Shuman et al. 2011). In addition to motor deficits, cognitive impairments are extremely prevalent in PD

and can substantially affect the quality of patient's life (Murray et al. 2014). In PD, non-motorized symptoms, such as; memory deficits, sleep disorders, depression and dementia are gradually caused by the spread of damage to other parts of the brain (Shuman et al. 2011; Chaudhuri and Martinez-Martin 2008). The etiology of the disease is not well-known but, various factors such as oxidative stress (Dias et al. 2013), mitochondrial dysfunction (Subramaniam and Chesselet 2013) and neuroinflammation (Zhang et al. 2018) are effective factors in the onset of this disease. Among these factors, oxidative stress plays an important role in the degeneration of dopaminergic neurons in PD. Based on the oxidative stress hypothesis, imbalance in oxidant and antioxidant agents leads to oxidative stress (Dias et al. 2013). During this process, enhancement of the production of reactive oxygen species (ROS) leads to neuronal damage and death through membrane lipid peroxidation, protein oxidation, and DNA damage

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(Sayre et al. 2007). Dopaminergic nigrostriatal neurons are highly vulnerable to oxidative stress, owing to the presence of oxidizable substances (dopamine, unsaturated fatty acids and iron) and its relatively low antioxidant content (Dias et al. 2013; Jenner and Olanow 2006; Liu et al. 2008; Sian-Hulsmann et al. 2011). ROS are produced in dopaminergic neurons by dopamine auto-oxidation or by dopamine metabolism via monoamine oxidase B enzyme (Dias et al. 2013). In addition, high levels of iron in the substantia nigra leads to the reduction of hydrogen peroxide (H_2O_2) and form a very toxic hydroxyl free radicals, which are extremely reactive with the membrane lipids of the neuron and the DNA contained in their nucleus, thereby damaging the neurons (Dexter and Jenner 2013; Demougeot et al. 2000). Moreover, oxidative stress impairs mitochondrial function, which causes the stimulation of apoptotic pathways and finally leads to the death of dopaminergic neurons in the substantia nigra pars compacta (Lin et al. 2012).

There is also evidence suggesting that inflammation plays a pathogenic role in PD (Hartmann et al. 2003). Immunohistochemical studies have demonstrated the presence of activated microglia, increased cytokine expression, and upregulation of inflammatory-associated factors in the striatum, substantia nigra and cerebrospinal fluid of PD patients (Olanow 2007; Nagatsu et al. 2000). Evidence has indicated that the levels of several cytokines, including tumor necrosis factor- (TNF) α are significantly elevated in the substantia nigra and striatum (Pieper et al. 2008; Ros-Bernal et al. 2011). TNF-α is known as an early and late player in the pathophysiology of PD (McCoy et al. 2011; Rocha et al. 2015; Gelders et al. 2018). TNF can activate the abundant numbers of microglia in the midbrain, potentiating inflammatory responses that lead to autoamplification of ROS, nitric oxide, and superoxide radicals and eventually oxidative damage of dopaminergic neurons (Niranjan 2014). In addition, several investigators have reported the presence of interleukins in the brain parenchyma or cerebrospinal fluid of PD patients (Rogers et al. 2007; Brodacki et al. 2008). Interleukin- (IL-) 10 is an anti-inflammatory cytokine, which is produced in the brain and inhibits microglial activation in PD (Zhu et al. 2016).

In spite of many advances in the treatment of PD, most drugs used only control disease symptoms and do not stop or delay the degeneration of dopaminergic neurons (De Lau and Breteler 2006). Treatment with dopamine replacement medications, such as; L-DOPA can effectively modify motor symptoms but cannot prevent progression of the disease. Furthermore, after several years of treatment with L-DOPA, severe side effects have been reported, which may be related to an increase in the number of free radicals due to L-DOPA and dopamine oxidative metabolism (Prasad et al. 1999). Moreover, synthetic glucocorticoids such as dexamethasone, was shown to be neuroprotective in parkinsonian rodents

(Kurkowska-Jastrzebska et al. 2004), however, chronic treatment with glucocorticoids induce serious side effects (Tentillier et al. 2016). Therefore, using the neuroprotective agents to reduce the complications of drugs and prevent or inhibit the neurodegenerative process has been proposed.

Saffron is a spice obtained from the stigmas of the flower of Crocus sativus L, which is widely cultivated in Iran and other countries, such as; India, Spain and Greece. Three major bioactive compounds in saffron are crocin, picrocrocin and safranal, which are responsible for saffron's exclusive color, taste and odor, respectively (Mollazadeh et al. 2015). Crocin possesses several pharmacological effects, including; antioxidant (Chen et al. 2008; Rajaei et al. 2013), anti-inflammatory (Nam et al. 2010), cancer cell proliferation inhibitor (Magesh et al. 2006), hypolipidemic (Lee et al. 2005) and hepatoprotective effects (Tseng et al. 1995). It has also been reported that crocin protects against oxidative damage to the brain vessels (Zheng et al. 2007), kidney tissues (Hosseinzadeh et al. 2005), heart (Goyal et al. 2010) and retina (Yamauchi et al. 2011) under different experimental conditions. In addition, the protective effect of crocin against oxidative damage to pheochromocytoma (PC-12) cells has been reported and it has been shown that the antioxidant effects of crocin are more potent than alpha-tocopherol (Ochiai et al. 2004).

Moreover, exercise and physical activity are among the non-pharmacological methods used to prevent neurodegenerative disorders. Although exercise alone has significant neuroprotective properties, the benefits of exercise and its neuroprotective effects in PD needs further research. Experimental studies showed that exercise increased the antioxidant activity in the striatum (Somani et al. 1995) and protected against oxidative conditions in Alzheimer's disease (Um et al. 2008). It has also been reported that exercise increased the antioxidant status and the level of neurotrophins in the central nervous system (Tuon et al. 2012; Cotman and Berchtold 2002; Aguiar Jr et al. 2008). It has been shown that exercise has benefit effects on gait and posture in PD (Mehrholz et al. 2015) and induced neuroplasticity (Cotman and Berchtold 2002), nevertheless, its neuroprotective effects in PD have been challenged. As reported, performing treadmill exercise for 6 weeks in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced mouse model of PD, does not prevent mitochondrial inhibition (Aguiar Jr et al. 2014) or nigrostriatal neurodegeneration (Gerecke et al. 2012). These findings indicate that the mechanism of beneficial effects of exercise for a patient suffering from PD is still unknown and requires more research.

The present study was undertaken to examine the neuroprotective effects of crocin at a dose of 100 mg/kg and treadmill exercise (alone and in combination) in 6-OHDA model of PD in rat. For this purpose, the effects of exercise and crocin on motor activity (as assessed by rotational behavior), aversive and spatial memories, striatal inflammatory biomarkers

(TNFα and IL-10) and hippocampal oxidative stress status (by measuring lipid peroxidation levels and total thiol concentration) were examined.

Materials and methods

Animals

Adult male Wistar rats (250-300 g, 3–4 months old), procured from Pasteur's Institute (Tehran, Iran) were used in this experiment. The rats were housed at a room temperature (22° ± 2 °C) under standard 12 h light/dark cycles (lights on at 07:00 a.m.). Food and water were made available ad libitum. The Ethics Committee for Animal Experiments at Isfahan University of Medical Sciences approved the study (Approval No. 395676) and all experiments were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (NIH Publication, 8th edition, 2011).

The animals were randomly assigned into 5 groups, including; 1) Normal saline sham-operated group (injection of ascorbate-saline 0.2% into the left medial forebrain bundle (MFB), saline ip, $n = 7$), 2) 6-OHDA-lesioned group (injection of 16 μg 6-OHDA into left MFB, saline ip, n = 7), 3) crocin group (injection of 16 μg 6-OHDA into left MFB, 100 mg/kg crocin ip, $n = 7$), 4) exercise group (injection of 16 μg 6-OHDA into left MFB, treadmill exercise, $n = 8$) and 5) crocin+exercise group (injection of 16 μg 6-OHDA into left MFB, 100 mg/kg crocin along with treadmill exercise, n = 8). It should be noted that the injection of crocin (100 mg/kg, ip) or normal saline and treadmill exercise started one week before the 6-OHDA or ascorbate-saline injection and continued for 6 weeks after surgery. In addition, we had 2 more subgroups, sedentary rats in sham-operated and 6-OHDA-lesioned groups. These animals did not exercise; however, they were transported daily to the training room and placed in the switched-off treadmill, so that they were exposed to the same environment as the exercised group of animals. Statistical analysis showed that there was no significant difference in behavioural and biochemical parameters between two sham-operated and 6-OHDA-lesioned subgroups. Therefore, we only presented data in results section for sham-operated and 6-OHDA-lesioned subgroups which were daily treated with saline during the experiment.

Induction of Parkinson's disease

Animals in the 6-OHDA-lesioned groups were anesthetized with chloral hydrate (450 mg/kg, ip) (Rajaei et al. 2005), then they were placed in a stereotaxic apparatus (Stoelting, USA). A 26-gauge needle was placed into the left MFB according to the coordinates: −1.8 mm lateral to midline, 3.6 mm anterior

to posterior, depth − 8.2 mm deep from the surface of the brain (Paxinos and Watson 2005). Then, 6-OHDA (16 μg/4 μl 0.2% ascorbate-saline), was microinjected at a rate of 1 μl/min by a Hamilton microsyringe connected to the microinjection pump. The needle remained in the coordinates for 5 min and then slowly drawn back. The sham-operated group animals also received an identical volume of the ascorbate-saline as vehicle. On day 42, the animals were sacrificed, the striatum and hippocampus were dissected out and homogenised in the NaCl 0.9% solution by a homogenizer (Heidolph, Germany).

Treadmill training

A five-lane motorized rat treadmill was employed for exercise training. One week before essential protocol, the exercised groups of animals were trained on the treadmill running at 5 days/week, 5 min/day with 10 m/min speed to reduce their stress to the new environment (Tuon et al. 2012). One week before surgery, the animals in the exercised group were trained on the treadmill running at 5 days/week, 40 min/day with a speed up to 17 m/min (5 min at 7 m/min, 30 min at 17 m/min, 5 min at 7 m/min). This protocol continued for 6 weeks after surgery. The untrained animals were placed on the switched-off treadmill for the same weeks as the exercise groups.

Passive avoidance memory

Passive avoidance learning and memory was investigated using the shuttle box at the end of week 6 after surgery. The apparatus is made up of two dark and light chambers. The bottom of the dark chamber is connected to a shock generator. On the first day of the test, the rats were placed in light chamber. After a minute, the guillotine door was opened and delay in entering the dark chamber was recorded. After entrance of the rat into the dark chamber, the door was closed and a 0.5 mA foot electric shock was delivered for 3 s through the grid floor. The current intensity for foot shock was selected based on previous studies in our laboratory. After disconnection of the electric current, the door was opened and the shocked rat was removed from the device and transferred to the cage. In the test session (24 h later), animals were again placed individually in light chamber. The step-through latency to enter the dark compartment was measured as a positive index of memory performance, with a 300 s cut-off time (Haddadi et al. 2018).

Spatial memory

Elevated plus maze test was used to evaluate spatial memory at week 6. Elevated plus maze contains two open arms (50 × 10 cm) and two closed arms (10 × 10 × 50 cm), connected with a central platform (10 × 10 cm). It was located 80 cm above the ground. On the first day and at the acquisition stage,

the animal was placed at the end of the open arm facing away from the central platform. Then, transfer latency (TL1) was recorded as time taken by the rat to move into one of the enclosed arm with all its four legs. The animal was then allowed to move freely within the maze for 10 s. At reminder stage, 24 h later, the animal was again placed at the end of the open arm and latency to move into the closed arm was recorded (TL2). Less time spent entering the closed arm was considered as a positive criterion for spatial memory function (Mutlu et al. 2015).

Rotational test

Animals were injected apomorphine hydrochloride (2 mg/kg, ip) at the end of the 6th week after surgery. The hemiparkinsonian rats were recognized by observing the rotational behavior after injection of apomorphine hydrochloride (Sigma-Aldrich, USA). To evaluate rotation, the animals were placed in a transparent plexiglass container (28 × 28 × 50 cm) for 10 min to habituate. One minute after the injection of apomorphine, the number of rotations were counted at 10 min intervals for 30 min in a dimly-lit and quiet room. The number of ipsilateral rotations was counted as negative scores and that of contralateral rotations as positive scores. Net number of rotation was defined as the difference between the rotations in both directions (Hosseini et al. 2016).

Cytokine levels

After centrifugation of striatal homogenates at 3000 rpm for 5 min, the supernatants were assayed for TNF-α and IL-10 using commercially available ELISA kits (ebioscience Co, San Diego, CA, USA) according to the manufacturer's instructions. Results were shown as pg/ml.

Lipid peroxidation levels

The lipid peroxidation levels of hippocampus were measured as TBARS (thiobarbituric acid reactive substance). To measure, a mixture of trichloroacetic acid, thiobarbituric acid, and HCl were added to 1 ml of homogenate, and the mixture was heated for 45 min in a boiling water bath. After cooling, the samples were centrifuged at 1000 rpm for 10 min and the absorbance (A) were measured at the 535 nm. The level of TBARS was calculated by: $C(M) = A/1.65 \times 10^5$ (Ahmadi et al. 2017).

Total thiol concentration

2,2'-Dinitro-5,5'-dithiodibenzoic acid (DTNB) reagent was used to measure the total sulfhydryl groups. DTNB reacts with sulfhydryl groups and produces a yellow complex with an absorption peak at 412 nm. To measure, 50 μl of homogenate was added to 1 ml tris-EDTA buffer and then its absorption was read at 412 nm against the tris-EDTA buffer alone (A1). Then, 20 μl of DTNB reagent was added to the mixture and the sample absorbance was again read after 15 min (A2). The absorbance of the DTNB reagent was also read as a blank (B). The total thiol concentration (mM) was calculated by the equation: The total thiol concentration (mM) = (A2-A1-B) × 1.07/0.05 × 13.6 (Ahmadi et al. 2017).

Histology

To ensure that the neurotoxin 6-OHDA has been injected into MFB, histological study was done. At the end of experiment (day 42), the brain was removed and stored in 10% formalin for 72 h. The brain was sectioned coronally at 40 μm by a freezing microtome (Leica, Germany). Sections were collected in a phosphate buffer solution and then mounted on gelatin-coated slides and studied using a light microscope. The track of the needle and injection site of 6-OHDA was determined by reference to a rat brain atlas (Paxinos and Watson 2005).

Statistical analysis

Statistical analysis was carried out using one-way ANOVA followed by the Tukey's post hoc test. The data were expressed as mean ± SEM. A statistical p value <0.05 was considered significant.

Results

Apomorphine-induced rotations

Unilateral injection of 6-OHDA into MFB significantly increased apomorphine-induced rotations in 6-OHDA-lesioned group as compared with sham group at the end of week 6 ($P < 0.001$, Fig. 1). Pretreatment with crocin alone and along with treadmill exercise reduced the total net number of rotations as compared with 6-OHDA-lesioned group ($P < 0.01$, P < 0.01, respectively). However, treadmill exercise alone did not change rotations as compared with 6-OHDA- lesioned group (Fig. 1).

Passive avoidance memory

As shown in Fig. 2, the step-through latency of 6-OHDA-lesioned rats was shorter than sham group rats at the end of week 6 ($P < 0.05$, Fig. 2). Moreover, pretreatment with crocin along with treadmill exercise significantly increased the latency as compared with 6-OHDA-lesioned group (P < 0.05). Treatment with crocin and treadmill exercise alone did not significantly change the latencies as compared with 6-
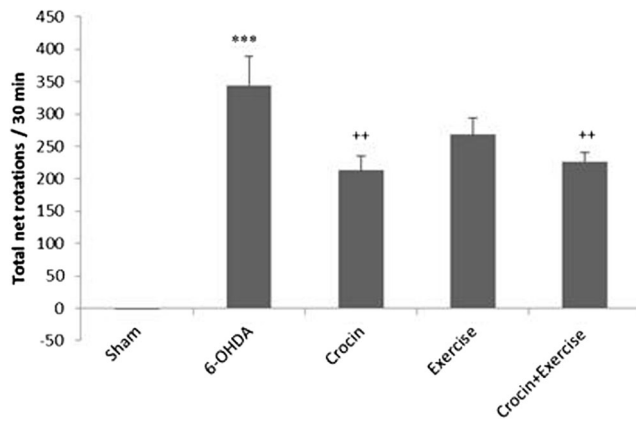
Fig. 1 Total net number of rotations (mean ± SEM) induced by apomorphine (2 mg/kg, ip) over a period of 30 min among the experimental groups at the end of week 6. Crocin was administered daily at a dose of 100 mg/kg for 7 weeks. $n = 7–8$ for each group. ***$P < 0.001$ vs sham group, ++$P < 0.01$ vs 6-OHDA-lesioned group

Fig. 3 Transfer latency (mean ± SEM) among the experimental groups at week 6. Crocin was administered daily at a dose of 100 mg/kg for 7 weeks. n = 7–8 for each group. *P < 0.05 vs sham group, +P < 0.05 vs 6-OHDA-lesioned group

OHDA-lesioned group, though there was a tendency toward an increase in latencies in both groups (Fig. 2).

Spatial memory in elevated plus maze

Statistical analysis showed that 6-OHDA injection into MFB significantly prolonged the transfer latency on the second day as compared with the sham group (P < 0.05, Fig. 3). Moreover, pretreatment with crocin along with treadmill exercise significantly decreased the transfer latency as compared with 6-OHDA-lesioned group ($P < 0.05$). Treatment with crocin and treadmill exercise alone did not significantly change the latencies as compared with 6-OHDA-lesioned

group, though there was a tendency toward a decrease in latencies in both groups (Fig. 3).

TNF-α levels

A significant increase in the TNF-α levels was found in the striatum of 6-OHDA-lesioned rats ($P < 0.01$, Fig. 4) as compared with the sham group. In addition, crocin at a dose of 100 mg/kg and treadmill exercise, alone ($P < 0.01$, $P < 0.001$, respectively) and in combination ($P < 0.01$) reduced the TNF-α levels in the striatum at the end of week 6, as compared with 6-OHDA-lesioned group (Fig. 4).
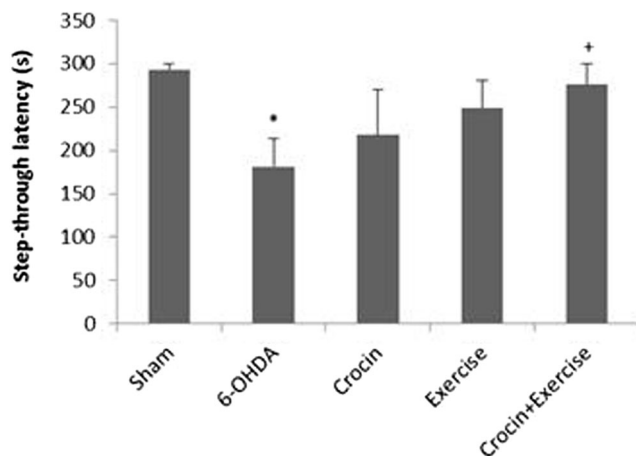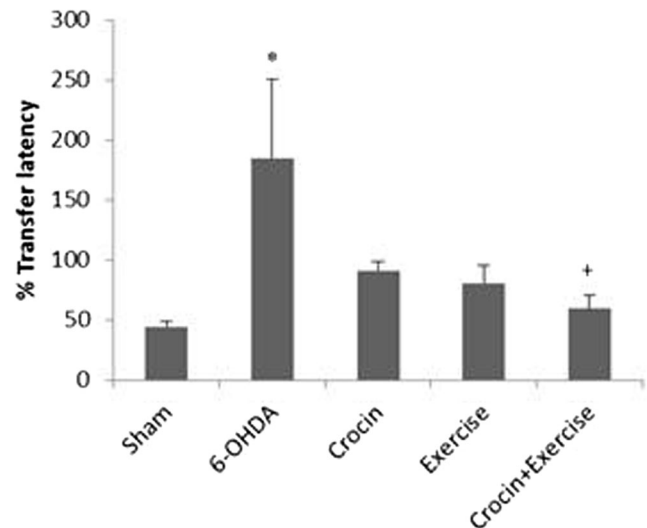

Fig. 2 Step-through latency (mean ± SEM) among the experimental groups at the end of week 6. Crocin was administered daily at a dose of 100 mg/kg for 7 weeks. n = 7–8 for each group. *P < 0.05 vs sham group, +P < 0.05 vs 6-OHDA-lesioned group
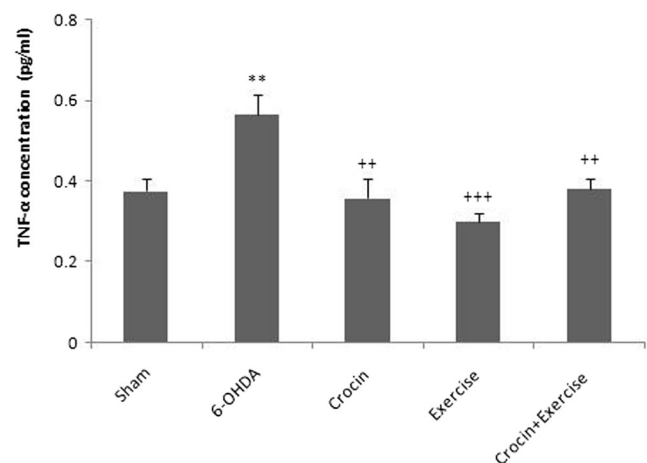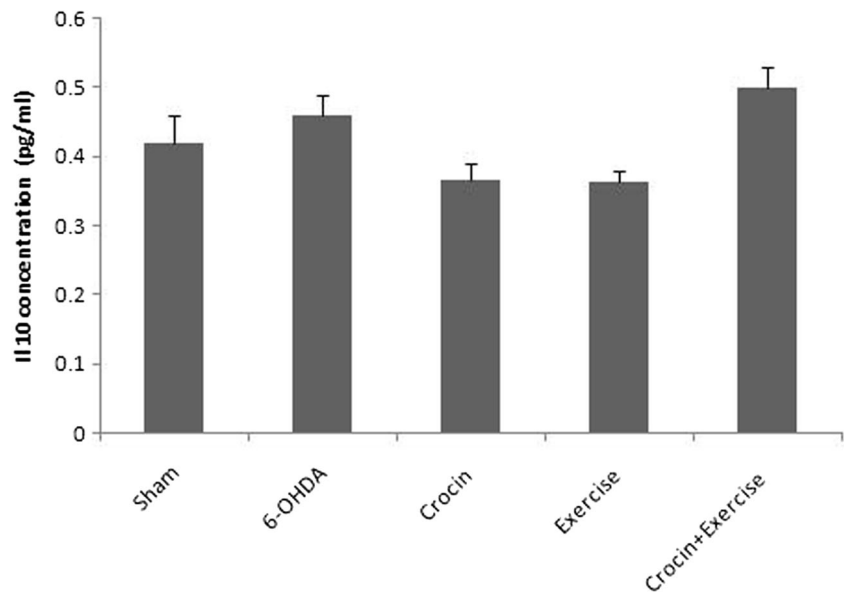

Fig. 4 TNF-α levels (mean ± SEM) in the striatum among the experimental groups at the end of week 6. Crocin was administered daily at a dose of 100 mg/kg for 7 weeks. n=7-8 for each group. **P<0.01 *vs* sham group, ++ P<0.01, +++ P<0.001 *vs* 6-OHDA-lesioned group

IL-10 levels

Statistical analysis showed that there was no significant
change in IL-10 levels in the striatum of sham and experimen-
tal groups at the end of week 6 (Fig. 5).

Lipid peroxidation levels

Lipid peroxidation levels in the hippocampus was quantified
by evaluating TBARS levels. A significant increase in the
levels of TBARS was found in the hippocampus of 6-
OHDA-lesioned rats ($P < 0.05$, Fig. 6) as compared with the
sham group. Moreover, pretreatment of lesioned rats with
crocin at a dose of 100 mg/kg reduced the TBARS levels in
the hippocampus at the end of week 6 ($P < 0.05$). However,
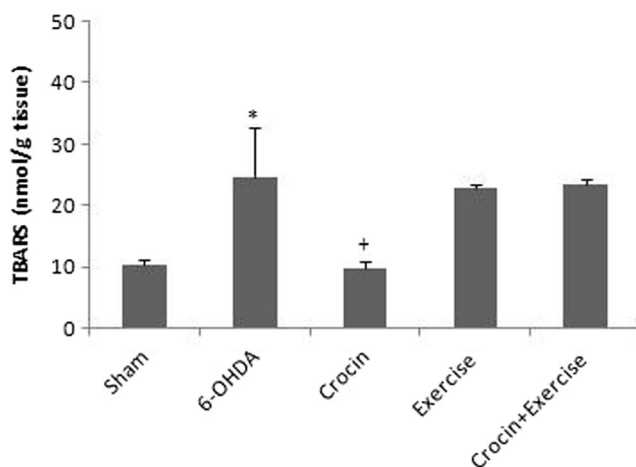treatment with crocin along with treadmill exercise and

exercise alone did not change the TBARS levels in the hippo-
campus at the end of week 6 (Fig. 6).

Total thiol concentration

Statistical analysis showed the total thiol concentration in
the hippocampus significantly decreased in 6-OHDA-
lesioned group as compared with sham group ($P < 0.01$,
Fig. 7). Pretreatment with crocin along with treadmill
exercise and exercise alone significantly increased total
thiol concentration as compared with 6-OHDA-lesioned
group ($P < 0.01$). However, treatment with crocin at a
dose of 100 mg/kg alone did not significantly change
the total thiol concentration as compared with 6-
OHDA-lesioned group (Fig. 7).

Fig. 6 Lipid peroxidation levels (mean ± SEM) in the hippocampus
among the experimental groups at the end of week 6. Crocin was
administered daily at a dose of 100 mg/kg for 7 weeks. n = 7–8 for each
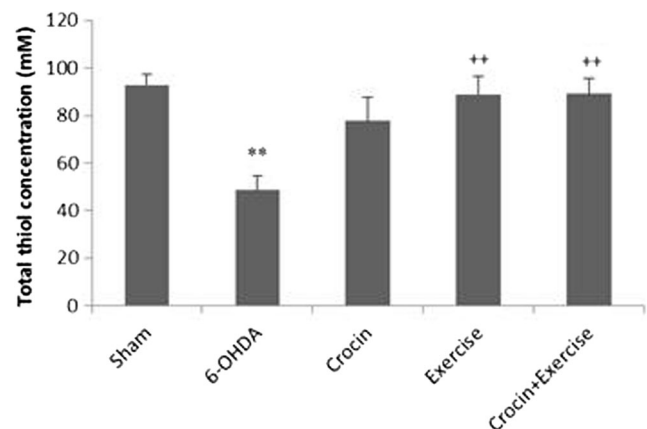group. *$P < 0.05$ vs sham group, +$P < 0.05$ vs 6-OHDA-lesioned group

Fig. 7 Total thiol concentration (mean ± SEM) in the hippocampus
among the experimental groups at the end of week 6. Crocin was
administered daily at a dose of 100 mg/kg for 7 weeks. n = 7–8 for each
group. **$P < 0.01$ vs sham group, ++$P < 0.01$ vs 6-OHDA-lesioned
group

Discussion

Parkinson's disease is a progressive neurodegenerative disorder with motor symptoms, which is due to the death of dopaminergic neurons in the substantia nigra and dysfunction of basal ganglia (Shuman et al. 2011). Studies have shown that PD is a multifactorial disease and several factors, such as; oxidative stress (Olanow 2007), mitochondrial dysfunction (Subramaniam and Chesselet 2013), and neuroinflammation (Tansey et al. 2007) are considered to be effective factors in the onset and progression of this disease. The neurotoxin 6-OHDA was used in the present study to induce Parkinson's animal model. 6-OHDA significantly increased apomorphine-induced rotations as compared with sham group animals. 6-OHDA possesses pro-oxidant activity which causes neurotoxicity. As a result of the auto-oxidation of this toxin in the extracellular space, reactive oxygen species are produced (Hanrott et al. 2006). Excessive amounts of ROS and subsequent oxidative stress leads to neuronal damage and death through membrane lipid peroxidation, protein oxidation, and DNA damage (Sayre et al. 2007). Interestingly, 6-OHDA lesion also results in an inflammatory response (Cicchetti et al. 2002) that can only be controlled approximately 12 days after 6-OHDA lesion (Sanchez-Pernaute et al. 2004). Microgliosis and increased pro-inflammatory mediators in the brain are found in the 6-OHDA PD model (Cicchetti et al. 2002; Nagatsu and Sawada 2005; McCoy et al. 2006). Our results showed that microinjection of 6-OHDA into MFB increased TNF-α levels in the striatum. This is in line with previous studies which have reported the increased levels of cytokines, in particular TNF-α, in the substantia nigra and striatum of 6-OHDA-lesioned rats (Ros-Bernal et al. 2011). Pharmacological evidence implicates TNF-dependent events in death of dopaminergic neurons. For instance, it has been reported that inhibition of soluble TNF signaling for 2 weeks with a dominant negative TNF inhibitor attenuated 6-OHDA induced dopaminergic neuron loss (McCoy et al. 2006).

In the current study, injury to the nigrostriatal dopaminergic neurons following injection of 6-OHDA into MFB was assessed by injection of apomorphine, a dopamine receptor agonist. Following injury, dopamine levels in the striatum is decreased and subsequently dopamine postsynaptic receptors are up-regulated at the lesioned-side. These changes lead to a marked performance and motor asymmetry that can be assessed by apomorphine as a rotational behavior (Schwarting and Huston 1997). Our findings also showed a significant decrease of the apomorphine-induced rotations in crocin group as compared with 6-OHDA group. The improvement effect of crocin on motor deficits could be partly attributed to anti-inflammatory effect of crocin. As shown in results, crocin reduced TNFα levels in striatum. To our knowledge, this is the first study reporting the anti-inflammatory effects of crocin by reducing TNF-α levels in a 6-OHDA

model of PD. In agreement with this, it has been reported that crocin reduced the LPS-stimulated production of TNFα, interleukin-1β, and intracellular reactive oxygen species from activated microglia (Nam et al. 2008). Moreover, anti-inflammatory effects of crocin by reducing TNF-α have been reported in other inflammatory insults, such as; rheumatoid arthritis (Li et al. 2017) and hemorrhagic shock (Yang and Dong 2017). Accordingly, we can conclude that the neuroprotective effect of crocin in the striatum of parkinsonian rats could be partly due to its anti-inflammatory activity.

Our data also showed that treadmill exercise reduced TNFα levels in the striatum of 6-OHDA-lesioned rats, reinforcing reports which have shown anti-inflammatory effects of exercise in PD model (Tuon et al. 2015; Koo et al. 2017). For instance, Tuon and colleagues have reported that 8 weeks of treadmill exercise improved motor deficits and decreased TNF-α and interleukin-1β expression in the substantia nigra pars compacta and striatum in MPTP-induced mouse model of PD (Tuon et al. 2015).

Behavioural analysis showed that treadmill training for 7 weeks did not significantly attenuate the apomorphine-induced rotations in hemiparkinsonian rats, however, there was a tendency toward a decrease in rotations in the exercise group (Fig. 1), suggesting that exercise could have a modest neuroprotective effect against this neurotoxin insult. In this context, several studies have shown that treadmill exercise improved motor deficits in 6-OHDA-lesioned rat (Tuon et al. 2012), while others have reported that treadmill training does not ameliorate locomotor deficits in the 6-OHDA model of PD (Poulton and Muir 2005; Pothakos et al. 2009). This discrepancy in experimental results could be due to differences in experimental method, including; severity of the nigrostriatal lesion, duration and intensity of the applied exercise regimen. For instance, Petzinger and colleagues have shown that the degree of nigrostriatal neurons lesion could alter the influence of exercise on dopamine level (Petzinger et al. 2007). Furthermore, Lau and colleagues reported that exercise performance 1 week before, 5 weeks during, and 12 weeks (a total of 18 weeks) after injury improved motor activity (Lau et al. 2011). According to our results, treatment with crocin along with exercise significantly reduced the number of rotations as compared with 6-OHDA-lesioned group. Collectively, it could be concluded that improvement of motor activity in this group could be due to anti-inflammatory effects of crocin and exercise in 6-OHDA model of PD.

In addition to motor deficits, PD patients also suffer from cognitive impairments (Lang and Lozano 1998). Cognitive impairments gradually occur due to the spread of damage to the other parts of the brain (Shuman et al. 2011; Chaudhuri and Martinez-Martin 2008). In the present study, 6-OHDA injections also produced memory deficit, which acts by increasing oxidative stress within the brain of rats. Previous studies have also demonstrated that 6-OHDA could produce

cognitive impairments in animals, and oxidative stress has been shown to play an important role in memory impairment (Hritcu et al. 2008). Reactive oxygen species induced by 6-OHDA can react with biological target molecules and contribute to increased neuronal damage and death through protein oxidation, DNA damage, and peroxidation of membrane lipids. In our study, the passive avoidance and elevated plus maze tests were used to examine whether crocin and treadmill exercise could improve aversive and spatial memories of parkinsonian rats. The first task is based on the motivation of passive avoidance from the fear of foot shock. The principle of elevated plus maze test is based on aversion of rodents to open spaces and heights. The animals prefer the enclosed, protected areas of the maze. Our results showed that treatment with crocin along with exercise training improved spatial and aversive memory deficits induced by 6-OHDA. However, crocin treatment and exercise alone partially improved memory deficits. Memory-enhancing effects of these agents could be due to their synergism effects on redox system in the hippocampus. As shown in results section, crocin at a dose of 100 mg/kg significantly reduced TBARS levels in the hippocampus. Also, treadmill exercise for 7 weeks increased total thiol concentration, as an index of total antioxidant potential, in the hippocampus. In agreement with this, it has been reported that aerobic exercise for 4 weeks increased the level of antioxidant enzymes, such as; superoxide dismutase and catalase in the hippocampus of young rats (Macêdo et al. 2017). Also, it has been reported that crocin possesses remarkable radical scavenging activity (Assimopoulou et al. 2005) and its antioxidant effects stronger than those of alpha-tocopherol (Ochiai et al. 2004). Therefore, the beneficial effect of crocin along with exercise on memory function could be partly due to its antioxidant activity, so that they can overcome the destructive effects of 6-OHDA and improve memory deficits.

In conclusion, our findings indicated that pretreatment with crocin along with treadmill exercise ameliorated motor and memory deficits induced by 6-OHDA. These effects could be due to antioxidant and anti-inflammatory activities of crocin and exercise in a 6-OHDA model of PD. The results suggest that combined therapy with crocin and exercise may be protective for motor and memory functions in PD patients.

Compliance with ethical standards

Conflict of interest None.

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