Crocin prevents haloperidol-induced orofacial dyskinesia: possible an antioxidant mechanism

Marzyeh Kamyar [1], Bibi Marjan Razavi [2], Faezeh Vahdati Hasani [1], Soghra Mehri [3], Amir Foroutanfar [1], Hossein Hosseinzadeh [3]*

[1] School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
[2] Targeted Drug Delivery Research Center, Department of Pharmacodynamy and Toxicology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
[3] Pharmaceutical Research Center, Department of Pharmacodynamy and Toxicology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran

A R T I C L E I N F O	A B S T R A C T
	Objective(s): Long-term treatment with antipsychotics causes serious side effects such as tardive dyskinesia that characterized by abnormal movements in the orofacial region. Oxidative stress in the brain specific area is implicated in the pathophysiology of tardive dyskinesia. In this study the protective effect of crocin on haloperidol-induced orofacial dyskinesia was evaluated. ***Materials and Methods:*** Haloperidol (1 mg/kg, IP) and crocin (10, 20 and 40 mg/kg, IP) were administrated to rats for 21 days. Behavioral assessments such as orofacial dyskinesia movements, open field test and elevated plus maze (EPM) were evaluated every week. Malondealdehyde (MDA) and glutathione (GSH) levels in the hippocampus, cortex and striatum were also measured. ***Results:*** Haloperidol increased vacuous chewing movements (VCMs) and tongue protrusions (TPs) in rats and co-administration of crocin (20 and 40 mg/kg) significantly reduced them. Furthermore, haloperidol decreased the locomotor and exploratory activities (rearing) in the open field test and decreased the percentage of entries into open arms and the percentage of the time spent on open arms in the EPM. Pretreatment with crocin (10 mg/kg) modified haloperidol effects on these behavioral parameters. Haloperidol induced lipid peroxidation in three brain regions, whereas crocin co-administration reduced the MDA and restored the decreased GSH levels. ***Conclusion:*** Our finding suggests that oxidative stress has an important role in the development of tardive dyskinesia. Crocin showed protective effect against haloperidol induced tardive dyskinesia and as a potent naturally antioxidant could be a new and useful drug and a possible therapeutic option for the treatment of tardive dyskinesia.

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Introduction

Haloperidol is a typical antipsychotic belongs to the group of butyrophenone that is highly potent and commonly used in the treatment of both acute and chronic schizophrenia. Therapeutic effect of haloperidol and other typical antipsychotics is related to the blockade of dopamine D_2 receptors especially in mesocortical and mesolimbic system in the brain (1, 2). Blockade of D_2 receptors in the striatum and nigrostriatal system can also occur and cause movement disorders including tardive dyskinesia (2-4). TD is a potentially irreversible syndrome consisting of athetoid, repetitive, rhythmic and abnormal involuntary movements in the face, especially in the mouth region (5-7). TD is an important clinical problem that develops during antipsychotic treatment (8). The mechanisms involved in tardive dyskinesia development poorly understood, but several hypothesis have been proposed to explain TD pathophysiology include dopamine receptors supersensitivity, dysfunction of striatonigral GABAergic neurons, imbalance between dopaminergic and cholinergic systems, maladaptive synaptic plasticity and striatal neurodegenerative hypothesis (7, 9-13). It has been reported that oxidative stress and free radical production induced by chronic treatment with antipsychotics have an important role in TD pathophysiology (14, 15). In previous studies, rats treated with haloperidol showed higher levels of TBARS in striatum and some regions of the brain (15-17). Treatment with haloperidol causes an increase in dopamine turnover by blocking the dopamine receptors that leads to overproduction of free radicals by enzymatic

Corresponding author: Hossein Hosseinzadeh. Pharmaceutical Research Center, Department of Pharmacodynamy and Toxicology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran. Tel: +98-51-38819042; Fax: +98-51-38823251; email: hosseinzadehh@mums.ac.ir

dopamine metabolism (18). Furthermore, haloperidol through its toxic metabolites such as RHPP[+] and HP[+] inhibits complex I in mitochondrial respiration chain that is associated with generation of reactive oxygen species (ROS) and oxidative stress (16, 19).

Crocin is a water-soluble bioactive carotenoid constituent of *Crocus sativus* L. (saffron) (20-23). Pharmacological studies have demonstrated that crocin possesses various beneficial effects such as antidepressant (24-26), hypolipidemic (27), anti-inflammatory (28, 29), antitumor (30), radical scavenger (31), neuroprotective (32, 33) and antioxidant effect (34-36). It has been reported that crocin prevented the death of PC-12 cells deprived of serum/glucose by inhibition of lipid peroxidation, increasing SOD activity and increasing GSH synthesis by enhancing the GR and γ-GCS activities (37, 38). The antioxidant effect of crocin was more potent than α-tocopherol (37). Crocin can inhibit aggregation and deposition of amyloid β- peptide fibrils and development of Alzheimer's disease by its antioxidant effects (39). Crocin also caused decreasing MDA level and increasing total thiol and glutathione peroxidase activity and attenuated renal oxidative stress and nephrotoxicity induced by cisplatin in rats (40). Crocin has shown protective effects against chronic stress-induced oxidative stress damage to the hippocampus and impairment of learning and memory in rats (34). Furthermore preventive effects of crocin on diazinon, acrylamide and acrolein induced oxidative stress have been studied (41, 42).

It has been observed that natural antioxidants such as *Bauhinia forficata (43)* and curcumin (44) have protective effect against orofacial dyskinesia induced by haloperidol. Therefore, the aim of this study was to investigate the protective effect of crocin on orofacial dyskinesia and oxidative stress markers in different brain regions induced by haloperidol in rats.

Materials and Methods

Animals
Male Wistar albino rats weighing between 200-250 g bred in Animal House of Mashhad University of Medical Sciences, School of Pharmacy, Iran, were used. The animals were kept in standard plastic cages in a colony room maintained on a 12-h light/dark cycle with a temperature of 21 ± 2°C and 40-50% humidity conditions and were allowed free access to water and food except in the time of behavioral assessment. The experimental protocols was approved by the ethical committee of Mashhad University of Medical Sciences and the animals were maintained and used in accordance to the institutional guidelines for the use and care of laboratory animals.

Crocin preparation
Stigmas of saffron were purchased from Novin Saffron Co. (Mashhad, Iran). Crocin was extracted and purified as previously described (45). 25 ml ethanol 80% was added to 10 g saffron stigmas powders at 0 °C and was suspended by vortex (2 min). The suspension was centrifuged at 1500 g for 10 min and then the supernatant was separated. The extraction was repeated 6 other times by addition of 25 ml ethanol 80% to precipitate. The resulting extract was kept in a thick walled glass container which placed at temperature of −5 °C in darkness for 24 days. The formed crystals were separated from the solution and washed with acetone to remove remaining water and then were dissolved in 120 ml ethanol 80% and kept at −5 °C for 20 extra days in darkness. The final amount of obtained crocin was 1.02 g and the purity of yielded crystals was determined by spectrophotometry and HPLC and was more than 97%.

Drugs and treatment schedules
Haloperidol obtained from Caspian tamin, Rasht, Iran and was dissolved in saline. Crocin was suspended in distilled water. All drugs and agents were administered once daily intraperitoneally (IP) for a period of 21 days.

The rats were randomly divided into 7 groups, consisted of six rats in each group: First group (negative control) received saline as the vehicle (1 ml/kg), second group received haloperidol (1 mg/kg), third group received haloperidol (1 mg/kg) plus crocin (10 mg/kg), fourth group received haloperidol (1 mg/kg) plus crocin (20 mg/kg), fifth group received haloperidol (1 mg/kg) plus crocin (40 mg/kg) (26), sixth group received only crocin (40 mg/kg) and last group (positive control) received haloperidol (1 mg/kg) plus vitamin E (200 mg/kg).

Induction of orofacial dyskinesia
For the induction of oral dyskinesia, haloperidol (1 mg/kg; IP) was administered to rats for a period of 21 days (46-48).

Behavioral assessments
All the behavioral assessments were carried out in the first day (before the haloperidol administration), 11th day of study, and last behavioral assessment was done 24 hr after the last dose of haloperidol (22nd day) between 09:00 AM and 02:00 PM. The interval between different behavioral tests was 1 hr.

Assessment of orofacial dyskinesia
Rats were placed individually in a small transparent cage for the measurement of VCMs and TP in which mirrors were placed in the floor and back wall to enable better observation of VCM and TP when the rat was faced away from the observer. Rats were allowed 10 min to get acclimatized to the cage before behavioral assessments were carried out. The number of VCMs and TP were counted continuously during a 5 min observation period after adaptation and counting was stopped when the rat began grooming. VCMs are defined to as single mouth openings which resembled

chewing, with or without TP, not directed toward any particular physical stimulus (48).

Open field test

This test was carried out to analyze changes in spontaneous locomotors and exploratory activity caused by haloperidol and/or crocin administration. Each rat was placed in the center of an open field arena (100× 100 cm). The apparatus had a white floor divided by black lines into 25 squares and black plywood walls. The number of line crossings and rearing was measured during a period of 5 min (49).

Elevated plus maze test

The elevated plus maze was used to evaluate the anxiety like state caused by haloperidol and crocin treatment. The apparatus consisted of two open arms and two closed arms and was located 50 cm above the floor. The rat was placed at the center of the plus maze and the number of the entries into the open arms and the time spent on the open arms were recorded during a period of 5 min by Maze router Software. The percentage of the entries into the open arms and the percentage of the time spent on the open arms were calculated by the below formula:

Number of the entries into the open arms and the time spent on the open arms/ total number or total time of the entries into open and closed arms × 100 (50).

Tissue dissection

On the 22 nd day of haloperidol treatment, 24 h after the last haloperidol injection and after last behavioral assessments, the rats were sacrificed by decapitation. The brain was removed and placed on ice. The striatum, hippocampus and cortex were rapidly separated, washed by ice-cold saline and stored at – 80 °C for subsequent biochemical analysis.

Lipid peroxidation assay

The amount of MDA, as an index of lipid peroxidation, was measured by the reaction with thiobarbituric acid (TBA) at 532 nm using spectrophotometer. The tissues weighed and were immediately homogenized on ice with cold 1.15% KC1 to prepare a 10% tissue homogenate. 500 µl of homogenate was pipetted into a tube and 3 ml 1% phosphoric acid and 1 ml 0.6% TBA was added to the tube. Then the mixture placed in a boiling water bath for 45 min. The tubes were cooled and 4 ml n-butanol was added and mixed by vortex for 1 min. The n-butanol phase was separated by centrifugation at 3000 g for 20 min and absorbance of colored layer was measured at 532 nm. The standard curve of MDA was constructed over the concentration range of 0-100 nmol/ml and levels of MDA were expressed as nmol/g tissue (51, 52).

Estimation of reduced glutathione

The tissues weighed and were immediately homogenized on ice with phosphate buffer (pH 7.4) to prepare a 10% tissue homogenate. 0.5 ml 10% TCA was added to 0.5 ml of tissue homogenate to precipitate it. After vortex, the mixture was centrifuged for removing the precipitate. 2.5 ml phosphate buffer (pH 8) and 0.5 ml DTNB (5-5'-DithioBis-(2-Nitrobenzoic acid)) was added to supernatant and absorbance of the sample was read immediately at 412 nm. The standard curve of GSH was constructed over the concentration range of 0-150 nmol/ml and the amount of GSH was expressed as nanomol/g tissue (53).

Statistical analysis

The data had normal distribution and were analyzed using Graph Pad Prism version 6.00 by analysis of variance (ANOVA) followed by Tukey-Kramer post test for multiple comparisons. All results are expressed as mean±SD and the differences between groups were considered statistically significant when $P<0.05$.

Results

Behavioral assessments results

Effect of crocin on VCMs and TPs induced by haloperidol treatment
Haloperidol (1 mg/kg, IP) administration significantly increased VCMs and TPs as compared to control group on day 22nd of experiment ($P<0.001$ and $P<0.01$). Crocin co-administration (20 and 40 mg/kg) and Vit. E attenuated the VCMs and tongue protrusions as compared to haloperidol group ($P<0.01$, $P<0.05$) on day 22nd of experiment. Crocin (40 mg/kg) alone did not cause any significant increase in VCMs and tongue protrusions as compared to control group (Figure 1, 2).
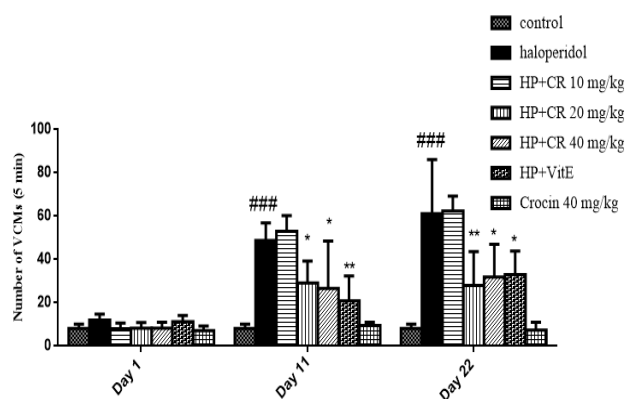

Figure 1. Effect of crocin (CR) on haloperidol (HP) induced vacuous chewing movements (VCMs) recorded on day 1, 11, 22 in rats. Data analyzed by ANOVA followed by Tukey-Kramer post test and is expressed as mean±SD (n=6). ### $P<0.001$ as compared to control group and * $P<0.05$ and ** $P<0.01$ as compared to haloperidol group
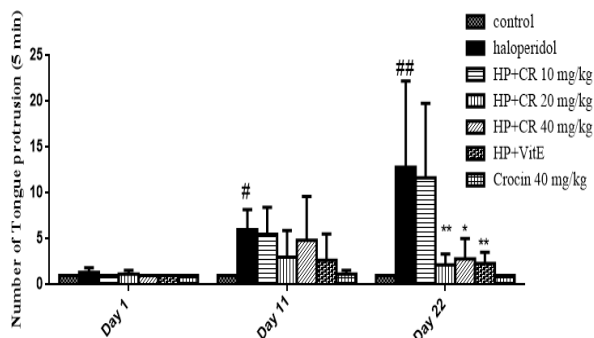
Figure2. Effect of crocin (CR) on haloperidol (HP) induced tongue protrusions (TPs) recorded on day 1, 11, 22 in rats. Data analyzed by ANOVA followed by Tukey-Kramer post test and is expressed as mean±SD (n=6). #P<0.05 and ## P<0.01 as compared to control group and * P<0.05 and ** P<0.01 as compared to haloperidol group

Effects of haloperidol and crocin on locomotor activity

Haloperidol (1 mg/kg, IP) treatment caused a decrease in number of crossings in the open field test as compared to control group on day 22nd of experiment (*P*<0.05). Pretreatment with crocin (10 mg/kg) prevented this decrease (*P*<0.01). Crocin (40 mg/kg) alone did not cause any significant change in the number of crossing as compared to control group (Figure 3).

Effects of haloperidol and crocin on stereotypic rearing behavior

Haloperidol (1 mg/kg, IP) treatment resulted in a decrease in rearing in the open field test as compared to control group on day 22nd of experiment (*P<0*.001). Pretreatment with crocin (10 mg/kg) inhibited this decrease in rearing (*P*<0.01). Crocin (40 mg/kg) alone did not cause any significant change in rearing as compared to control group (Figure 4).

Effect of haloperidol and crocin on elevated plus maze test

Haloperidol (1 mg/kg, IP) treatment significantly decreased the percentage of the time spent on the open
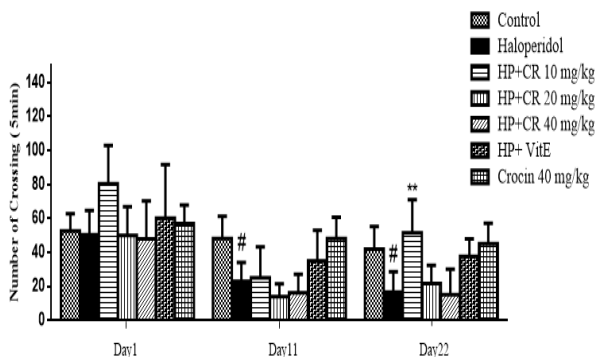

Figure 3. Effect of haloperidol (HP) and crocin (CR) on number of crossing. Data analyzed by ANOVA followed by Tukey-kramer post test and is expressed as mean ± SD (n=6). # P<0.05 as compared to control group and ** P<0.01 as compared to haloperidol group
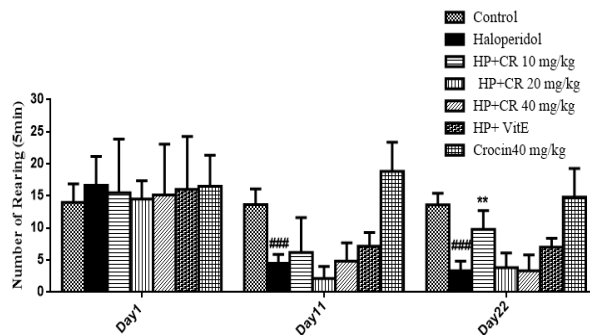

Figure4. Effect of haloperidol (HP) and crocin (CR) on number of stereotypic rearing behavior. Data analyzed by ANOVA followed by Tukey-kramer post test and is expressed as mean±SD (n=6). ### *P*<0.001 as compared to control group and ** *P*<0.01 as compared to haloperidol group

arms as compared to control group (*P*<0.001). Co-administration of crocin could not prevent this effect of haloperidol. Crocin (40 mg/kg) alone did not cause any significant change in the % time spent on the open arms as compared to control group (Figure 5).

Furthermore, haloperidol administration caused a significant decrease in the percentage of entries into the open arms as compared to control group (*P*<0.01). Crocin (10 mg/kg) prevented this decrease as compared to haloperidol group (*P*<0.05) on day 22nd of experiment. Crocin (40 mg/kg) alone did not cause any significant change in the % entries into the open arms as compared to control group (Figure 6).
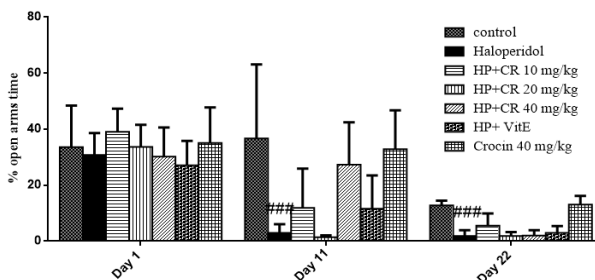

Figure5. Effect of haloperidol (HP) and crocin (CR) on % time spent on the open arms. Data analyzed by ANOVA followed by Tukey-kramer post test and is expressed as mean±SD (n=6). ### *P*<0.001 as compared to control group
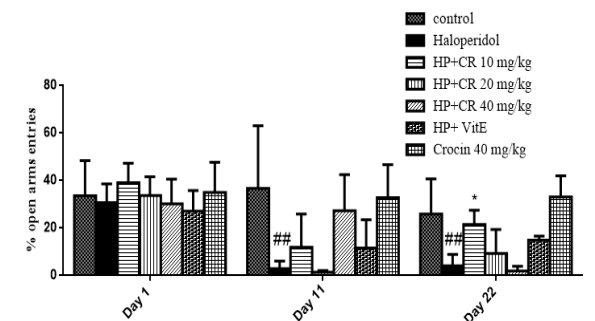

Figure 6. Effect of haloperidol (HP) and crocin (CR) on % entries into the open arms. Data analyzed by ANOVA followed by Tukey-kramer post test and is expressed as mean±SD (n=6). ## *P*<0.01 as compared to control group and * *P*<0.05 as compared to haloperidol group
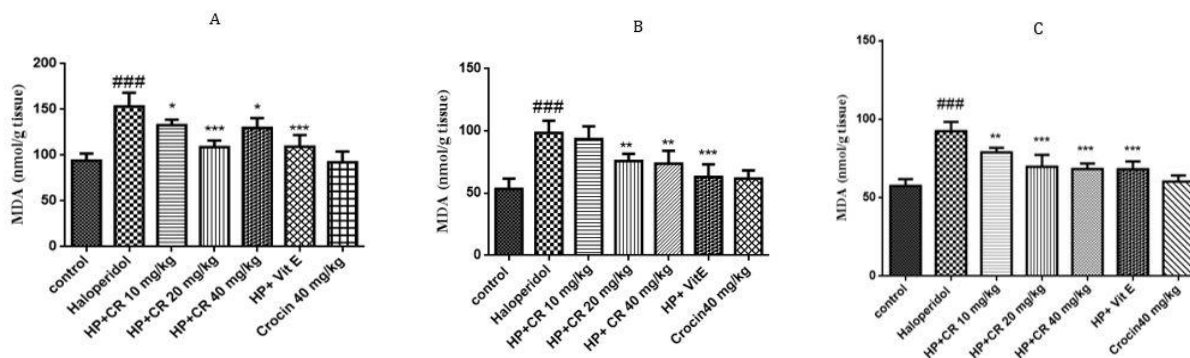
Figure 7. Effect of haloperidol (HP) and crocin (CR) on MDA level in cortex (A), striatum (B) and hippocampus (C) of rat's brain. Data analyzed by ANOVA followed by Tukey-kramer post test and is expressed as mean±SD (n=6). ### $P<0.001$ as compared to control group and * $P<0.05$, **$P<0.01$ and *** $P<0.001$ as compared to haloperidol group

Biochemical analysis results

Effect of haloperidol and crocin on MDA levels in striatum, cortex and hippocampus

Haloperidol treatment (1 mg/kg, IP) caused a significant increase in MDA level in cortex, striatum and hippocampus of the rat's brain as compared to control group ($P<0.001$). Co-administration of crocin (10, 20 and 40 mg/kg) and Vit. E decreased the elevated level of MDA in three regions of the brain as compared to haloperidol group. Crocin (40 mg/kg) alone did not cause any significant change in MDA level as compared to control group (Figure 7).

Effect of haloperidol and crocin on glutathione (GSH) levels in striatum, cortex and hippocampus

Haloperidol treatment (1 mg/kg, IP) resulted in a significant decrease in GSH level in cortex, striatum and hippocampus of the rat's brain as compared to control group ($P<0.001$). Co-administration of crocin (10,20 and 40 mg/kg) and Vit. E increased GSH level in three regions of the brain as compared to haloperidol group. Crocin (40 mg/kg) alone did not cause any significant change in GSH level as compared to control group (Figure 8).

Discussion

Oxidative stress defines as an imbalance between production of oxidant (such as ROS and RNS) and antioxidant defense system. Lipids are the main bio-

logical target of oxidative stress and oxidation of lipids produces a number of secondary products. MDA is the main and principal product of lipid peroxidation and is a highly toxic molecule (54, 55). In the present study to evaluate the effect of haloperidol and crocin in induction of oxidative stress, the amount of MDA (as marker of lipid peroxidation) and glutathione (GSH) levels were measured in three region of the brain (striatum, cortex and hippocampus). Haloperidol treatment caused an increase in MDA level and decrease in GSH content in three region of the brain as compared to control group. Crocin decreased the elevated level of MDA and increased the glutathione content in haloperidol-treated rats. Administration of haloperidol also increased VCMs and TPs in rats. Co-administration of crocin significantly attenuated the induction of haloperidol-induced VCMs and TPs. In several studies using animal model of tardive dyskinesia (orofacial dyskinesia), administration of haloperidol caused an increase in MDA level and induction of VCMs and TPs (48, 56). Treatment with antioxidant compounds such as melatonin seems to be effective in the reduction of orofacial dyskinesia (46, 47, 57, 58). Crocin is a potent antioxidant and its antioxidant activity is responsible for the various pharmacological effects (59).

Existing evidences indicate that oxidative stress
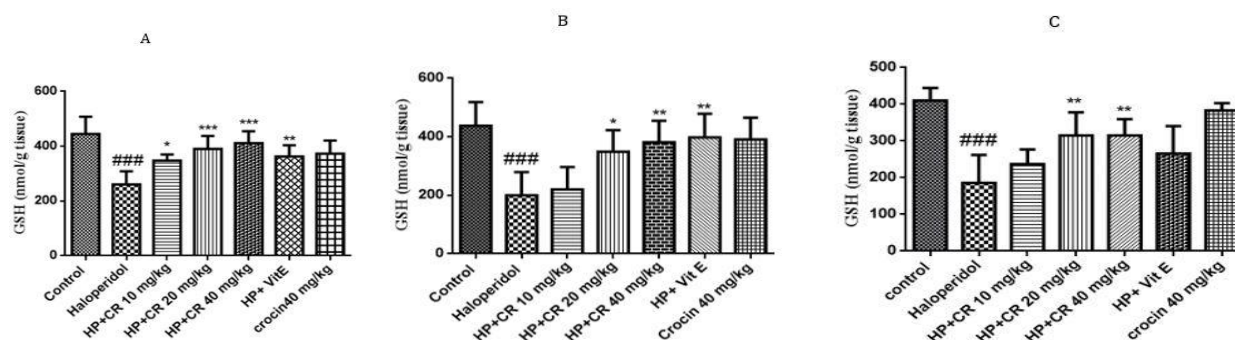

Figure 8. Effect of haloperidol (HP) and crocin (CR) on reduced glutathione (GSH) level in cortex (A), striatum (B) and hippocampus (C) of rat's brain. Data analyzed by ANOVA followed by Tukey-kramer post test and is expressed as mean±SD (n=6). ### $P<0.001$ as compared to control group and * $P<0.05$, ** $P<0.01$ and *** $P<0.001$ as compared to haloperidol group

induced by antipsychotic drugs contributes in the pathogenesis of tardive dyskinesia (60, 61). The brain is particularly vulnerable to oxidative stress damage because it uses high amount of energy and oxygen and contains large amount of oxidizable substrates such as catecholamines such as dopamine and polyunsaturated fatty acids which are targets for activity of lipid peroxidation cascades. Low levels of antioxidant enzymes such as catalase and high concentrations of transition metals such as iron, manganese and copper (which involve in free radical formation) in the brain also lead to vulnerability of brain to free radical damages (62, 63). Blockade of dopamine D_2 receptors by typical antipsychotic such as haloperidol led to a secondary increase in synthesis and metabolism of dopamine. This excess dopamine is metabolized by MAO which leads to overproduction of hydrogen peroxide and hydroxyl radicals or can auto-oxidize to form dopamine quinones that are free radicals. It has been demonstrated that basal ganglia, a region of the brain that involves in motor function, is the main region implicated in tardive dyskinesia pathophysiology. The basal ganglia are rich of dopamine, oxygen, polyunsaturated membrane lipids and transition metals such as iron and produces excessive free radicals that leads to weakening of antioxidant brain system (5, 7, 14, 64).

It was reported that resveratrol, a potent antioxidant and monoamine oxidase enzyme activity inhibitor, showed protective effect against reserpine-induced orofacial dyskinesia (65). De Monte *et al* (2014) reported that crocin inhibited both MAO enzyme isoforms with non-competitive mechanism (66). Therefore, the protective effect of crocin on haloperidol-induced TD may be due to its inhibitory effect on MAO enzyme and decrease in dopamine metabolism. Dopamine quinones, cytotoxic metabolites of dopamine, also generate by enzymatic oxidation of dopamine by cyclooxygenase (COX) enzymes. NSAIDs drugs prevent oxidation of dopamine to dopamine quinone by inhibition of COX enzymes like prostaglandin H synthase (67). Recently it was reported that indomethacin, a nonselective cyclooxygenase enzyme inhibitor, reversed haloperidol-induced orofacial dyskinesia in rats (68). Crocin exhibited an inhibitory effect on COX-1 and COX-2 enzymes and its protective effect may be due to its COX inhibitory activity (69).

The involvement of nitric oxide (NO) in development of haloperidol-induced orofacial dyskinesia has been reported. L-NAME, a nitric oxide synthase inhibitor, suppressed haloperidol-induced VCMs (70). Crocin decreased in NO content and inhibited nitric oxide synthase (NOS) activity and inducible nitric oxide synthase (iNOS) expression (71, 72) So, the beneficial effect of crocin might be due to its inhibitory effect on NO and NOS activity.

Pharmacological evidence has demonstrated that glutamergic system participated in pathophysiology of tardive dyskinesia. The reuptake of glutamate from the extracellular space is mediated by transporter proteins which contain reactive thiol groups in their structure. Oxidation of these groups leads to reduction in glutamate uptake. Reduction in glutamate transporter activity and glutamate uptake by haloperidol leads to extracellular increase in glutamate level which resulted in excitotoxicity and development of tardive dyskinesia (73). Further blockade of the presynaptic dopamine D_2 receptors, which prevents the release of glutamate from excitatory cortical striatal pathway, by antipsychotic drugs such as haloperidol leads to an increase in synaptic release of glutamate and aspartate in the striatum. It has been known that persistent activation of glutamate ionotropic receptors leads to neuronal degeneration (74). The glutamate neurotoxicity damages many cell components that lead to cell death. In this process reactive oxygen species (ROS) are generated and oxidative stress is involved in excitotoxicity induced by glutamate (73, 75). It was reported that dizocilpine, a non-competitive N-methyl-D-aspartate (NMDA) receptor antagonist, attenuated the haloperidol-induced orofacial dyskinesia (70). It has been demonstrated that safranal caused a reduction in extracellular glutamate concentration and saffron inhibited glutamatergic synaptic transmission (76, 77). The inhibitory effect of saffron and crocin on glutamatergic system has been indicated previously (78). As crocin hasn't any interaction with NMDA receptors (79), beneficial effect of crocin on haloperidol-induced TD may be due to reduction in extracellular glutamate concentration trough protection of glutamate transporter from oxidative stress damages or other mechanisms.

It was proposed that dysfunctional striatal γ-aminobutyric acid (GABA) containing neurons leading to decrease in inhibitory function of GABA in the basal ganglia and imbalance between direct and indirect basal ganglia pathways is implicated in the pathophysiology of tardive dyskinesia (10). Administration of haloperidol also caused a reduction in nigral L-glutamic acid decarboxylase (GAD) activity and GABA synthesizing enzyme, in rat with oral dyskinesia. This movement disorder is related to hypofunction in GABA-ergic neurons in substania nigra (80). Peixoto *et al* (2003) was reported that valproic acid, a GABA-mimetic drug, attenuated the orofacial dyskinesia in rats (81). In other study, co-administration of progabide (a GABA agonist) inhibited haloperidol-induced orofacial dyskinesia in rats (82). Saffron and its constituent crocin have neuroprotective effects and protected cells from apoptosis and death (83, 84). Crocin may protect GABA-ergic neurons from death and beneficial effect against TD may be due to its effects on GABA-ergic system.

In the present study, vitamin E decreased oxidative stress as well as VCMs and TPs induced by haloperidol administration. Vitamin E as an antioxidant and free radical scavenger was effective in the treatment of

tardive dyskinesia (85). Co-administration of vitamin E with haloperidol improved PC12 cell viability after haloperidol exposure and suggested the potential benefit of vitamin E for preventing of tardive dyskinesia (64). Abilio *et al* (2003) reported that vitamin E attenuated the reserpine-induced orofacial dyskinesia and increased ratio of oxidized/reduced glutathione ratio (GSSG/GSH) in striatum of rats (86).

The effects of haloperidol and crocin on locomotor activity and stereotypic rearing in the rats were also evaluated in an open field test. Haloperidol administration resulted in a significant decrease in locomotor activity (total crossing) and rearing and crocin co-administration caused an increase in these parameters. It is known that blockade of dopamine D_2 receptors and reduction of dopaminergic pathway function in nucleus accumbens led to suppression of locomotion and sedation by haloperidol (25, 87-89). It was reported that crocin increased stereotypic activity and locomotion in open field test which revealed that crocin can affect the dopaminergic system (25).

Effect of haloperidol in induction of anxiety was evaluated in this study by an elevated plus maze test. In this test anxiolytic compounds decrease the natural aversion of animals to the open arms. Therefore, increased the number of entry or time spent on the open arms reflect the anxiolytic effect of a compound (90). In the present study, administration of haloperidol decreased the percentage of time spent on the open arms. Crocin couldn't improve alteration of this parameter. Haloperidol also decreased the percentage of entry to the open arms which increased by crocin. Karl *et al* (2006) reported that haloperidol administration increased anxiety levels in rats (87). The anxiolytic-like effect of crocin has demonstrated previously (91) and therefore positive effect of crocin on the parameters studied in this study may be due to its anxiolytic effect that needs to more investigation.

Conclusion

The major findings of the present study suggested that oxidative stress plays an important role in the development of haloperidol-induced orofacial dyskinesia and crocin co-administration reverses the behavioral and biochemical changes induced by haloperidol. Crocin, a naturally antioxidant, could be considered as a useful agent for treatment of tardive dyskinesia.

Acknowledgment

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