Protective Effects of Crocin against Streptozotocin-Induced Oxidative Damage in Rat Striatum

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Abstract- The study aimed to investigate the protective effects of crocin (Cro) against streptozotocin (STZ)-induced oxidative damage in rat striatum. Animals were randomly divided into four groups (five each). Group 1 (sham) were treated with normal saline (2 ml/kg, p.o.). Group 2 (STZ-lesioned or lesion) were injected with ICV-STZ (3 mg/kg bilaterally, on day 1 and 3) and treated with normal saline (2 ml/kg, p.o.) respectively, for 21 days. Group 3 (sham+Cro) were injected ICV on day 1 and 3 with artificial CSF and treated with crocin (100 mg/kg, p.o.) for 21 days. Group 4 (lesion+Cro) were injected with ICV STZ (3 mg/kg bilaterally, on day 1 and 3) and treated with crocin (100 mg/kg, p.o.) for 21 days. The homogenized striatum was used for measuring malondialdehyde (MDA), and total thiol contents besides glutathione peroxidase (GPx) activity. Crocin treatment resulted in a significant reduction in MDA concentration as compared to the STZ-lesioned rats. Moreover, crocin produced a significant elevation in total thiol content and GPx activity, as compared with STZ-lesioned group. The present findings provide evidence that crocin may have a therapeutic significance for neurodegenerative diseases such as Alzheimer's disease (AD).

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Keywords: Crocin; Streptozotocin; Oxidative stress; Striatum; Rat

Introduction

Free radicals and oxidative stress have been implicated as the prime candidates mediating the behavioral impairments and memory deficits in age related neurodegenerative disorders such as Parkinson's disease and Alzheimer's disease (AD). Some of this damage may include lipid and protein peroxidation, increase in DNA oxidation products, and deficits in calcium regulatory mechanisms that may eventually lead to cell death (1).

Since oxidative damage is implicated in the etiology of neurological complications, treatment with antioxidants has been used as a therapeutic approach in various types of neurodegenerative diseases (2).

Intracerebroventricular (ICV) injection of streptozotocin (STZ), in a sub diabetogenic dose in the rat, has been found to cause prolonged impairment of brain glucose and energy metabolism and presence of oxidative stress. This is accompanied by impairment in learning and memory, in addition to decreased choline acetyltransferase, levels in the hippocampus (3).

Crocin is one of the active constituents of saffron (Crocus sativus) (4). Crocin exhibits a variety of pharmacological effects including inhibition of skin tumour growth (5), improvement of learning behavior previously impaired by ethanol or scopolamine (6,7), prevention of long-term potentiation inhibition caused by ethanol (8), anti-hyperlipidemic effect (9), treatment of colon adenocarcinoma (10), anti-atherosclerotic property (11), anti-oxidant effect in PC-12 cells by increasing GSH synthesis (4), the protective effect against inflammation-induced neurotoxicity (12), attenuation of reperfusion-induced oxidative/nitrative injury to cerebral microvessels after global ischemia (13) and protective effects against cisplatin-induced oxidative stress and nephrotoxicity (14,15).

It has been observed that the use of antioxidants, as well as dietary improvements with regard to the consumption of fruits and vegetables high in antioxidant activity and neuroprotective agents, may decrease the risk of memory deficits of AD (2). Thus, the present study was

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designed to investigate the protective effects of crocin on biochemical markers of oxidative stress induced by ICV STZ administration in the striatum of rats.

Materials and Methods

Animals

Adult male Wistar Albino rats weighing 250-300 g were used throughout the study. All animals were obtained from the Animal House of the Ahvaz Jundishapur University of Medical Sciences (Ahvaz, Iran). Animals were put separately in the cages in an air-conditioned unit and allowed free access to standard laboratory chow and water, ad libitum. Animals were housed in colony room 12/12 h light/dark cycle at 22 ± 2°C and had free access to water and food ad libitum. All animal experiments were performed in accordance with the Ahvaz University of Medical Sciences (Ahvaz, Iran), Ethical Committee Acts.

Chemicals

1,1,3,3- tetramethoxypropane (purity: 99%), Streptozotocin, DTNB (5, 5'-dithiobis-2-nitrobenzoic acid), 2-Thiobarbituric acid (TBA), n-butanol, Tris–HCl, Na_2EDTA, phosphoric acid and potassium chloride were obtained from Sigma (England). Crocin and GPx kit were purchased from Fluka (Japan) and Randox Company (England), respectively. All other chemicals were of analytical grade and purchased from Merck Company (Germany).

Experimental design

Animals were randomly divided into four groups (5 each). In group 1 (sham) rats were treated with normal saline (2 ml/kg, p.o.). In group 2 (lesion), rats were injected with ICV STZ (3 mg/kg bilaterally, on day 1 and 3) and treated with normal saline (2 ml/kg, p.o.). In group 3 (sham+Cro), rats were injected ICV with artificial CSF and treated with crocin (100 mg/kg, p.o.). In the last group (lesion+Cro), rats were injected with ICV STZ and treated with crocin (100 mg/kg, p.o.) once a day for 21 consecutive days as mentioned. On the day of ICV injections (days 1 and 3), crocin or normal saline was administered one hour prior to ICV injection. The dose of crocin (100 mg/kg), used in this study, has been obtained from previous experiments in our lab (15).

Intracerebroventricular administration of streptozotocin

Rats were anesthetized with the combination of ketamine/xylazine (60/6 mg/kg, i.p.). The head was positioned in a stereotactic frame, and a midline sagittal incision was made in the scalp. Burr holes were drilled in the skull on both sides over the lateral ventricles using the following coordinates: 0.8 mm posterior to bregma, 1.5 mm lateral to sagittal suture, and 3.6 mm beneath the surface of the brain. STZ (3 mg/kg) was injected ICV bilaterally on day 1 and 3 of the experiment (18). In the sham group, artificial CSF: 147 mM NaCl, 2.9 mM KCl, 1.6 mM MgCl2, 1.7 mM CaCl2 and 2.2 mM dextrose was injected (20 µl on each site) on the same days as STZ group.

STZ was dissolved in artificial CSF. Each rat was given 20-µl injection on each site. After euthanizing the animals, brains were removed, and the striatum was dissected for the biochemical studies. The tissues were homogenized in cold KCl solution (1.5%) to give 10% homogenate suspension used for biochemical assays including determination of malondialdehyde (MDA) and total thiol contents and measurement of glutathione peroxidase (GPx) activity.

Thiobarbituric acid reactive Thiobarbituric acid reactive species (TBARS) measurement

Malondialdehyde (MDA) levels, an index of lipid peroxidation, produced with free radicals were measured. MDA reacts with thiobarbituric acid as a thiobarbituric acid reactive substance to produce a red colored complex that has the peak absorbance at 532 nm. Briefly, 3 ml phosphoric acid (1%) and 1 ml TBA (0.6%) was added to 0.5ml of homogenate in a centrifuge tube and the mixture was heated for 45 min in a boiling water bath. After cooling, 4 ml n-butanol was added to the mixture and vortex-mixed for 1 min followed by centrifugation at 2000 rpm for 20 min. The colored layer was transferred to a fresh tube, and its absorbance was measured at 532 nm. The standard curve of MDA was constructed over the concentration range of 0-20 µM (16).

Total thiol (-SH) groups assay

Total -SH groups were measured using DTNB (5, 5´-dithiobis- 2-nitrobenzoic acid) as the reagent (17). This reagent reacts with the SH groups to produce a yellow colored complex which has a peak absorbance at 412 nm. Briefly, 1 ml Tris-EDTA buffer (pH 8.6) was added to 50 µl of homogenate in 2 ml cuvettes and absorbance was read at 412 nm against Tris-EDTA buffer alone (A1). Then, 20 µl DTNB reagents (10 mM in methanol) was added to the mixture and after 15 min (stored in laboratory temperature), the sample absorbance was read again (A2). The absorbance of

DTNB reagent was also read as a blank (B). Total thiol concentration (mM) was calculated from the following equation:

Total thiol concentration (mM) = (A2-A1-B) × 1.07/0.05 × 13.6

Determination of GSH peroxidase concentration

GSH peroxidase concentration was measured with the GSH peroxidase kit (Randox Company, England).

Statistical analysis

Results were presented as mean±SEM. Statistical differences were analyzed using ANOVA followed by Tukey's test. The p-value < 0.05 was considered statistically significant.

Result

Effect of Crocin on MDA levels in the striatum

The degree of free radical damage following STZ injection was assessed using lipid peroxidation (LPO), which was measured as MDA levels. According to Figure 1, there was an increase in MDA levels of STZ-lesioned group ($p<0.01$) as compared to sham-operated rats in the striatum. Oral administration of crocin resulted in a significant reduction of MDA levels in the striatum of lesion+Cro animals as compared to STZ-lesioned group ($p<0.05$).

Effect of Crocin on total thiol levels in the striatum

The total thiol concentration (mM) was measured to evaluate the non-enzymatic defense potential of the cell against the oxidative stress. According to Figure 2, total thiol levels in STZ-lesioned animals were found to be significantly depleted as compared to sham group animals in the striatum ($p<0.01$). Chronic treatment with crocin in lesion+Cro group was able to raise total thiol levels significantly as compared to STZ-lesioned animals ($p<0.05$).

Effect of Crocin on GPx activity in the striatum

GPx activity (u/L) was measured as the enzymatic defense potential of the cells against the oxidative stress. According to Figure 3, GPx activity significantly ($p<0.001$) decreased in STZ-lesioned group as compared to sham-operated group in the striatum. On the other hand, the decrease of GPx activity was significantly restored by crocin treatment in the striatum of lesion+Cro group ($p<0.001$).

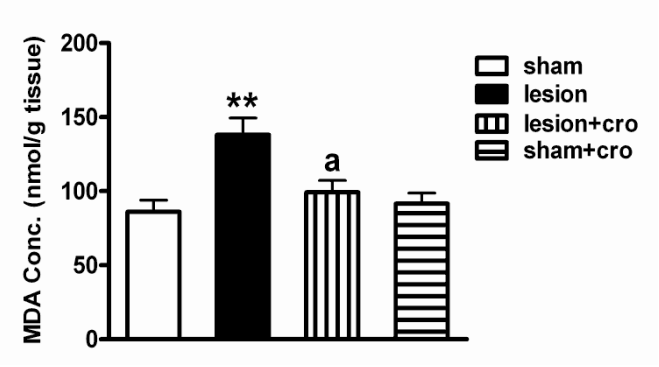

Figure 1. Effect of crocin on concentration of MDA in the striatum of STZ-lesioned rats. Values are expressed as mean±S.E.M. (n=5). **p<0.01 and [a]p<0.05 as compared to sham and STZ-lesioned groups, respectively (one-way ANOVA followed by Tukey's test).

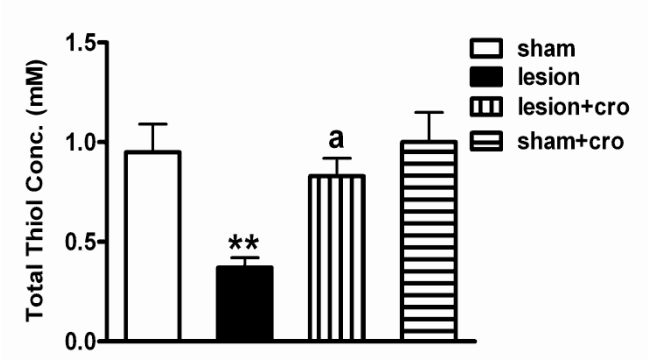

Figure 2. Effect of crocin on concentration of total thiol in the striatum of STZ-lesioned rats. Values are expressed as mean±S.E.M. (n=5). **p<0.01 and [a]p<0.05 as compared to sham and STZ-lesioned groups, respectively (one-way ANOVA followed by Tukey's test.

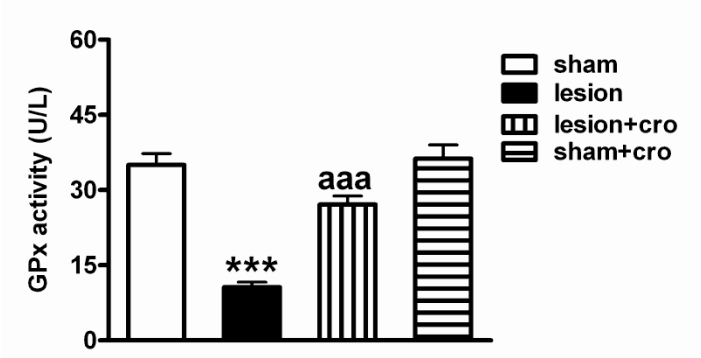

Figure 3. Effect of crocin on GPx activity in the striatum of STZ-lesioned rats. Values are expressed as mean±S.E.M. (n=5). ***p<0.001 and [aaa]p<0.001 as compared to sham and STZ-lesioned groups, respectively (one-way ANOVA followed by Tukey's test).

Discussion

The present study shows that the oral administration of crocin effectively attenuated oxidative stress in rat striatum caused by ICV STZ.

In our previous studies, we have shown the protective effects of crocin against cisplatin-induced acute renal failure and relative oxidative stress (14).

The ICV STZ model in rat has been described as an appropriate animal model for sporadic Alzheimer type

dementia characterized by a progressive deterioration of memory, and presence of oxidative stress in the brain of rats (14, 18).

Certain regions of central nervous system (CNS), such as the striatum, may be particularly sensitive to oxidative stress because of their low endogenous levels of vitamin E, an important biochemical antioxidant, relatively to other brain regions. Such a depressed defense system may be adequate under normal circumstances. However, in pro-oxidative conditions, these low antioxidant defenses can predispose the brain to oxidative stress (19).

Free radicals and oxidative stress have been implicated as the prime candidates mediating the behavioral impairments and memory deficits in age related neurodegenerative disorders such as AD and Parkinson's disease. Though it is not clear precisely how oxidative stress exerts its deleterious effects, but some of this damage may include lipid and protein peroxidation, increase in DNA oxidation products, and deficits in calcium regulatory mechanisms that may eventually lead to cell death (1). Brain is particularly susceptible to peroxidation due to the simultaneous presence of high levels of polyunsaturated fatty acids and iron, which is the target of free radicals (19).

In this study, the results from the biochemical experiments showed a significant increase in MDA levels and a simultaneous decrease in total thiol contents and GPx activity in STZ-lesioned rats indicating neuronal damage caused by oxidative stress. While, crocin restored the oxidative damages via reduction in MDA levels and increment in total thiol content and GPx activity. The reduction of MDA level in the brain with crocin indicates attenuation of LPO.

The tripeptide glutathione (GSH), the non-specific endogenous mitochondrial and the cytosolic antioxidant, fulfills a variety of physiological functions, such as the reduction of various peroxides and free radicals, thereby resulting in the enzymatic (via GPx) or nonenzymatic conversion of GSH to GSSG. The thiol/disulfide (GSH/GSSG) ratio has, therefore, an important effect on the redox status of the protein thiols with modulation of protein conformation and enzyme activity. Moreover, oxygen-derived free radicals may overwhelm the radical scavenging potential of nerve cells, leading to lipid peroxidation and destruction of cell membranes, which in turn may result in cellular depletion of GSH, with subsequent increases of GSSG (20).

According to our findings, chronic treatment with crocin significantly restored total thiol contents and GPx activity. Crocin has been demonstrated to have antioxidant potential and reported to scavenge free radicals (14, 15) which suggests that its ameliorating effects on STZ-induced oxidative stress may be associated with this antioxidant effect. Carotenoids are well known as highly efficient scavengers of oxygen radicals and other excited species. Thus, crocin may protect against oxidation of lipids, proteins and DNA (21).

The present findings provide evidence that crocin has a possible neuroprotective effect against oxidative stress induced by STZ. Taken together, crocin may havetherapeutic significance for neurodegenerative diseases such as AD.

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References

1. Sharma M, Gupta YK. Chronic treatment with trans resveratrol prevents intracerebroventricular streptozotocin induced cognitive impairment and oxidative stress in rats. Life Sci 2002;71(21):2489-98.
2. Tiwari V, Kuhad A, Bishnoi M, et al. Chronic treatment with tocotrienol, an isoform of vitamin E, prevents intracerebroventricular streptozotocin-induced cognitive impairment and oxidative–nitrosative stress in rats. Pharmacol Biochem Behav 2009;93(2):183-9.
3. Sharma M, Gupta YK. Effect of chronic treatment of melatonin on learning, memory and oxidative deficiencies induced by intracerebroventricular streptozotocin in rats. Pharmacol Biochem Behav 2001;70(2-3):325-31.
4. Ochiai T, Soeda SH, Ohno S, et al. Crocin prevents the death of PC-12 cells through sphingomyelinase-ceramide signaling by increasing glutathione synthesis. Neurochem Int 2004;44(5):321-30.
5. Konoshima T, Takasaki M, Tokuda H, et al. Crocin and crocetin derivatives inhibit skin tumour promotion in mice. Phytother Res 1998;12(6):400-4.
6. Abe K, Saito H. Effects of saffron extract and its constituent crocin on learning behavior and long-term potentiation. Phytother Res 2000;14(3):149-52.
7. Pitsikas N, Zisopoulou S, Tarantilis PA, et al. Effects of the active constituents of Crocus sativus L., crocins on recognition and spatial rats' memory. Behav Brain Res 2007;183(2):141-6.
8. Sugiura M, Shoyama Y, Saito H, e al. The effects of

ethanol and crocin on the induction of long-term potentiation in the CA1 region of rat hippocampal slices. Jpn J Pharmacol 1995;67(4):395-7.

9. Lee IA, Lee JH, Baek NI, et al. Antihyperlipidemic effect of crocin isolated from the fractus of Gardenia jasminoides and its metabolite crocetin. Biol Pharm Bull 2005;28(11):2106-210.
10. Garcia-Olmo DC, Riese HH, Escribano J, et al. Effects of long-term treatment of colon adenocarcinoma with crocin, a carotenoid from saffron (Crocus sativus L.): an experimental study in the rat. Nutr Cancer 1999;35(2):120-6.
11. He SHY, Qian ZY, Tang FT, et al. Effects of crocin on experimental atherosclerosis in quails and its mechanisms. Life Sci 2005;77(8):907-21.
12. Nam KN, Park YM, Jung HJ, et al. Anti-inflammatory effects of crocin and crocetin in rat brain microglial cells. Eur J Pharmacol 2010;648(1-3):110-6.
13. Zheng YQ, Liu JX, Wan JN, et al. Effects of crocin on reperfusion-induced oxidative/nitrative injury to cerebral microvessels after global cerebral ischemia. Brain Res 2007;1138(1):86-94.
14. Naghizadeh B, Boroushaki MT, Vahdati Mashhadian N, et al. Protective effects of crocin against cisplatin-induced acute renal failure and oxidative stress in rats. Iran Biomed J 2008;12(2):93-100.
15. Naghizadeh B, Mansouri MT, Vahdati Mashhadian N. Crocin attenuates cisplatin-induced renal oxidative stress in rats. Food Chem Toxicol 2010;48(10):2650-5.
16. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95(2):351-8.
17. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959;82(1):70-7.
18. Sharma M, Gupta YK. Intracerebroventricular injection of streptozotocin in rats produces both oxidative stress in the brain and cognitive impairment. Life Sci 2001;68(9):1021-9.
19. Militao GC, Ferreira PM, de Freitas RM. Effects of lipoic acid on oxidative stress in rat striatum after pilocarpine-induced seizures. Neurochem Int 2010;56(1):16-20.
20. Rodriguez-Martinez E, Camacho A, Maldonado PD, et al. Effect of quinolinic acid on endogenous antioxidants in rat corpus striatum. Brain Res 2000;858(2):436-9.
21. Stahl W, Sies H. Antioxidant activity of carotenoids. Mol Aspects Med 2003;24(6):345-51.