Protective effect of aqueous saffron extract (Crocus sativus L.) and crocin, its active constituent, on renal ischemia-reperfusion-induced oxidative damage in rats

Hossein Hosseinzadeh1, Hamid R. Sadeghnia2, Toktam Ziaee3 and Aghdas Danaee4

1- Pharmaceutical Research Center, Faculty of Pharmacy, Mashhad University of Medical Sciences, Mashhad, I. R. Iran
2- Department of Pharmacology, Faculty of Medicine, Mashhad University of Medical Science, Mashhad, I.R. Iran
3- Faculty of Pharmacy, Mashhad University of Medical Sciences, Mashhad, I. R. Iran
4- Faculty of Veterinary, Ferdowsi University, Mashhad, I. R. Iran

Received March 13, 2005; Revised August 1, 2005; Accepted August 15, 2005, Published August 22, 2005

Abstract PURPOSE. The generation of reactive oxygen species and lipid peroxidation are associated with tissue injury following ischemic insult; therefore, the use of antioxidants appears rational in the improvement of kidney diseases therapy. The aim of the present study was to assess the effect of aqueous saffron extract (Crocus sativus L.) and its active constituent, crocin, on oxidative stress following renal ischemia-reperfusion injury (IRI) in rats. METHODS. The cellular redox status (thiobarbituric acid reactive species (TBARS) and total thiol levels) and antioxidant power (using ferric reducing/antioxidant power test) were assessed in control and ischemic groups. The left kidney was exposed to warm ischemia for 60 min followed by reperfusion for 90 min. The macerated aqueous extract of saffron (with doses of 5, 20 and 80 mg/kg, i.p.) and crocin (with doses of 50, 200 and 400 mg/kg, i.p.) were administrated prior to induction of ischemia. Normal saline (10 ml/kg, i.p.) was injected to control group and a sham group that did not have ischemia-reperfusion. RESULTS. Ischemia-reperfusion (IR) caused a significant increase in TBARS levels (p<0.001) and decrement in both antioxidant power (FRAP value) (p<0.05) and total thiol concentration (p<0.001) in kidney homogenate samples. In crocin pretreated groups, a reduction in TBARS levels (from 85.8 ± 5.4 to 20.9 ± 1.5 nmol/g tissue, p<0.001; 400 mg/kg) and elevation in antioxidant power (FRAP value) (from 3.05 ± 0.16 to 4.15 ± 0.16 µmol/g tissue, p<0.001; 400 mg/kg) and total thiol concentrations (from 0.38 ± 0.03 to 0.62 ± 0.03 mM, p<0.001; 200 mg/kg), as compared with control group, were observed. The aqueous extract also reduced lipid peroxidation products (from 85.8 ± 5.4 to 15.9 ± 2.6 nmol/g tissue, p<0.001; 80 mg/kg) and increased antioxidant power (from 2.98 ± 0.11 to 5.97 ± 0.56 µmol/g tissue, p<0.001; 80 mg/kg) in ischemia-reperfusion injured rat kidneys. CONCLUSION. This study therefore suggests that the aqueous saffron extract (Crocus sativus L.) and its active constituent, crocin, may be useful agents for the prevention of renal ischemia-reperfusion (IR)-induced oxidative injury in rats.

INTRODUCTION

The cellular depletion of ATP, the initial pathophysiologic event and hallmark of ischemic injury, lead to a series of morphologic, biochemical and physiologic derangements. Free oxygen radical (ROS) generation is an important mechanism of cellular injury in ischemic and reperfused tissues that causes oxidative damage to cellular macromolecules including membrane lipids, proteins and nucleic acids [1, 2]. Renal ischemia is a major cause of acute renal failure. Ischemic renal failure occurs following an episode of severe hemorrhagic shock, endotoxin sepsis, thermal burns, or transplantation surgery [3]. ROS per se have also been shown to compromise renal function, depress glomerular filtration, impair glomerular sieving function [4-6], and induce apoptosis in renal cells [7].

Cellular defense against free radical injury is provided by enzymatic (catalase, superoxide dismutases, and glutathione peroxidase) and nonenzymatic (GSH, α-tocopherol, vitamin C, and urate) free radical scavenging systems, present in the cell [3]. Recent overwhelming attention to plant products and alternative medicine has encouraged plant chemists, pharmacologists, biochemists, and molecular biologists to combine their efforts in a search for natural agents that can limit free radical-mediated injuries during and following ischemia-reperfusion, for better therapeutic management of IRI.

Crocus sativus L., commonly known as saffron, is used in folk medicine as an antispasmodic, eupeptic, gingival sedative, antcatarrhal, nerve sedative, carminative, diaphoretic, expectorant, stimulant, stomachic, aphrodisiac and emmenagoge [8]. Furthermore,
modern pharmacological studies have demonstrated that saffron extract or its active constituents have anticonvulsant [9], antidepressant [10], anti-inflammatory [11] and antitumour effects, radical scavenger as well as learning and memory improving properties [8, 12-15] and promote the diffusivity of oxygen in different tissues [8]. Saffron extract also has chemopreventive and showed protective effects on genotoxins-induced oxidative stress in Swiss albino mice [16-19].

The aim of present study was to assess the protective effects of aqueous saffron extract (Crocus sativus L.) and its active constituent, crocin, on renal IR-induced oxidative injury in rats.

MATERIALS AND METHODS

Animals
Adult male Wistar rats weighing 200-300 g were used throughout the study. All of them were kept in the same room under a constant temperature (22 ± 2 °C) and illuminated 7:00 a.m. to 7:00 p.m., with food pellets and water available ad libitum. The animals were divided into eight groups, each of which contained 6-8 rats. Group 1 was the sham group in which only surgery was done without induction of ischemia. Group 2 was the control group in which saline solution (10 ml/kg) was given intraperitoneally. In groups 3-8 aqueous saffron extract (5, 20 and 80 mg/kg, i.p) and crocin (50, 200 and 400 mg/kg, i.p.) were administrated prior to induction of ischemia.

Chemicals
DTNB (2, 2´-dinitro-5, 5´-dithiodibenzoic acid), TPTZ (2, 4, 6-tri (2-pyridyl)-1, 3, 5-triazine), TBA (2-thiobarbituric acid), n-butanol, tris, Na 2EDTA, sodium acetate, glacial acetic acid, phosphoric acid, potassium chloride, tetramethoxypropane (TMP), ferric chloride (FeCl 3.6H2O), ferrous sulfate and hydrochloric acid was obtained from Merck. Crocin was purchased from Fluka.

Preparation of aqueous saffron extract
*Crocus sativus* L. stigmas were collected from Ghaen (Khorasan province, Northeast of Iran). In the maceration method, 3g of stigmas were macerated in 400 ml distilled water for three days. The mixture was subsequently filtered and concentrated under reduced pressure at 35ºC.

Induction of renal ischemia-reperfusion injury (IRI)
The animals were subjected to left renal warm ischemia for 60 min, and reperfusion for 90 min. Briefly, under intraperitoneal ketamine/xyazine anesthesia (60 mg/kg and 10 mg/kg, respectively) and through a midline incision; the abdominal contents were displaced to the right side. The left renal artery and vein were dissected and the perirenal fat was preserved. The vascular pedicle was temporarily ligated with 2–0 silk before the abdominal contents were replaced and the incision was covered with a moistened pad. At the end of the ischemic period, the abdominal cavity was reentered, the ligature was removed and reperfusion was supplied. Throughout the experiments, body temperature was kept at 36-38 °C by placing the rats under light source. At the 90th min of reperfusion, left kidney was removed and maintained at -80 °C until analysis [20]. At the day of analysis, the kidney tissues was homogenized in cold KCl solution (1.5%) to give a 10% homogeny suspension and used for biochemical assays.

The aqueous saffron extract and crocin were dissolved in physiologic saline and administrated prior to induction of ischemia.

*Tiobarbituric acid reactive species (TBARS) measurement*
Malondialdehyde (MDA) levels, as an index of lipid peroxidation, were measured. MDA reacts with thiobarbituric acid (TBA) as a thiobarbituric acid reactive substance (TBARS) to produce a red colored complex that has peak absorbance at 532 nm [21].

3 ml phosphoric acid (1%) and 1 ml TBA (0.6%) was added to 0.5 ml of homogenate in a centrifuge tube and the mixture was heated for 45 min in a boiling water bath. After cooling, 4 ml of n-butanol was added the mixture and vortex-mixed for 1 min followed by centrifugation at 20000 rpm for 20 min. The organic 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 0-40 µM [22].

*Ferric Reducing / Antioxidant Power (FRAP) assay*
The FRAP assay measures the change in absorbance at 593 nm owing to the formation of a blue colored Fe II-tripyridyltriazine compound from the colorless oxidized Fe III form by the action of electron donating antioxidants [23].

The FRAP reagent consist of 300 mM acetate buffer (3.1 g sodium acetate + 16 ml glacial acetic acid, made up to 1 liter with distilled water; pH=3.6), 10 mM TPTZ in 40 mM HCl and 20 mM FeCl3.6H2O in the ratio of 10:1:1.

Briefly, 50 µl of kidney homogenate was added to 1.5 ml freshly prepared and prewarmed (37 ºC) FRAP reagent in a test tube and incubated
at 37 ºC for 10 min. The absorbance of the blue colored complex was read against reagent blank (1.5 ml FRAP reagent + 50 µl distilled water) at 593 nm. Standard solutions of Fe II in the range of 100 to 1000 mM were prepared from ferrous sulphate (FeSO₄·7H₂O) in distilled water. The data was expressed as mmol ferric ions reduced to ferrous form per liter (FRAP value) [24].

Total sulfhydryl (SH) groups assay
Total SH groups were measured using DTNB (2, 2´-dinitro-5, 5´-dithiodibenzonic acid) as the reagent. This reagent reacts with the SH groups to produce a yellow colored complex which has a peak absorbance at 412 nm [25].

Briefly, 1 ml Tris-EDTA buffer (pH=8.6) was added to 50 µl kidney homogenate in 2 ml cuvettes and sample absorbance was read at 412 nm against Tris-EDTA buffer alone (A₁). Then 20 µl DTNB reagent (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

Statistical analysis
Data are expressed as mean ± SEM. Statistical analysis was performed using one-way ANOVA followed by Tukey-Kramer post-hoc test for multiple comparisons. The p-values less than 0.05 were considered statistically significant.

RESULTS

Modulation of MDA levels by crocin and saffron extract
There was an increase (50 %) in the MDA levels following IRI as compared with sham-operated animals (85.8 ± 5.4 vs. 42.9 ± 4.3 nmol/g tissue, p<0.001) (Figure 1).

Crocin and the saffron extract pretreatment resulted in a significant reduction in the free radical-mediated lipid peroxidation as indicated by a decrease in the MDA levels, at various dose levels (Figure 1, 2).

In crocin-pretreated groups, a reduction in TBARS levels (from 85.8 ± 5.4 to 20.9 ± 1.5 nmol/g tissue, p<0.001; 400 mg/kg) was observed. The aqueous saffron extract also reduced lipid peroxidation products (from 85.8 ± 5.4 to 15.9 ± 2.6 nmol/g tissue, p<0.001; 80 mg/kg) in ischemia-reperfusion injured rat kidneys.

Figure 1: Effect of aqueous saffron extract on lipid peroxidation following renal IRI. MDA levels were measured in 10% homogenates of kidney samples from rats subjected to 60 min of ischemia and 90 min of reperfusion. All drugs were administrated intraperitoneally prior to induction of ischemia. Values are mean ± SEM (n=6). ***p<0.001 as compared with vehicle (normal saline) treated animals (One-way ANOVA followed by Tukey-Kramer test)

Figure 2: Effect of crocin on lipid peroxidation following renal IRI. MDA levels were measured in 10% homogenates of kidney samples from rats subjected to 60 min of ischemia and 90 min of reperfusion. All drugs were administrated intraperitoneally prior to induction of ischemia. Values are mean ± SEM (n=8). ***p<0.001 as compared with vehicle (normal saline) treated animals (One-way ANOVA followed by Tukey-Kramer test)

Modulation of FRAP value by crocin and saffron extract
IRI caused a significant reduction in FRAP value (30.2 %) of kidney homogenate samples as compared with sham-operated animals (4.37 ± 0.10 vs. 3.05 ± 0.16 µmol/g tissue, p<0.001) (Figure 3).
Crocin pretreatment increased antioxidant power (FRAP value) of kidney homogenate samples, dose dependently (from 2.98 ± 0.11 to 4.15 ± 0.16 μmol/g tissue, p<0.001; 400 mg/kg). The aqueous saffron extract also increased antioxidant power (from 2.98 ± 0.11 to 5.97 ± 0.56 μmol/g tissue, p<0.001; 80 mg/kg) in ischemia-reperfusion injured rat kidneys (Figure 3, 4).

Crocin pretreatment caused a significant and dose dependently elevation in total thiol concentration, as compared with control group (from 0.38 ± 0.03 to 0.62 ± 0.03 mM, p<0.001; 200 mg/kg). In contrast, the saffron extract failed to increase total SH groups following ischemia-reperfusion injury (Figure 5, 6).

**Effect of crocin and saffron extract on total thiol concentration**

Following ischemia-reperfusion injury a significant reduction (37.7 %) in total SH groups (0.61 ± 0.03 vs. 0.38 ± 0.03 mM, p<0.001) in kidney homogenate samples were observed (Figure 5).
DISCUSSION

A great deal of effort has been directed toward searching for compounds that can be used for better management of the clinical consequences arising from renal ischemia–reperfusion injuries, without much success. The results obtained in the present investigation suggest that the saffron extract and its active constituent, crocin, have an overall protective effect against kidney ischemia/reperfusion injury in a rat model. The observed protective effects can be attributed to the water soluble chemical constituents of saffron which would include mainly constituents such as crocin and picrocrocin [13].

A number of processes have been implicated in the pathogenesis of oxygen deprivation–induced cell injury. These include the disturbances of cell calcium homeostasis, depletion of adenine nucleotides, activation of enzymes like phospholipases with production of toxic lipid metabolites, proteases and endonucleases and generation of free radicals (ROS) that can cause oxidative damage to cellular macromolecules [3, 26]. ROS have been shown to play a major role in IRI [27, 28] and collectively are instrumental in impairing overall renal function [4-6]. ROS can induce damage to endothelial, glomerular mesangial and tubular epithelial cells (especially S3 segment of proximal tubule) [27, 28] and induce apoptosis in renal cells [7]. Cellular death following renal ischemia-reperfusion injury is well associated with ROS production and lipid peroxidation and antioxidant therapy has been well documented to help in the improvement of organ functions [29].

We assessed the effect of crocin and the aqueous saffron extract by studying their effects on lipid peroxidation, which was measured in terms of MDA, a stable metabolite of the free radical-mediated lipid peroxidation cascade. MDA levels increased significantly following renal IRI. Crocin and the saffron extract reversed the increase of MDA levels to a considerable extent, thereby confirming its antioxidant role in IRI.

Sulfhydryl (SH) groups known to be sensitive to oxidative damage and depleted following ischemic insult [30], therefore we studied the effect of these agents on total thiol concentration during IRI. Similarly, in our studies, total sulfhydryl groups were decreased following ischemic-reperfusion injury. Crocin pretreated rats exhibited higher SH contents than their respective controls in the dose related pattern, indicating that crocin helped in replenishing the total thiol pool. However saffron-mediated SH replenishment was not as impressive as expected. Saffron pretreatment slightly increased total thiol concentration following ischemic insult, but this elevation was not significant as compared with control group. Premkumar et al showed oral pretreatment with the saffron aqueous extract (40 and 80 mg/kg) for five consecutive days inhibits genotoxins-induced oxidative stress in mice liver. In this study, an increase in the levels of glutathione (GSH) concentration as well as the activities of glutathione S-transferase (GST), glutathione peroxidase (GPx), catalase and superoxide dismutase (SOD) were observed, however, normal levels of GSH could not be attained [19]. In contrast, in the study conducted by El Daly oral pretreatment with saffron extract (50 mg/kg 30 min prior to cisplatin administration for five alternative days, ip) had no significant effect on the activities of enzymes such as alkaline phosphatase, glutation reductase, isocitrate dehydrogenase, malate dehydrogenase, glucose-6-phosphate dehydrogenase, etc. in the kidney of male albino rats as compared with cisplatin treated animals [31]. It has been postulated that the nephrotoxic mode of action of drug cisplatin is similar to that of the other heavy metals, and is related to the decrease in the intracellular concentrations of glutathione and protein-bound SH groups, which are required for normal cellular, function [32]. The possibility that cisplatin itself has the inhibitory effect on the enzyme activities was excluded by the fact that in vitro addition of the drug to the reaction media did not affect the reaction rates [33].

Under acute and chronic pathologic conditions such as ischemia, the balance between oxidant and antioxidant systems has been interrupted [2, 34]. Therefore we evaluate the antioxidant or reducing potential of kidney homogenate samples following IRI, using FRAP assay. As expected following IRI, a significant reduction in antioxidant power, as indicated by FRAP value, was observed. Instead, crocin and the saffron extract increased the antioxidant power of kidney homogenate samples.

In rats, crocin dyes are known to exert protective effects against acute hepatic damage induced by aflatoxin B1 and dimethylnitrosamine [35]. Saffron has chemopreventive effects and its extract inhibits tumor growth in vivo and in vitro [12, 16, 17]. Escribano et al showed that the saffron extract and its constituents, crocin, safranal and picrocrocin inhibit the growth of human cancer cells (Hella cells) in vitro [13]. The saffron extract also has radical scavenger properties [8] and protects from genotoxicity as well as genotoxins-induced oxidative stress in mice [18, 19]. These observations indicate that more than one mechanism of protection is operating. It is well
known that many naturally occurring compounds exhibit discrete mechanisms of protection. Therefore, further extensive work is needed to identify the mechanisms of action of saffron and its constituents.

In this study, the saffron extract was more potent than crocin. This may be due to the fact that the aqueous saffron extract consists of many constituents such as crocins (water soluble carotenoids which are glycosyl esters of crocetin), crocetin, dimethyl crocetin and flavonoids which quenching of free radicals and antioxidant effects and of these compounds have established and may have role in protective effect of saffron on IRI. In addition, saffron contains proteins, sugars, vitamins (especially riboflavin), amino acids, minerals and gums [36].

In conclusion, the present study showed saffron extract and its constituent, crocin, have protective effect on IRI-induced oxidative stress in rats kidney that at least partly due to antioxidant properties of saffron.

ACKNOWLEDGEMENTS

The authors are thankful to the Vice Chancellor of Research, Mashhad University of Medical Sciences for financial support.

REFERENCES


