PHARMACOLOGICAL PROFILE OF CROCUS SATIVUS- A COMPREHENSIVE REVIEW

Vijender Kumar, Z A Bhat, Dinesh Kumar, N A Khan, I A Chashoo, MY Shah

Department of Pharmaceutical Sciences, University of Kashmir, Srinagar-190006

***Corresponding Author**
Dr. Z.A.Bhat
Sr. Asst. Professor
Department of Pharmaceutical Sciences
University of Kashmir, Srinagar-190006
Email: zabhat2000@gmail.com; vijenderpareek24@yahoo.com

Summary

Crocus sativus L. (Iridaceae) is an herbaceous perennial-cormous, stemless herb comprises of the dried red stigma with a small portion of the yellowish style attached. It is cultivated in Azerbaijan, France, Greece, Iran, Italy, Spain, China, Israel, Morocco, Turkey, Egypt, Mexico and Kashmir in India. The method for the cultivation of saffron contributes greatly to its high price. Saffron is a spice of great economic value, as one kilogram of good quality saffron produced from *Crocus sativus* L. can cost over 2,000 US dollars. Approximately 150,000 flowers are needed to produce one kilogram of dried saffron, and to grow this amount one would typically need some 2,000 m^2 under cultivation per kg harvest. The cost of Indian saffron, which has been reported to contain up to 26 times as much crocin as commercial (Sigma) saffron samples. The value of saffron is determined by the existence of three main secondary metabolites: crocin, picrocrocin, and safranal. The stigma of this plant is also a well known traditional Chinese medicine and is used as safflower to stimulate blood flow and relieve pain by removing stagnated blood. It is also used in the treatment of amenorrhea, menostasis, melancholia, chest and abdominal pain, painful swellings due to blood stasis, hepatomegaly, splenomegaly, convulsion, and pain of traumatic wounds and as a sedative. It is used in folk medicine as antispasmodic, carminative, stomachic, expectorant, aphrodisiac, cardiotonic and stimulant. In traditional medicine this plant is utilized as an exhilarant and curative of anxiety. In ayurveda saffron is used to cure chronic diseases such as asthma, arthritis, skin diseases, spasmodic disorders, digestive disorders, digestive disorders, kidney disorders. Also it has been recently reported that ethanol extract of Saffron petals possesses antidepressant activity. The present review explores its important and scientifically proven pharmacological activities.

Keywords: Saffron, *Crocus sativus*, crocin, picrocrocin, safranal, crocetin.

Introduction

Crocus sativus L. var. *cashmirianus,* (Iridaceae) commonly has known as kesar (Hindi), avarakta (Sanskrit) and saffron (English). It comprises of the dried red stigma with a small portion of the yellowish style attached. It is cultivated in Azerbaijan, France, Greece, Iran, Italy, Spain, China, Israel, Morocco, Turkey, Egypt, Mexico and Kashmir in India [1]. The height of the plant is about 0.25 to 0.5 foot and the width is about 0.25 to 0.5 foot. The best season for the growth saffron is

early spring. It prefers sunny to part shady, moderately dry, well-drained, ordinary garden soil. The flower styles are commonly used as a flavoring and yellow coloring for various foods such as bread, soups, sauces, rice and puddings .Yields per plant are extremely low, about 4000 stigmas yield 25g of saffron. The corms are toxic to young animals, so this report of edibility should be treated with some caution [2]. The method for the cultivation of saffron contributes greatly to its high price. It is picked during 3-4 weeks in October-November. The value of saffron is determined by the existence of three main secondary metabolites: crocin, picrocrocin, and safranal [3] .It is used in folk medicine as antispasmodic, carminative, stomachic, expectorant, aphrodisiac, cardio tonic and stimulant [4]. In traditional medicine this plant is utilized as an exhilarant and curative of anxiety [5]. It is also used in the treatment of amenorrhea, menostasis, melancholia, chest and abdominal pain, painful swellings due to blood stasis, hepatomegaly, splenomegaly, convulsion, and pain of traumatic wounds and as a sedative [6]. Also it has been recently reported that ethanol extract of Saffron petals possesses antidepressant activity [5] .Test-tube and animal studies hint that saffron and its constituents may help prevent or treat cancer, reduce cholesterol levels, protect against side effects of the drug cisplatin, and enhance mental function. It is beneficial in the treatment of several digestive disorders. Its use has been found especially valuable in flatulent colic. It is also used in the fevers, melancholia and enlargement of the liver and spleen. It is used in medicines that reduce inflammation. A combination of saffron and ghee is used to treat diabetes. Saffron also merits usage in disorders of Brain. It has been found beneficial in the treatment of kidney disorders. It acts as a diuretic, if soaked overnight in water and administered with honey [2].

Pharmacological evidence of *Crocus sativus* L.

Saffron has been traditionally used as an acrid, aphrodisiac, analgesics ,anodyne, antispasmodic, bitter, cephalgia, diuretic, depression, epilepsy, fragrant, fever, galactogogue , inflammations, Kapha, laxative, stimulant, stomachic and as a tonic [2]. C. sativus also possesses a number of therapeutic activities such as antihypertensive, anticonvulsant, antitussive, antigenototoxic, anticancer, cytotoxic effects, anxiolytic, antioxidant, antidepressant, anti-inflammatory, and relaxant activity [7]. *Crocus sativus* commonly known as saffron (Kesar) is used in folk medicine as an anti-spasmodic, eupeptic, gingival, sedative, anticatarrhal, carminative, diaphoteric, expectorant, stimulant, stomachic, aphrodisiac and emmenagogue.

Protective effect on retina: Crocetin is a carotenoid (aglicone of crocin) which is the active components of *Crocus sativus* L. Crocetin prevents oxidative stress induced retinal degeneration via inhibition of caspase activity. The retinal damage was induced by exposure to white light at 8000 lx for 3 h after dark adaptation. Crocetin at 100 mg/kg, p.o. significantly inhibited photoreceptor degeneration and retinal dysfunction. These results indicate that crocetin has protective effects against retinal damage in vitro and in vivo, suggesting that the mechanism may inhibit increase in caspase-3 and -9 activities after retinal damage [8].

Protective effect against ethanol-induced memory impairment

Crocin from *Crocus sativus* L. has antagonizes the inhibitory effect of ethanol on long-term potentiation in the rat hippocampus. Crocin prevented both ethanol-induced impairment of learning in mice and ethanol-induced inhibition of hippocampal LTP in rats. The effects of ethanol and crocin on synaptic potentials mediated by *N*-methyl-D-aspartate NMDA receptors in the dentate gyrus of rat hippocampal slices. Crocin 10 mM did not affect the inhibition of non-NMDA response by 100 mM ethanol, but significantly blocked the inhibition of NMDA response by 10-50 mM ethanol [9].

Inhibitory effect on skin cancer

Saffron treatments were given both before and after the induction of skin carcinogenesis. Standard histological examination of mice skin demonstrated that saffron ingestion inhibited the formation of skin papillomas and reduced their size also. Saffron extract treated early inhibits skin carcinoma due to the induction of cellular defense systems in mice [10].

Cytotoxic effect

Crocin is major constituent of *Crocus sativus* extract and it's significantly inhibited the growth of colorectal cancer cells while not affecting normal cells [11].The inhibitory effect of ethanol extract of *C. sativus* shown on macromolecular synthesis (DNA and RNA) in three human malignant cell lines: A-549 cells, WI-38 cells and VA-13 cells[12]. Crocetin caused a dose-dependent inhibition of nucleic acid and protein synthesis, inhibition of growth of human chronic myelogenous leukaemia and promyelocytic leukaemia by dimethyl-crocetin, crocetin, and crocin with 50% inhibition reached at concentrations of 0.8 and 2 mM, respectively [12]. Crocetin also had a dose-dependent inhibitory effect on DNA and RNA synthesis in isolated nuclei and suppressed the activity of purified RNA polymerase II [13].Cytotoxicity of dimethyl crocetin and crocin to various tumors cell lines (DLA, EAC, S-180, L1210 leukemia, and P388 leukemia) and to human primary cells from surgical specimens (osteosarcoma, fibrosarcoma, and ovarian carcinoma) has been reported. The inhibitory effect of the ethanolic saffron extract on the in vitro growth of HeLa cells was mainly due to crocin, where picrocrocin and safranal played a minor role in the cytotoxicity of saffron extracts [14]. Sugars might play a key role in cytotoxic effect of crocin, since its deglucosylated derivative crocetin did not cause cell growth inhibition even at high doses. These findings are in accordance with the results [15] which found no effect of crocetin on colony formation in HeLa cells and two other solid tumor cell lines. An ID50 of 0.4 and 1.0 mM was reported for crocin on the rat adenocarcinoma DHD/K12-PROb cells and human colon adenocarcinoma HT-29 cells, respectively [12].

Effect on neuronal injury

The carotenoid pigments of saffron consist of crocetin-dicrocin, tricrocin, crocin and picrocrocin. The effect of crocin showed as neuroprotective, antioxidant and GSH-synthetic activities in PC12 cells under serum-free and hypoxic conditions. Measurements of cell viability, peroxidized membrane lipids and caspase-3 activity showed that the rank order of the neuroprotective potency at a concentration of 10 μM was crocin>tricrocin>dicrocin and picrocrocin. Crocin most effectively promotes mRNA expression of γ-glutamylcysteinyl synthase, which contributes to GSH synthesis as the rate-limiting enzyme. Saffron carotenoid can significantly reduce infracted areas caused by occlusion of the middle cerebral artery in mice [16].

Inhibitory effect on tumor

Saffron produced a significant inhibitory effect on the growth of transplanted tumor cells in mice [17]. Crocetin effective in treating certain types of cancer treatable with all-trans retinoic acids and it's a safer alternative to treat all-trans retinoic acids sensitive cancers in women of childbearing age[18].The oral administration of the saffron ethanolic extract increased the life span of Swiss albino mice intraperitoneally transplanted with sarcoma-180(S-180) cells, Ehrlich ascites carcinoma (EAC) or Dalton's lymphoma ascites tumors. Liposome encapsulation of saffron effectively enhanced its antitumor activity against S-180 and EAC solid tumors in mice, promoting significant inhibition in the growth of these tumors [19]. Crocin treatment significantly increased their survival time rats bearing colorectal tumors, induced by rat adenocarcinoma DHD/K12-PROb

cells injected by subcutaneously, decreased tumor growth rate more intensely in females. The selective action of crocin in female rats as compared with male rats suggests that the effects of crocin in animals might be partially dependent on hormonal factors. An increase in the levels of b-carotene and Vitamin A in the serum of laboratory animals under oral administration of saffron extracts was detected [20]. Saffron carotenoids possessed provitamin-An activity according to the hypothesis that the action of carotenoids was dependent upon its conversion to retinal (Vitamin A), because most of the evidence supporting the anticancer effects of carotenoids was referred to b-carotene [12].

Effect on cisplatin-induced nephrotoxicity

Reactive oxygen species and oxidative damage are the most important factors in cisplatin-induced acute renal failure. Blood urea and creatinine and urinary glucose and protein concentrations in crocin-treated groups were significantly lower compared to the cisplatin-treated group. Histopathological studies showed massive damage in the S-3 segment of proximal tubules in cisplatin-treated group but not in crocin-treated groups. Crocin treatment resulted in a significant reduction in malondialdehyde (MDA) concentration and produced a significant elevation in total thiol and glutathione peroxidase concentrations. There was a significant elevation in the mRNA expression of glutathione peroxidase in crocin-treated groups. The results suggest that crocin attenuates cisplatin-induced renal oxidative stress in rats [21].

Neuroprotective activity

Crocin is a carotenoid pigment, which prevents the death of rat pheochromocytoma (PC-12) cells when death is induced by tumor necrosis factor (TNF)-α.Crocin suppresses the TNF-α-induced expression of latent ICE and Bcl-Xs mRNAs and simultaneously restores the cytokine-induced reduction of Bcl-XL mRNA expression. Crocin also suppressed the death of serum/glucose deprived PC-12 cells by increasing glutathione (GSH) synthesis and thus inhibiting neutral sphingomyelinase (nSMase) activity present in the cell membrane. The hypoxic or ischemic death of PC-12 cells is mediated by ceramide produced through activation of nSMase. The cell death process also includes then reduction of GSH and Bcl-XL. These facts imply that it is crocin's antioxidant effect that prolongs the survival of PC-12 cells deprived of serum/glucose. The antioxidant effect of crocin is stronger than that of α-tocopherol and suppresses the activation of caspase-8, an initiator-caspase.Crocin showed neuroprotective effect against oxidative stress-induced death of neurons [22].

Effect on memory

Crocins (15 and 30 mg/kg) counteracted delay-dependent recognition memory deficits in the normal rat, suggesting that these carotenoids modulate storage and/or retrieval of information. In a subsequent study, treatment with crocins (30 mg/kg and to a lesser extent also 15 mg/kg) attenuated scopolamine (0.2 mg/kg) induced performance deficits in the radial water maze test. Scopolamine-treated rats exhibited severe impairments in the rate of acquisition by committing more errors while finding the escape platform suggesting that the aforementioned muscarinic receptor antagonist disrupted both reference and working memory. Crocins (30 mg/kg and to some extent also 15 mg/kg) significantly reduced reference memory errors produced by scopolamine. The higher dose of crocin (30 mg/kg) was able to attenuate scopolamine induced working memory deficits. Scopolamine plus crocins 15 mg/kg-treated animals committed fewer working memory errors with respect to scopolamine plus vehicle-treated animals. Both scopolamine plus crocins groups displayed a lower performance as compared to the respective cohorts treated with crocins

plus vehicle. The lower doses of crocins antagonized extinction of recognition memory and attenuated scopolamine-induced spatial memory performance deficits in the rat [23].

Anxiolytic activity

Rats were treated with crocins 15 mg/kg; 30 mg/kg and 50 mg/kg; and compared with diazepam (1.5 mg/kg). Dose of diazepam was selected based on prior study in which it was found active in the same behavioral paradigm and did not produce adverse side effects .Vehicle and crocins were injected i.p., 60 min before testing. Diazepam was administered i.p., 20 min before undertaking the experiment. Either crocins, at a dose which did not influence animal's motor activity (50 mg/kg), or diazepam (1.5 mg/kg), increased the latency to enter the dark compartment and prolonged the time spent in the lit chamber in the rats. Crocins, given at lower doses (15 and 30 mg/kg), did not influence animals behavior. It has been demonstrated that the time spent in light compartment is a more sensitive parameter to indicate the anxiolytic action of drugs than the number of transitions between the lit and dark compartments of the apparatus. In addition, the number of rearings displayed by rats in the lit area of the light/dark box was not different among the various groups of animals (data not reported). Based on the present results it could be concluded that crocins reduced the anxiety of animals exposed to the light/ dark procedure without influencing rodents' motor activity [24].

Antigenotoxic effect

Topical administration of saffron extracts (100 mg/kg body weight) inhibited the initiation/ promotion of 7, 12-dimethylbenz [a] anthracene (DMBA) - induced skin tumors in mice, delaying the onset of papilloma formation and reducing the mean number of papillomas per mouse [12]. The oral administration of the same dose of saffron extracts restricted tumor incidence of 20-methylcholanthrene (MCA)-induced soft tissue sarcomas in mice. Extracts from saffron stigmas prolonged the life span of cisplatin-treated mice and partially prevented the decrease in body weight, leukocyte count and hemoglobin levels. Pretreatment with the aqueous extract of saffron in experiments with Swiss albino mice significantly inhibited the genotoxicity of cisplatin, cyclophosphamide, mitomycin, and urethane. It was suggested that saffron rich in carotenoids might exert its chemopreventive effects by the modulation of lipid peroxidation, antioxidants, and detoxification systems .Crocetin from saffron also ameliorates bladder toxicity of the anticancer agent cyclophosphamide without altering its antitumor activity .The treatment of animals with cysteine (20 mg/kg bodyweight) together with saffron extract (50 mg/kg body weight) significantly reduced the toxic effects caused by cisplatin, such as nephrotoxicity and changes in enzyme activity [12] .

Effect on sexual behavior

The aphrodisiac activities of *C. sativus* stigma aqueous extract and its constituents, safranal and crocin, were evaluated in male rats. The aqueous extract (80, 160, and 320 mg/kg body wt.), crocin (100, 200, and 400 mg/kg body wt.), safranal (0.1, 0.2, and 0.4 ml/kg), sildenafil (60 mg/kg body wt., as a positive control), and saline were administered intraperitoneally to male rats. Mounting frequency (MF), mount latency (ML), intromission latency (IL), and ejaculation latency (EL) were the factors evaluated during the sexual behavior study. Crocin, at all doses, and the extract, especially at doses 160 and 320 mg/kg b.w., increased MF, IF, and EF behaviors and reduced EL, IL, and ML parameters. Safranal did not show aphrodisiac effects. This study exhibited an aphrodisiac activity of saffron aqueous extract and its constituent crocin [25].

Antidepressant activity

The efficacy of petal of *C. sativus* was assessed in the treatment of mild-to-moderate depression in a 6-week double-blind, placebo-controlled and randomized trial. Forty adult outpatients who met the Diagnostic and Statistical Manual (DSM) of Mental Disorders, fourth edition for major depression based on the structural clinical interview for DSM IV, participated in the trial. In this double-blind, placebo controlled and randomized trial, patients were randomly assigned to receive capsule of petal of *C. sativus* 30 mg/day (Group 1) and capsule of placebo (Group 2) for a 6-week study. At 6 weeks, petal of *C. sativus* produced a significantly better outcome on Hamilton Depression Rating Scale than placebo (d.f. = 1, $F = 16.87$, $P<0.001$). There were no significant differences in the two groups in terms of observed side-effects. The results of this study indicate the efficacy of petal of *C. sativus* in the treatment of mild-to-moderate depression. In addition, in a recent pre-clinical study, it has been reported that petal of *C. sativus*, the part of this herb that is very cheap compared to stigma of *C. sativus* (saffron), has antidepressant effect [26].

Antinociceptive and anti-inflammatory activity

Antinociceptive activity was examined using the hot plate and writhing tests. The effect of aqueous and ethanolic macerated extracts against acute inflammation was studied using xylene induced ear edema in mice. The activity of the extracts against chronic inflammation was assessed by formalin-induced edema in the rat paw. In the hot plate tests, intraperitoneal injection of both extracts showed no significant antinociceptive activity in mice. The extracts exhibited antinociceptive activity against acetic acid induced writhing. Naloxone partially blocked only the antinociceptive activity of the stigma aqueous extract. Only the stigma extracts showed weak to moderate effect against acute inflammation. In chronic inflammation, both aqueous and ethanolic stigma extracts, as well as ethanolic petal extract, exerted anti-inflammatory effects [27].

Stimulatory effect on β2 – adrenoreceptors

The effect of aqueous-ethanolic extracts of *C. sativus* and its constituent, safranal was examined on β- adreno receptors in tracheal chains of guinea pigs. The β2 -adrenergic a stimulatory effect was tested by performing the cumulative concentration-response curves of isoprenaline-induced relaxation of pre contracted isolated guinea pig tracheal chains. The studied solutions included two concentrations of aqueous ethanolic extracts from *C. sativus* (0.1 and 0.2 g %), safranal (1.25 and 2.5 μg), 10 nM propranalol, and saline. The study was done in two different conditions including non-incubated (group 1, $n = 9$) and incubated tissues with 1 μM chlorpheniramine (group 2, $n = 6$). The results showed clear leftward shifts in isoprenaline curves obtained in the presence of only higher concentration of the extract in group 1 and its both concentrations in group 2 compared with that of saline. The EC50 (the effective concentration of isoprenaline, causing 50% of maximum response) obtained in the presence of both concentrations of the extract (0.17 ± 0.06 and 0.12 ± 0.02) and safranal (0.22 ± 0.05 and 0.22 ± 0.05) in group 1 and only in the presence of two concentrations of the extract (1.16 ± 0.31 and 0.68 ± 0.21) in group two was significantly lower compared to saline. The maximum responses obtained in the presence of both concentrations of the extract and safranal in group 1 were significantly lower than that of saline. The results indicated a relatively potent stimulatory effect of the extract from *C. sativus* on β2 - adrenoreceptors, which is partially due to its constituent, safranal. A possible inhibitory effect of the plant on histamine (H 1) receptors was also suggested [28].

Antihypertensive activity

The effects of petals extracts of Crocus *sativus* was investigated on blood pressure in anaesthetized rats and also on responses of the isolated rat vas deferens and guinea-pig ileum induced by electrical field stimulation (EFS). Aqueous and ethanol extracts of *C. sativus* petals reduced the blood pressure in a dose-dependent manner. Administration of 50 mg/100 g of aqueous extract changed the blood pressure from 133.59 3.9 to 1179 2.1 (mmHg). EFS of the isolated rat vas deferens and guinea-pig ileum evoked contractions were decreased by aqueous and ethanol extracts of *C. sativus* petals. The aqueous extract (560 mg/ml) significantly reduced the contractile responses of vas deferens to epinephrine (1 mM) without any change in contraction induced by KCl (300 mM). The present results may suggest that the relaxatory action of C. sativus petals extract on contraction induced by EFS in the rat isolated vas deferens is a postsynaptic effect [29].

Anti-tussive effect

The anti-tussive activity of *Crocus sativus* stigma and petal extracts and its components, safranal and crocin, was evaluated using the nebolized solution of citric acid 20% in guinea pigs. The extract and agents were injected intraperitoneally. The ethanolic extract of C. sativus (100–800 mg/kg) and safranal (0.25–0.75 ml/kg) were used. Safranal and the ethanolic extract of saffron stigma significantly reduced cough numbers. The ethanolic and aqueous extracts of petal and crocin did not show antitussive activity [30].

Anticonvulsant activity

The anticonvulsant activities of *Crocus sativus* stigma constituents, safranal and crocin, were evaluated in mice using pentylenetetrazole (PTZ)-induced convulsions in mice. Safranal (0.15 and0.35 ml/kg, i.p.) reduced the seizure duration, delayed the onset of tonic convulsions and protected mice from death. Crocin (200 mg/kg, i.p.) did not show anticonvulsant activity. This study indicates that safranal has an anticonvulsant activity in PTZ-induced seizures in mice [31]. Safranal has protective effects in both the clonic and tonic phases of PTZ-induced seizures and the $GABA_A$-benzodiazepine receptor complex may play an important role in the effects of drug [32].

Immunomodulator activity

Saffron corms contain a proteoglycan that is highly cytotoxic on human tumor cells and present work was undertaken to study the possible immunomodulatory and anti-invasive properties of this compound. Non-cytotoxic concentrations of this glycoconjugate promoted significant macrophage activation, detected by the release of nitric oxide. A rapid activation of protein kinase C and NF-kB was obtained after proteoglycan treatment, which could explain the induction of nitric oxide synthase. Proteoglycan concentrations ranging from 10±1000 ng/ml specially promoted apoptosis of macrophages, probably triggered by their activation. This molecule did not inhibit in vitro migration or invasion of human tumor cells. Altogether these results support a plausible immuno-modulating activity for this saffron Crocus compound. Our data indicate that low doses of proteoglycan activate macrophages in vitro, as revealed by the release of NO in treated cells. Thus, this compound may be useful in activating macrophages to defend against tumors [33].

Cardioprotective activity

The effects of crocin, a pharmacologically active constituent of Crocus sativus L. was investigated in isoproterenol (ISO)-induced cardiotoxicity with reference to hemodynamic, antioxidant, histopathological and ultra structural parameters. Rats were administered crocin (5, 10 and 20mg / kg / day) or vehicle orally for 21days along with ISO (85mg/kg, subcutaneously, at 24h interval) on 20^{th} and 21^{st} day. On 22nd day ISO-control rats showed cardiac dysfunction as indicated by

lowering of systolic, diastolic and mean arterial blood pressures. In addition, a significant decrease in maximum positive and negative rate of developed left ventricular pressure (7LVdp/dt max) and an increase in left ventricular end-diastolic pressure (LVEDP) were observed. Furthermore, a marked reduction in the activities of myocardial creatine kinase-MB (CK-MB) isoenzyme, lactate dehydrogenase (LDH), superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) levels along with an increase in content of malondialdehyde (MDA) were observed. Myocardial necrosis, edema and inflammation were evident from the light microscopic and ultra structural changes. Crocin at the dose of 20mg/kg/day significantly modulated hemodynamic and antioxidant derangements. The preventive role of crocin on ISO-induced MI was reconfirmed by histopathological and ultra structural examinations. The effect at the dose of 20 mg/kg/day of crocin was more pronounced than that of other two doses (5 and 10 mg/kg/day). The results suggest that crocin may have cardio protective effect in ISO-induced cardiac toxicity through modulation of oxidative stress in such a way that maintains the redox status of the cell. To conclude, the results that crocin has cardio protective potential. Crocin pretreatment improved cardiac functions, the effect which can be attributed to its ability of maintaining redox status which is disturbed by ISO challenge, via restoration of endogenous antioxidants, controlling lipid peroxide formation and preserving activities of CK-MB, LDH enzymes[34].

Effect on body weight

Snacking is an uncontrolled eating behavior, predisposing weight gain and obesity. It primarily affects the female population and is frequently associated with stress. We hypothesized that oral supplementation with Satiereal (Inoreal Ltd, Plerin, France), a novel extract of saffron stigma, may reduce snacking and enhance satiety through its suggested mood-improving effect, and thus contribute to weight loss. Healthy, mildly overweight women (n= 60) participated in this randomized, placebo-controlled, double-blind study that evaluated the efficacy of Satiereal supplementation on body weight changes over an 8-week period. Snacking frequency, the main secondary variable, was assessed by daily self-recording of episodes by the subjects in a nutrition diary. Twice a day, enrolled subjects consumed 1 capsule of Satiereal (176.5 mg extract per day (n = 31) or a matching placebo (n = 29). Caloric intake was left unrestricted during the study. At baseline, both groups were homogeneous for age, body weight, and snacking frequency. Satiereal caused a significantly greater body weight reduction than placebo after 8 weeks ($P<0.1$). The mean snacking frequency was significantly decreased in the Satiereal group as compared with the placebo group ($P<0.5$). Other anthropometric dimensions and vital signs remained almost unchanged in both groups. No subject withdrawal attributable to a product effect was reported throughout the trial, suggesting a good tolerability to Satiereal. Our results indicate that Satiereal consumption produces a reduction of snacking and creates a satiating effect that could contribute to body weight loss. The combination of an adequate diet with Satiereal supplementation might help subjects engaged in a weight loss program in achieving their objective [35].

Antioxidant activity

Saffron is the red dried stigmas of Crocus sativus L. flowers and used both as a spice and as a drug in traditional medicine. It's an antioxidant and anticancer agent are due to its secondary metabolites and their derivatives (safranal, crocetin, dimethyl crocetin).The antioxidant activity of saffron carotenoids is more effective than safranal. However the synergistic effect of all the bioactive constituents gives to saffron spice a significant antioxidant activity. The antioxidant activity of saffron compounds can protect DNA and tRNA from harmful chemical reaction in this ligand–polynucleotide complexes [36].Crocins is water-soluble carotenoids responsible for the color

of saffron. In this study, we isolated and identified three major crocins from gardenia, and then evaluated their antioxidant potential using four in vitro antioxidant tests in comparison with saffron ethanol extract (SE). The crocins appeared to possess antioxidant activity when tested by four in vitro antioxidant models. However, in anti-hemolysis, DPPH radical-scavenging and lipid peroxidation assays. The present study shows that crocins possess antioxidant capacities assayed in four models, which confirm their roles as antioxidant agents. However, these pigments, the main chemicals considered responsible for various pharmacological effects of gardenia fruit, seemed not to be major contributors to the antioxidant activity of gardenia in anti-hemolysis, DPPH radical-scavenging and lipid peroxidation assays [37].

Effect against breast cancer

Saffron (Crocus sativus), widely used as a spice in Middle Eastern cuisine and is known for anti-cancer properties. The mechanism of saffron-induced cytotoxicity, in tumor cells has not been adequately explored. Therefore, we investigated the role of caspases and Bax protein in saffron-induced apoptosis in MCF-7 cells, a commonly used cell culture system for in vitro studies on breast cancer. Cells were incubated with different concentrations of saffron extract. Cell viability was quantitated by MTT assay. Apoptotic cells were determined using PI staining of DNA fragmentation by flow cytometry (sub-G1 peak). Role of caspase were studied using the pan-caspase inhibitor. Bax protein expression was analysed by western blotting. Saffron extract (200–2000 lg/ml) decreased cell viability in MCF-7 cells as a concentration- and time dependent manner with an IC50 of 400 ± 18.5 lg/ml after 48 h. Analysis of DNA fragmentation by flow cytometry showed apoptotic cell death in MCF-7 cell treated with saffron extract. Saffron-induced apoptosis could be inhibited by pan-caspase inhibitors, indicating caspase-dependent pathway was induced by saffron in MCF-7 cells. Bax protein expression was also increased in saffron-treated cells. Thus saffron exerts proapoptotic effects in a breast cancer-derived cell line and could be considered as a potential chemotherapeutic agent in breast cancer [38].

Calcium channel antagonistic effects

Coronary and other diseases in cardiac or brain blood vessels are considered to be due to the excessive influx of Ca^{2+} into cytoplasm. If Ca^{2+} channels in cell membrane are blocked by medicines or other substances with considerable calcium antagonistic effects, these diseases might be cured or controlled. Crocus sativus on Ca^{2+} influx in isolated rat aortas was investigated by using 45Ca as a radioactive tracer, and their calcium antagonistic effects were evaluated. Ca^{2+} uptake in isolated rat aorta rings in normal physiological status was not markedly altered by these drugs, whereas the Ca^{2+} influxes induced by norepinephrine of 1.2 mmol/L and KCl of 100 mmol/L were significantly inhibited by Crocus in a concentration-dependent manner. The results show that extracellular Ca^{2+} influx through receptor-operated Ca^{2+}channels and potential-dependent Ca^{2+}channels can be blocked by Crocus [39].

Effect on brain against oxidative stress

The effects of crocin, a pharmacologically active component of Crocus sativus L. were studied on ischemia/reperfusion (I/R) injury in mice cerebral microvessels. Transient global cerebral ischemia (20 min), followed by 24 h of reperfusion, significantly promoted the generation of nitric oxide (NO) and malondialdehyde (MDA) in cortical micro vascular homogenates, as well as markedly reduced the activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH-px) and promoted the activity of nitric oxide synthase (NOs). Reperfusion for 24 h led to serous edema with substantial micro villi loss, vacuolation, membrane damage and mitochondrial injuries in

cortical micro vascular endothelial cells (CMEC). Furthermore, enhanced phosphorylation of extracellular signal-regulated kinase 1/2 (ERK1/2) and decreased expression of matrix metalloproteinase-9 (MMP-9) were detected in cortical microvessels after I (20 min)/R (24 h). Reperfusion for 24 h also induced membrane (functional) G protein-coupled receptor kinase 2 (GRK2) expressions, while it reduced cytosol GRK2 expression. Pretreatment with crocin markedly inhibited oxidizing reactions and modulated the ultrastructure of CMEC in mice with 20 min of bilateral common carotid artery occlusion (BCCAO) followed by 24 h of reperfusion in vivo. Furthermore, crocin inhibited GRK2 translocation from the cytosol to the membrane and reduced ERK1/2 phosphorylation and MMP-9 expression in cortical microvessels. We propose that crocin protects the brain against excessive oxidative stress and constitutes a potential therapeutic candidate in transient global cerebral ischemia [40].

Neuromodulatory activity

Reactive oxygen species (ROS) are implicated as the leading biochemical cause of neuronal death in various neurologic disorders, including Parkinson's disease. In the present study, neuro modulatory effects of crocetin (active constituent of Crocus sativus) in a 6- hydroxyl dopamine (6-OHDA) model of rat Parkinsonism were investigated. Male Wistar rats were pre-treated with crocetin (25, 50 and 75 Ag/kg body weight) for 7 days and subjected to unilateral intrastriatal injection of 10 Ag 6-OHDA on day 8. Locomotion and rotation were observed on day 23 post-injection, and after 4 weeks, striatum and substantia nigra were dissected out by decapitation. Activity of antioxidant enzymes and content of dopamine (DA) and its metabolites were estimated in striatum, whereas glutathione (GSH) content and thiobarbituric acid reactive substance (TBARS) were evaluated in substantia nigra. Levels of GSH and dopamine were protected, while TBARS content was attenuated in crocetin-treated groups. The activity of antioxidant enzymes was decreased in the lesion group, but protected in the crocetin-treated groups. These findings were supported by the histopathologic findings in the substantia nigra that showed that crocetin protects neurons from deleterious effects of 6-OHDA. This study revealed that crocetin, which is an important ingredient of diet in India and also used in various systems of indigenous medicine, is helpful in preventing Parkinsonism and has therapeutic potential in combating this devastating neurologic disorder [41].

Protective effect on kidney and urinary disorders

Traditional medicine of clod desert Ladakh has large potential to treat various ailments among tribal communities inhabited in the remotest region of Indian subcontinent. This study was conducted to document the new ethno medico botanical information and traditional use of medicinal plants against kidney and urinary disorders, and thus to conserve the rapidly disappearing traditional knowledge system of Amchis of Ladakh. The information was collected from 105 villages of Leh and Kargil districts of Ladakh region by involving 47 Amchis (the herbalists), village heads and old aged persons including women population through on spot interview and repeated queries among other interviewees over a period of 3 years from 2004–2006. The use crocus sativus with 68 medicinal plants belonging to 29 families and 58 genera of clod desert was documented against the treatment of problem in urine discharge, burning sensation and painful urination, inflammation and bleeding in the kidney, irritable condition of bladder, haemorrhage of kidney and removal of blocked urine and kidney stone were the frequently reported disorders in the tribal communities of Ladakh region in India [42].

Antisecretory and antiulcer activity

An aqueous suspension of saffron was subjected for evaluating gastric antiulcer activity induced by pylorus ligation (Shay rats), indomethacin and various necrotizing agents including (80% ethanol, 0.2 M NaOH and 25%NaCl) in rats. Gastric wall mucus and non-protein sulfhydryl contents were also estimated in rats. Histopathological assessment of rat stomach was carried out. The saffron aqueous suspension at doses (250 and 500 mg/kg) exhibited decrease in basal gastric secretion and ulcer index in Shay rats and indomethacin treated groups. Gastric wall mucus elevation was observed. No significant histopathological changes were noted. A large margin of safety was observed in animals after acute and chronic treatment.) Saffron exhibited significant antisecretory and antiulcer activities without causing any deleterious effects on acute and chronic toxicity in rodents [43].

References

1. Zargari A. Medicinal Plants. Tehran University Press, Tehran 2007; 1:574.
2. Kataria D, Kumar C, Nerkar N, Gadiya RV, Abhyankar MM. Detail profile of *Crocus sativus*. International Journal of Pharma and Bio Sciences 2011; 2 (1):530-540.
3. Negbi M. *Saffron* cultivation: past, present and future prospects. Harwood Academic Publishers, Amsterdam 1999; 1: 1-19.
4. Abe K, Saito H. Effects of *saffron* and its constituent crocin on learning behavior and long-term potentiation. Phytother Res. 2000; 14:149-52.
5. Salomi MJ, Nair SC, Panikkar KR. Inhibitory effects of *Nigella sativa* and *Crocus sativus* on chemical carcinogenesis in mice. Nutr Cancer 1991; 16:67-72.
6. Chia-Ying Li, E-Jian Lee, Tian-Shung Wu. Antityrosinase Principles and Constituents of the Petals of *Crocus sativus*. Journal of Natural Products 2004; 67:437-440.
7. Srivastava R, Ahmed H, Dixit RK, Dharamveer, Saraf SA. *Crocus sativus* L.: A comprehensive review. Pharmacognosy Review **2010;** 4(8); 200-208.
8. Yamauchi M, Tsuruma K, Imai S, Nakanishi T, Umigai N, Shimazawa M, Hara H. Crocetin prevents retinal degeneration induced by oxidative and endoplasmic reticulum stresses via inhibition of caspase activity. European Journal of Pharmacology 2011; 650; 110–119.
9. Kazuho A, Minoru S, Yukihiro S, Hiroshi S. Crocin antagonizes ethanol inhibition of NMDA receptor-mediated responses in rat hippocampal neurons. Brain Research 1998; 787:132–138.
10. Das I, Das S, Saha T. Saffron suppresses oxidative stress in DMBA- induced skin carcinoma: A histopathological study. Acta histochemica 2010; 112: 317-327.
11. Aung HH, Wang CZ, Ni M, Fishbein A, Mehendale SR, Xie JT, Shoyama CY, Yuan CS. Crocin from *Crocus sativus* possesses significant anti-proliferation effects on human colorectal cancer cells. Exp Oncol 2007; 29 (3):175–180.
12. Abdullaev FI, Espinosa-Aguirre JJ. Biomedical properties of saffron and its potential use in cancer therapy and chemoprevention trials. Cancer Detection and Prevention 2004; 28:426–432.
13. Abdullaev FI. Inhibitory effect of crocetin on intracellular nucleic acid and protein synthesis in malignant cells. Toxicology Letters 1994; 70:243-251.
14. Escribano J, Alonso GL, Prados MC, Fernandez JA. Crocin, safranal and picrocrocin from saffron (*Crocus sativus* L.) inhibit the growth of human cancer cells in vitro. Cancer Lett 1996; 100: 23–30.

15. Morjani H, Tarantilis P, Polissiou M, Manfait M. Growth inhibition and induction of crythroid differentiation activity by crocin, dimethylcrocetine and b-carotene on K562 tumor cells. Anticancer Res1990; 10:1398–406.
16. Takashi O, Hiroshi S, Ken-ichi M, Katsunori I, Michihiro F, Hiroyuki T, Yukihiro S, Akihisa T, Reiko E, Shinji S. Protective effects of carotenoids from saffron on neuronal injury in vitro and in vivo. Biochimica et Biophysica Acta 2007; 1770:578–584.
17. Nair SC, Salomi MJ, Varghese CD, Panikkar B, Panikkar KR. Effect of saffron on thymocute proliferation, intracellular glutathione levels and its antitumor activity. Bio Factors 1992; 4(1):51–4.
18. Martin G, Goh E, Neff AW. Evaluation of the developmental toxicity of crocetin on Xenopus. Food Chem Toxicol 2002; 40(7):959–64.
19. Nair SC, Kurumboor SK, Hasegawa JH. Saffron chemoprevention in biology and medicine: a review. Cancer Biother 1995; 10:257–64.
20. Daly ES. Protective effect of cysteine and vitamin E, *Crocus sativus* and *Nigella sativa* extracts on cisplatin-induced toxicity in rats. J Pharm Belg 1998; 53:87–93.
21. Naghizadeh B, Mansouri SMT, Mashhadian NV. Crocin attenuates cisplatin-induced renal oxidative stress in rats. Food and Chemical Toxicology 2010; 48: 2650–2655.
22. Ochiai T, Ohno S, Soeda S, Tanaka H,Shoyama Y, Shimeno H. Crocin prevents the death of rat pheochromyctoma (PC-12) cells by its antioxidant effects stronger than those of α - tocopherol. Neuroscience Letters 2004; 362: 61–64.
23. Pitsikas N, Zisopoulou S, Tarantilis PA, Kanakis CD, Polissiou MG, Sakellaridis N. Effects of the active constituents of *Crocus sativus* L., crocins on recognition and spatial rats' memory. Behavioural Brain Research 2007; 183:141–146.
24. Pitsikas N, Boultadakis A, Gergiadou G, Tarantilis PA, Sakellaridis N. Effects of the active constituents of *Crocus sativus L.* in an animal model of anxiety. Phytomedicine 2008; 15:1135-9.
25. Hosseinzadeh H, Ziaee T, Sadeghi A. The effect of saffron, Crocus sativus stigma, extract and its constituents, safranal and crocin on sexual behaviors in normal male rats. Phytomedicine 2008; 15:491–495.
26. Akhondzadeh B A, Ghoreishi SA, Noorbala AA, Akhondzadeh SH, Rezazadeh SH. Petal and Stigma of *Crocus sativus* L. in the treatment of depression: A Pilot Double - blind Randomized Trial. Journal of Medicinal Plants 2008; 7 (4):29-36.
27. Hosseinzadeh H, Younesi H. Petal and stigma extracts of *Crocus sativus L.* have antinoceceptive and anti-inflammatory effects in mice. BMC Pharmacol 2002; 2:7.
28. Nemati H, Boskabady MH, Ahmadzadef Vortakolaei H. Stimulatory effects of *Crocus sativus* (Saffron) on β-*2* adrenoreceptors of guinea pig tracheal chains. Phytomedicine 2008; 15:1038-45.
29. Fatehi M, Rashidabady T, Fatehi-Hassanabad Z. Effects of *Crocus sativus* petals extract on rat blood pressure and on response induced by electrical field stimulation in the rat isolated vas deferens and guinea-pig ileum. J Ethnopharmacology 2003; 84:199-203.
30. Hosseinzadeh H, Ghenaati J. Evaluation of the antitussive effect of stigma and petals of saffron (Crocus sativus) and its components, safranal and crocin in guinea pigs. Fitoterapia 2006; 77:446–448.
31. Hosseinzadeh H., Talebzadeh F.Anticonvulsant evaluation of safranal and crocin from Crocus sativus in mice. Fitoterapia 2005; 76:722–724.

32. Hosseinzadeha H, Sadeghnia HR. Protective effect of safranal on pentylenetetrazol-induced seizures in the rat: Involvement of GABAergic and opioids systems. Phytomedicine 2007; 14:256–262.
33. Escribano J, Diaz-Guerra JMM, Riese HH, Ontanon J,Olmo DG, Olmo DCG, Rubio A, Fernandez J. A. In vitro activation of macrophages by a novel proteoglycan isolated from corms of Crocus sativus L. Cancer Letters 1999; 144:107-114.
34. Goyal SN, Arora S, Sharma AK, Joshi S, Ray R, Bhatia J, Kumari S, Arya DS. Preventive effect of crocin of *Crocus sativus* on hemodynamic, biochemical, histopathological and ultra structural alterations in isoproterenol-induced cardiotoxicity in rats. Phytomedicine 2010; 17: 227–232.
35. Gout B, Bourges C, Dubreuil SP. Satiereal, a Crocus sativus L extract, reduces snacking and increases satiety in a randomized placebo-controlled study of mildly overweight, healthy women. Nutrition Research 2010; 30:305–313.
36. Kanakis CD, Tarantilis PA, Pappas C, Bariyanga J, Riahi HAT, Polissiou MG. An overview of structural features of DNA and RNA complexes with saffron compounds: Models and antioxidant activity. Journal of Photochemistry and Photobiology B: Biology 2009; 95: 204–212.
37. Chen Y, Zhang H, Tian X, Zhao C, Cai L, Liu Y, Jia L,Yin HX, Chen C. Antioxidant potential of crocins and ethanol extracts of *Gardenia jasminoides* ELLIS and *Crocus sativus* L.: A relationship investigation between antioxidant activity and crocin contents. Food Chemistry 2008; 109:484–492.
38. Mousavi SH, Afshari JT, Brook A, Anarkooli IJ. Role of caspases and Bax protein in saffron-induced apoptosis in MCF-7 cells. Food and Chemical Toxicology2009; 47:1909–1913.
39. Liu N, Yang Y, Mo S, Liao J, Jin J.Calcium antagonistic effects of Chinese crude drugs: Preliminary investigation and evaluation by ^{45}Ca. Applied Radiation and Isotopes 2005; 63:151–155.
40. Zhen YQ, Liu JX, Wang JN, Xu L. Effects of crocin on reperfusion-induced oxidative/nitrative injury to cerebral microvessels after global cerebral ischemia. Brain Research.2007; 1138:86-94.
41. Ahmad AS, Ansari MA, Ahmad M, Saleem S, Yousuf S, Hoda MN, Islam F. Neuroprotection by crocetin in a hemi-parkinsonian rat model. Pharmacology, Biochemistry and Behavior 2005; 81:805-813.
42. Ballabh B, Chaurasia OP, Ahmed Z, Singh SB. Traditional medicinal plants of cold desert Ladakh-Used against kidney and urinary disorders. Journal of Ethnopharmacology2008; 118: 331-339.
43. Mofleh LA, Alhaider AA, Mossa JS, Sohaibani MOA, Qureshi S, Rafatullah S. Antigastric Ulcer Studies on 'Saffron' *Crocus sativus* L. in Rats. Pakistan Journal of Biological Sciences 2006; 9 (6): 1009-1013.