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Review

Bioactivity assessment and toxicity of crocin: A comprehensive review

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ABSTRACT

Since ancient times, saffron, the dried stigma of the plant *Crocus sativus* L. has been extensively used as a spice and food colorant; in folk medicine it has been reputed to be efficacious for the alleviation and treatment of ailments. In addition to the three founded major constituents including crocin, picrocrocin and safranal, presence of carotenoids, carbohydrates, proteins, anthocyanins, vitamins and minerals provide valuable insights into the health benefits and nutritional value of saffron. Of the carotenoids present in saffron, highly water-soluble crocin (mono and diglycosyl esters of a polyene dicarboxylic acid, named crocetin) is responsible for the majority of its color, and appears to possess various health-promoting properties, as an antioxidant, antitumor, memory enhancer, antidepressant, anxiolytic and aphrodisiac. It is also worth noting that the crocin principle of saffron exhibited high efficacy along with no major toxicity in experimental models. We would be remiss to not consider the great potential of saffron and crocin, which benefits the cuisine and health of human life throughout the world. The present study provides a comprehensive and updated report of empirical investigations on bioactivities and biological characteristics of crocin.

Contents

Abbreviations: AMD, age-related macular degeneration; CE, capillary electrophoreis; CPP, conditioned place preference; CK, creatine kinase; *C. sativus* L., *Crocus sativus* L.; FAB-MS, fast atom bombardment-mass spectrometry; *G. fructus*, *Gardenia fructus*; *G. jasminoides*, *Gardenia jasminoides Elliss*; GC, gas chromatography; GSH, glutathione peroxidase; IC_{50}, half maximal inhibitory concentration; HPLC, high performance liquid chromatography; i.p, intraperitoneal; i.c.v., intracerebroventricular; IR, ischemia-reperfusion; LDH, lactate dehydrogenase; MDA, malondialdehyde; MES, maximal electroshock seizure; MMS, methyl methanesulfonate; NMR, nuclear magnetic resonance; NO, nitric oxide; PTZ, pentylenetetrazole; ROS, reactive oxygen species; SOD, superoxide dismutase; TLC, thin layer chromatography; TBA, 2-thiobarbituric acid method; TBARS, thiobarbituric acid reactive species; TG, triglyceride; TNF, tumor necrosis factor; (UV/visible), ultraviolet/visible.

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1. Introduction

The genus *Crocus* includes roughly 88 species among which *Crocus sativus* L. (*C. sativus* L.), is the most studied. This perennial herb is a member of the Iridaceae family, the line of Liliaceae and is cultivated in Azerbaijan, France, Greece, India, Iran, Italy, Spain, China, Israel, Morocco, Turkey, Egypt, and Mexico (Xue, 1982). Saffron, the dried stigma of the plant *C. sativus* L., has a distinct color, flavor and smell. Among the estimated 150 volatile and nonvolatile compounds present in saffron, fewer than 50 constituents have been identified so far (Winterhalter and Straubinger, 2000). The volatiles consist of more than 34 components that are mainly terpenes, terpene alcohols and their esters among which safranal (2,6,6-trimethylcyclohexane-1,3-dien-1-carboxaldehyde) is the principle constituent (Liakopoulou-Kyriakides and Kyriakidis, 2002). Non-volatiles include crocins, which are mainly responsible for the red color of stigmas, together with carotenes, crocetin (also called α-crocetin or crocetin-I), picrocrocin and safranal (Liakopoulou-Kyriakides and Kyriakidis, 2002).

Saffron has three major characteristic components (a) crocins, the principle coloring pigment (mono and diglycosyl esters of a polyene dicarboxylic acid, named crocetin), at nearly 10% (Pfander and Wittwer, 1975; Tsimidou and Tsatsaroni, 1993), (b) the glycoside picrocrocin which is a precursor of safranal and responsible for its distinctly bitter flavor and (c) safranal, a monoterpen aldehyde which is the deglycosylated form of picrocrocin and the major organoleptic principle of the stigmas (Fig. 1). Studies have shown the presence of carbohydrates, proteins, anthocyanins, flavonoids, vitamins (especially riboflavin and thiamine), amino acids, mineral matters, gums and many other chemical compounds in saffron (Abdullaev, 1993; Liakopoulou-Kyriakides and Kyriakidis, 2002; Winterhalter and Straubinger, 2000).

Saffron has a long history of use in many specialty dishes around the world, known for its color, odor and taste; it also enhances perfumes and cosmetics, as well as medication to treat a wide variety of human health conditions (Winterhalter and Straubinger, 2000). At present, saffron is almost exclusively used as a natural flavoring in the food industry. Recent studies have boosted interest in its medicinal properties as antioxidants (Hosseinzadeh et al., 2009b; Ochiai et al., 2004; Soeda et al., 2007), antitumorigenic (Abdullaev, 2002; Aung et al., 2007; Escribano et al., 1996; Garc-Olmo et al., 1999; Mousavi et al., 2011), memory enhancers (Abe and Saito, 2000; Ghadrdoost et al., 2011; Hosseinzadeh and Ziaei, 2006; Pitsikas et al., 2007), antidepressants and anxiolytics (Hosseinzadeh et al., 2004, 2007; Hosseinzadeh and Noraei, 2009; Wang et al., 2010), aphrodisiac (Hosseinzadeh et al., 2008c; Shamsa et al., 2009; Sumalatha et al., 2010), genoprotectives (Hosseinzadeh and Sadeghnia, 2007a; Premkumar et al., 2001), antitussives (Hosseinzadeh and Ghenaati, 2006), cardioprotectives (Goyal et al., 2010; Imenshahidi et al., 2010; Xu et al., 2006; Zhang et al., 2009), and neuroprotectives (Essa et al., 2012; Mehri et al., 2012) see Fig. 2.

2. Chemistry of crocin

2.1. Chemical composition of crocin family

The crocins are a group of hydrophilic carotenoides that are either mono- or di-glycosyl polyene esters of crocetin in which D-glucose and/or D-gentiobiose occur as carbohydrate residues (Fig. 3). Other than crocins (glycosyl and gentiobiosyl esters of crocetin) and crocetin (a dicarboxylic 20-carbon carotenoid), a number of carotenoid compounds have been identified in saffron, including minor amounts of lycopene, alpha and beta carotene, zeaxanthin, phytoene and phytofluene, which are classified as the oil soluble color pigments of saffron (Pfander and Schurtenberger, 1982).

The crocin family includes various glycosyl esters of which six types have been detected in saffron. Sugars bound to the two acidic groups of the aglycone crocetin are provided in Table 1 (Liakopoulou-Kyriakides and Kyriakidis, 2002). Crocin analogues including crocins 1–4 are almost glycosides of trans-crocetin in

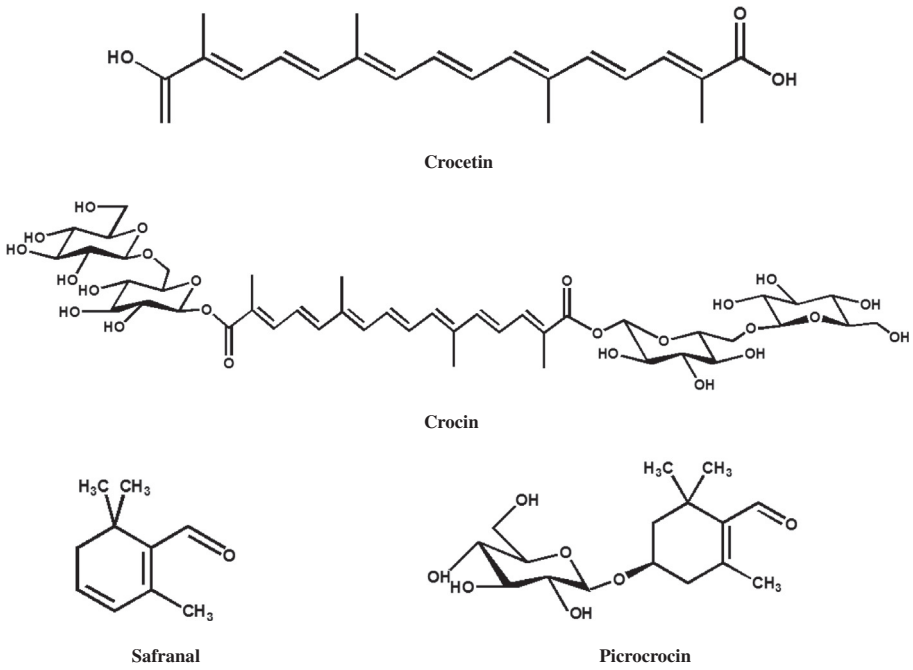

Fig. 1. Molecular structures of saffron polyene dicarboxylic acid (crocetin) and its diglycosyl ester (crocin), monoterpen aldehyde (safranal) and its glycoside form (picrocrocin).

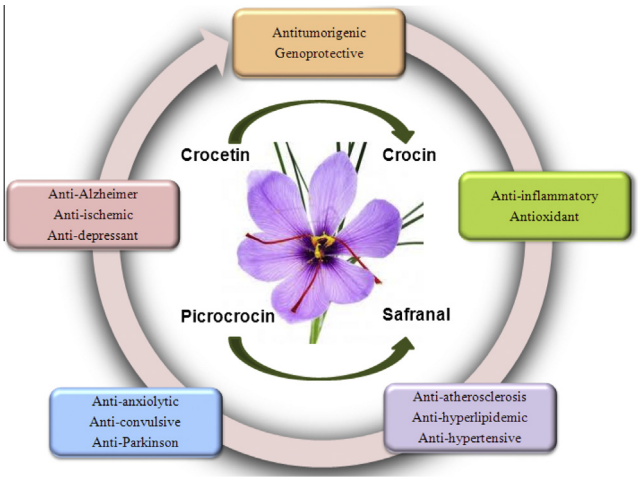

Fig. 2. Some of pharmacological effects of saffron and its constituents.

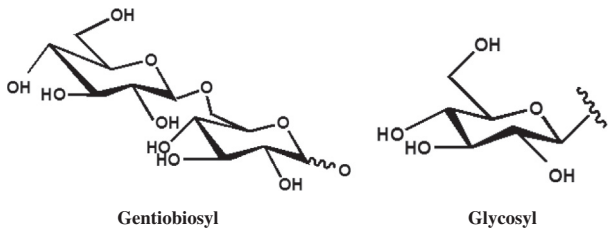

Fig. 3. Molecular structures of sugar moieties of crocin derivatives.

saffron among which trans-crocins 3 and 4 are the most abundant. In Spanish saffron, these crocins range between (0.01–9.44%) and (0.46–12.12%) respectively, (Alonso et al., 2001; Pfander and Wittwer, 1975) while, cis-crocetin and its glycosides are minor components (Li et al., 1999). It is reported that all crocin derivatives occur as pairs of cis–trans isomers except crocin-1 (Dhingra et al., 1975). A study by Speranza et al. (1984) indicated that trans-crocins undergo photoisomerization reactions and convert to cis-crocins; this process depends on the agricultural and environmental conditions in the area of the plants origin.

Compared to the other carotenoides of saffron, crocin or all-trans crocetin di-β-D-gentiobiosyl ester possess the highest coloring capacity due to its high water solubility (Tarantilis et al., 1995). Known for its quenching of free radicals and endowed with tumoricidal properties, crocin is regarded as the first choice water soluble food adjunct, although it is also soluble in methanol and ethanol.

2.2. Natural sources of crocin

It has been shown that geographical location and processing methods affect the resulting saffron samples; with respect to color, flavor and bitterness.

For example, Greek saffron has the highest concentration of components, followed by India and New Zealand. This variation may be the result of dissimilar drying processes, duration and storage, affecting the concentration of glycoside carotenoides (Caballero-Ortega et al., 2007). In addition, crocin is found in the fruit and flower of *Gardenia jasminoides* Elliss (*Rubiaceae*) which is a popular ornamental shrub grown worldwide (Table 2) (Wang et al., 2004).

Glycosylation of crocetin is crucial, since it confers hydrosolubility to the pigment. Limited information is available regarding the carotenoid glycosylation process, however; some reports state that the conversion of crocetin into crocetin glycosides naturally occurs in saffron stigmas and *gardenia* fruits through a multi-step pathway involving two different glycosyl transferases. While one enzyme renders formation of crocetin mono- and di-glycosyl esters (glucose transferase1), the other catalyzes conversion of glycosidic bonds to gentiobiosyl esters (glucose transferase 2) (Dufresne et al., 1999; Gresta et al., 2008). Plant cell cultures have been suggested as an efficient alternative for crocin derivatives production. The ability of *C. sativus* L. callus extract to transform crocetin into glycosyl esters (Dufresne et al., 1997) and saffron cell culture to

Table 1
Crocetin and crocin derivatives of saffron (Liakopoulou-Kyriakides and Kyriakidis, 2002).

Compound	Sugar moieties	Chemical formula	Isomer occurrence in saffron
Crocetin	R1 = R2 = OH	$C_{20}H_{24}O_4$	*cis–trans*
Crocin 1	R1 = β-D-glucosyl R2 = H	$C_{26}H_{34}O_9$	*trans*
Crocin 2	R1 = β-D-gentiobiosyl R2 = H	$C_{32}H_{44}O_{14}$	*cis–trans*
Crocin 2′	R1 = R2 = β-D-glucosyl	$C_{32}H_{44}O_{14}$	*cis–trans*
Crocin 3	R1 = β-D-gentiobiosyl R2 = β-D-glucosyl	$C_{38}H_{54}O_{19}$	*cis–trans*
Crocin 4	R1 = R2 = β-D-gentiobiosyl	$C_{44}H_{64}O_{24}$	*cis–trans*
Crocin 5	RI = 3 β-D-glucosyl R2 = β-D-gentiobiosyl	$C_{50}H_{24}O_{29}$	*cis–trans*

Table 2
Plants from which crocin is extracted.

Species	Part	References
Crocus sativus L. [Iridaceae]	Tissue culture	Sano and Himeno (1987)
Crocus sativus L. [Iridaceae]	Silk stigma style	Carmona et al. (2006)
Gardenia jasminoides J. ELLIS [Rubiaceae]	Fruit	Kamikura and Nakazato (1985) and Pfister et al. (1996)

glycosylate exogenously-added crocetin have been reported (Dufresne et al., 1999).

2.3. Crocin extraction and detection methods

Various methodologies and analytical tools have been applied to recover bioactive constituents of saffron (Castellar et al., 1993; Hadizadeh et al., 2010; Iborra et al., 1992; Sujata et al., 1992; Tarantilis et al., 1995). Analysis of saffron chemicals originating from various geographical areas indicate that the amount of constituents depends highly on processing; methods for extraction, drying and quantification. Extraction of saffron metabolites is optimized by the solvent, temperature, light and stirring time used in the process. Various solvents including water (Iborra et al.), ethanol (Hadizadeh et al., 2010; Iborra et al., 1992) and diethyl ether (Pitsikas et al., 2007) are used for crocin extraction. Recent studies indicate that a solution of methanol–water (50%, v/v) with magnetic stirring during 1 h in darkness at 25 °C is optimal for obtaining all the saffron components, including crocin (Castellar et al., 1993; Li et al., 1999; Tarantilis et al., 1994b, 1995; Tsimidou and Tsatsaroni, 1993). Methanolic extraction has also been used for isolation of crocin derived from the fruit of *G. jasminoides*; for structure identification techniques such as fast atom bombardment-mass spectrometry (FAB-MS), Ultraviolet/visible (UV/visible) and nuclear magnetic resonance (NMR) (Choi et al., 2001). In a study by Hadizadeh et al. (2010), a two-step crystallization procedure with 80% ethanol resulted in crocin of higher purity than that obtained with methanolic extraction when using UV–visible spectrophotometry and high performance liquid chromatography (HPLC). Using molecular imprinting, Mohajeri et al. (2010) designed a gentiobiose imprinted polymer which enabled selective and specific extraction of crocin from saffron stigmas with a significant recovery rate of 84%.

According to the international organization for standardization 3632 (ISO, 1993, ISIR), the measurement of picrocrocin, safranal and crocin is based on the absorbance of 1% aqueous solution of dried saffron at 257 (λ_{max} of picrocrocin), 330 (λ_{max} of safranal), and 440 nm (λ_{max} of crocins) respectively.

ISO 3632 (ISO, 1993, ISIR) which deals exclusively with saffron, established four empirical color intensity grades: IV, the poorest, III, II, and I as the highest quality based upon crocin contents by measurement of crocin-specific spectroscopic absorbance at 440 nm. Higher absorbance implies greater crocin content and a greater colorative intensity; the color quality of saffron samples on the market depends largely on the quantification of crocin (Alonso et al., 1990; Orfanou and Tsimidou, 1996). As the exact identification and quantification of each component in saffron extract cannot be accomplished by colorimetric methods alone, additional preparative and analytical procedures have been developed for quality control; including HPLC (Castellar et al., 1993; Li et al., 1999; Sujata et al., 1992; Tarantilis et al., 1994b, 1995; Tarantilis and Polissiou, 1997), thin layer chromatography (TLC) (Iborra et al., 1992; Sujata et al., 1992) and gas chromatography (GC) (Sujata et al., 1992; Zareena et al., 2001). Capillary electrophoresis (CE) has also been used as a simple but effective choice (Zougagh et al., 2005). Common extraction and quantification methods are summarized in Table 3.

3. Biological activities of crocin

Abundant research has been conducted concerning the biological and pharmacological properties of two saffron ingredients, safranal (Rezaee and Hosseinzadeh, 2013) and crocin. It has shown that crocin exhibits beneficial effects on many organs including the nervous system, the most studied, gastrointestinal, cardiovascular, genital, endocrine, immune systems, etc. (summarized in

Table 3
Methods for extraction and quantification of crocin from saffron.

Extraction methods (first step)	References	Quantification (as the second step)	References
Methanol extraction	Li et al. (1999), Lozano et al. (1999), and Zougagh et al. (2006)	UV–visible spectrophotometer	Hadizadeh et al. (2010) and Mohajeri et al. (2010)
MISPE (molecular imprinting solid phase extraction)	Mohajeri et al. (2010)	HPLC	Hadizadeh et al. (2010) and Mohajeri et al. (2010)
Crystallization method	Hadizadeh et al. (2010)	HPLC with photodiode array detection	Lozano et al. (1999), Alonso et al. (2001), and Caballero-Ortega et al. (2007)
Water extraction	Alonso et al. (2001)	HPTLC	Corti et al. (1996)
NACE (non-aqueous capillary electrophoresis)	Zougagh et al. (2005)	HPLC–UV	Li et al. (1999)

Table 4
Pharmacological effects of saffron in *in vivo* studies.

Targeted system and effect	Study design	Dose (mg/kg) animal route	Result	Proposed mechanism	References
CNS					
Learning and memory	Morris water maze, open field activity	50–200 Rats i.p.	Inhibited hyoscine-induced learning deficits and impaired acquisition/ performance activities at low and high doses		Hosseinzadeh and Ziaei (2006)
	Object recognition task, radial water maze test	15–30 Rats (n = 10) i.p.	Counteracted delay-dependent recognition memory deficits and Attenuated scopolamine-induced performance deficits	Modulation of storage and/or retrieval of information	Pitsikas et al. (2007)
	Water maze test	15–30 Rats (n = 20) s.c.	Blocked the ability of chronic stress to impair spatial learning and memory retention	Increasing activities of antioxidant enzymes	Ghadrdoost et al. (2011)
	Morris water maze	5–25 Wistar rats (n = 7) i.p.	Reduced the escape latency time and the traveled distance to find the platform and increased the spent time in the target quadrant at dose of 25	Probably due to Antioxidant effects	Hosseinzadeh et al. (2012)
Morphine withdrawal, conditioned place preference (CPP) and dependence	Acquisition and reinstatement of place preference	400–600 NMRI mice (n = 7) i.p.	Decreased the acquisition of morphine CPP (600 mg/kg) and blocked morphine-induced reinstatement of place preference (400–600 mg/kg)		Imenshahidi et al. (2011)
	Morphine-withdrawal syndrome	50–600 Mice (n = 8) i.p.	Crocin reduced withdrawal syndrome without reducing locomotor activity	Interaction with the opioid system	Hosseinzadeh and Jahanian (2010)
Cerebral ischemia	Transient global cerebral ischemia	5–20 C57BL/6JMice Oral	Inhibited oxidizing reaction, modulated the ultrastructure of CMEC and reduced ERK1/ 2 phosphorylation and MMP-9 expression in microvessels	Inhibition of reperfusion-induced oxidative/nitrative injury,	Zheng et al. (2007)
Depression and anxiety	Forced swimming test and open field activity test	50–600 Mice (n = 10) i.p.	Reduced immobility and increased climbing time	Uptake inhibition of dopamine and norepinephrine	Hosseinzadeh et al. (2004)
	Light/dark test	15–50 Wistar Rats (n = 10) i.p.	Increased the latency to enter the dark compartment and prolonged the time spent in the lit chamber		Pitsikas et al. (2008)
	Maze test, open field test and Rotarod test	Mice i.p.	No anxiolytic, hypnotic or myorelaxation effects		Hosseinzadeh and Noraei (2009)
Convulsion	PTZ-induced convulsions in mice	200 Mice i.p.	No anticonvulsant activity		Hosseinzadeh and Talebzadeh (2005)
	Penicillin-induced epileptiform activity	12.5–100 μg Wistar rats (n = 6) i.c.v.	Increased the latency time to onset of first spike wave, decreased the frequency and amplitude of spike waves at (25–100)	Involvement of GABAA-benzodiazepine receptor complex	Tamaddonfard et al. (2012)
Cardiovascular system					
Atherosclerosis	Prophylaxis effect of crocin on experimental atherosclerosis	25–100 Quails Oral	Inhibited the formation of atherosclerosis	Decrease the level of Ox-LDL	He et al. (2005)
Hyperlipidemia and hypertension	Hypolipidemic effect of crocin from *Gardenia jasminoides*	50 ICR mice (n = 6) Oral	Inhibited the increase of serum TG level, total and LDL cholesterol	Inhibition of pancreatic lipase activity	Lee et al. (2005)
	Hypolipidemic mechanism of crocin Hypolipidemic activity of crocin fermented with lactic acid bacteria	25–100 Sprague–Dawley rats-oral mice	Reduced serum triglyceride and total cholesterol Fermented crocin exhibited more potent hypolipidemic effects than crocin	Inhibition of pancreatic lipase Synergistically activated by lactic acid bacteria	Sheng et al. (2006) Lee et al. (2006)
	Hypotensive effect of crocin	50–200 Wistar rats i.v.	Reduced the mean arterial blood pressure in a dose-dependent manner		Imenshahidi et al. (2010)
Myocardial injury	Myocardial injury	12.5–50 i.v. 50–100 i.g. Rats	Only iv administration decreased the areas of myocardial injury	Management of myocardial ischemia	Du et al. (2005)
	Effect on cardiotoxicity	5–20 Wistar albino rats (n = 16) Oral	Modulated hemodynamic and antioxidant derangements at 20 mg/kg	Modulation of oxidative stress	Goyal et al. (2010)
Sexual function					
	The effect of crocin on sexual behavior	100–400 Wistar rats (n = 6) i.p.	Increased mounting frequency, intromission frequency, erection frequency and reduced mount latency, intromission latency and ejaculation latency		Hosseinzadeh et al. (2008c)
Tumor					
	Inhibition of carcinogenesis	Mice Oral	Delayed the formation of papillomas		Konoshima et al. (1998)

(*continued on next page*)

Table 4 (*continued*)

Targeted system and effect	Study design	Dose (mg/kg) animal route	Result	Proposed mechanism	References
	Antitumor effect of crocin	400 BD-IX rats (n = 12) s.c.	Extended life span and slowed tumor growth in female rats	Strong cytotoxic effect	Garc-Olmo et al. (1999)
	Antitumor activity of PEGylated nanoliposomes containing crocin	50–100 BALB/c mice (n = 6) i.v.	Decreased tumor size and increased survival rate		Rastgoo et al. (2013)
Kidney					
	Histopathological and biochemical examinations	100–400 Wistar Albino rats (n = 6) i.p.	Dose-dependent reduction in malondialdehyde and elevation in total thiol and glutathione peroxidase concentration	Antioxidant activity	Naghizadeh et al. (2008)
	Evaluation of antioxidant capacity	18.7–75 Kunming mice Oral	Enhanced SOD of liver and kidney and decreased MDA of serum	Antioxidant activity	Chen et al. (2010a)
Liver					
	Evaluation of serum enzymes as markers of aflatoxin B1-induced hepatotoxicity	50 Sprague–Dawley rats Oral	Suppressed enzyme elevation	Carotenoid contents converted metabolically to retinoid	Lin and Wang (1986)
	Biochemical changes induced by iron overload in rat liver	200 Wistar albino rats (n = 8) i.p.	Ameliorated most of the biochemical changes induced by iron overload in rat liver	Enhanced the recovery of cellular ATP and stimulated oxygen consumption	EL-Maraghy et al. (2009)
	Hematological and biochemical examinations of blood and tissue samples	200 Wistar rats (n = 8) i.p.	Restored hematological and biochemical parameters near to normal levels	Reduction of BeCl2-induced oxidative stress and mRNA expression of antioxidant genes	El-Beshbishy et al. (2012)
Inflammation					
	Cyclooxygenase (COX) inhibition assays, gastric lesion tests anti-inflammatory effect	Mice rats oral	Fewer stomach lesions, inhibitory activity against the COX-1 and 2 enzymes and inhibited edema in mice and rats		Xu et al. (2009)
	Neurobehavioral feature in experimental autoimmune encephalomyelitis	100 C57BL/6 wild type mice i.p.	Suppressed the induction of endoplasmic reticulum stress and immune gene transcripts *in vivo*		Deslauriers et al. (2011)
	Formalin test	50–200 Rats i.p.	Increased morphine-induced antinociception	Naloxone insensitive-induced analgesia	Tamaddonfard and Hamzeh-Gooshchi (2010a)
	Hypertonic saline-induced corneal pain	Rats i.p. i.c.v.	Decreased the number of eye wipes, increased Morphine-induced antinociception	Opioid receptors-independent analgesic effect	Tamaddonfard and Hamzeh-Gooshchi (2010a)
	The von Frey filament, acetone drop, radiant heat and open field test	12.5–50 Wistar Rats i.p.	Failed to produce any protective effect at a dose of 50 mg/kg	Limited permeability into the CNS	Amin and Hosseinzadeh (2012)
Ischemia					
	The cellular redox status evaluation and Ferric reducing /antioxidant power test	50–400 Wistar Rats (n = 6–8) i.p.	Significant reduction in cellular redox status and a subsequent elevation in antioxidant power	Quenching of free radicals and antioxidant effects	Hosseinzadeh et al. (2005b)
	TBARS, FRAP and total SH groups assay	50–400 Wistar rats (n = 8) i.p.	Elevated total SH contents and antioxidant capacity in muscle flap and declined MDA level	Antioxidant and anti-inflammatory activity	Hosseinzadeh et al. (2009a)
Antidote					
	Crocin-induced biomarker alteration in diazinon toxicity	50–200 Wistar rats (n = 6) i.p.	Prevented diazinon-induced enzymes elevation and some specific biomarkers i.e. TNF-a, 8-iso-prostaglandin F2a and soluble protein-100 b	Reduced lipid peroxidation and acted as an antioxidant	Hariri et al. (2010)
	Crocin-induced hematological indices alteration	50–200 Wistar rats (n = 6) i.p.	Reduced diazinon hematological toxicity, but did not prevent the genotoxicity		Hariri et al. (2011)
Genotoxicity					
	Comet assay	50–400 NMRI mice (n = 5–7) i.p.	Significantly decreased DNA damage by MMS (4.69 and 6.55-fold for liver and spleen (400 mg/kg)	Decreases DNA damage by protecting DNA nucleophilic sites	Hosseinzadeh et al. (2008a)
Miscellaneous					
	Evaluation the number of cough induced by citric acid 20%	50–600 guinea pigs (n = 6) i.p.	No antitussive activity		Hosseinzadeh and Ghenaati (2006)

Table 4 (*continued*)

Targeted system and effect	Study design	Dose (mg/kg) animal route	Result	Proposed mechanism	References
	Measurement of lipid and gastric mucosa GSH level	2.5–10 Wistar rats (n = 10) Oral	Prevented gastric lesions, increased lipid peroxidation and decreased glutathione levels induced by indomethacin in non-diabetic and diabetic rats		Kianbakht and Mozaffari (2009)
	Neutrophil adhesion test, cyclophosphamide induced neutropenia, etc.	9.69 Mice oral	Stimulated both cellular and humoral immune responses by increasing adhesion of neutrophils, attenuating neutropenia, reducing mice mortality and, etc.		Khajuria et al. (2010)
	Crocin-induced biochemical indices alteration by in diabetic rats	50–150 Wistar rats (n = 10) Oral	Reduced the fasting blood glucose and HbA1c and increased the blood insulin levels		Kianbakht and Hajiaghaee (2011)
	Crocin effect on non-rapid eye movement sleep	30–100 Mice i.p.	Increased the total time of non-rapid eye movement sleep by 60 and 170% during a 4 h period from 20:00 to 24:00		Masaki et al. (2012)
Toxicity					
	Reversible hepatic black pigmentation and enzyme alteration	50–100 Rats oral	Low toxicity in rats even in high experimental dosage	Gradual accumulation of crocin dyes	Wang et al. (1984)
	Acute and sub-acute toxicity of crocin	3 g Orally 0.5–3 g/kg i.p. Razi mice (n = 5) 50–180 Wistar rats (n = 6) i.p.	Crocin at pharmacological doses did not exhibit marked damages to all the major organs of the body		Hosseinzadeh et al. (2010)

Table 4). Below by class are recent advances regarding the effects of crocin on a number of organs by order of importance.

3.1. Impact of crocin on central nervous system

Crocin demonstrated several pharmacological effects on the nervous system including anxiolytic activity, antidepressant effects, aphrodisiac properties, learning and memory-enhancement and reduction of physical signs of morphine withdrawal which are categorized as follows.

3.1.1. Effect on learning and memory

3.1.1.1. Basic studies.

3.1.1.1.1. In vitro studies. It is generally known that neurodegenerative disorders often cause memory and learning impairments. It was reported that crocin significantly antagonized the suppression of long-term potentiation induced by ethanol in hippocampal neurons (10–30 μM). In addition, it was suggested that the mechanism is mainly through acting on *N*-methyl-D-aspartate receptors (Abe et al., 1998) in which the role of gentiobiose residue in the crocin structure is of crucial importance (Abe and Saito, 2000; Sugiura et al., 1994). However; the compound did not affect the baseline synaptic responses (Sugiura et al., 1995).

3.1.1.1.2. In vivo studies. Interestingly, Hosseinzadeh and Ziaei (2006) showed that crocin at both low and high doses (50–200 mg/kg) inhibited hyoscine-induced learning deficits and impaired acquisition/performance abilities. A similar study by Pitsikas et al. (2007) supported previous findings and suggested crocin involvement in the modulating storage and/or retrieval of information. Crocin also showed positive effects in preventing the impairment of learning and oxidative stress damage induced by chronic stress (Ghadrdoost et al., 2011). Another reported on the antioxidant properties of crocin, highlighting its role in spatial cognitive improvement following chronic cerebral hypoperfusion (Hosseinzadeh et al., 2012).

3.1.2. Effect of crocin on Alzheimer's disease

3.1.2.1. Basic studies.

3.1.2.1.1. In vitro studies. Alzheimer is a neurodegenerative disorder characterized by the formation of extracellular fibrous amyloid plaques and intracellular neurofibrillary tangles. Recently, it was reported that apoptotic cell death occurs in the brains of Alzheimer's patients. A study by Soeda et al. (2001) demonstrated the modulatory role of crocin on the expression of Bcl-2 family proteins; by suppressing the effect of tumor necrosis factor (TNF)-alpha, crocin and ethanolic extract prevented apoptotic morphological changes in PC-12 cells. It was shown that the water: methanolic (50:50, v/v) extract of *C. sativus* L. stigmas inhibited amyloid β-peptide fibrillogenesis possibly due to the presence of trans-crocin-4 and its digentibiosyl moiety (Ghahghaei et al., 2012; Papandreou et al., 2006). A similar study investigated the inhibitory effect of safranal and crocin on apo-α-lactalbumin, a model protein; crocin was found more efficacious than safranal possibly due to its amphiphilic character (Ebrahim-Habibi et al., 2010). Other mechanisms are also involved in the anti-Alzheimer action of saffron, including the inhibitory effect of crocin on acetylcholinesterase activity (Geromichalos et al., 2012) and the reduction of various proinflammatory and neurotoxic factors which are triggered by microglia chronic activation (Nam et al., 2010).

3.1.2.2. Clinical findings. Two 2010 studies by Akhondzadeh et al. highlighted the effectiveness of saffron in mild to moderate Alzheimer's disease (AD). In one study, 46 patients with probable AD received saffron capsules (15 mg) twice daily. Results suggest that using saffron capsules can be associated with a better outcome on cognitive functions compared to the placebo group in a 16-week study (Akhondzadeh et al., 2010a). A further study with similar dosing consisted of 54 adults, 55 years or older, evaluated the efficacy of saffron compared with donepezil (10 mg/day) in treating mild to moderate AD in a 22-week trial. Results demonstrate similarity in cases with adverse effects except vomiting, which occurred more frequently in the donepezil treated group (Akhondzadeh et al., 2010b).

While clinical studies demonstrated that saffron is efficacious in a short-term treatment of mild to moderate disease conditions, there is no clinical evidence for crocin in this regard.

3.1.3. Effect on morphine withdrawal, conditioned place preference and dependence

3.1.3.1. Basic studies.

3.1.3.1.1. In vivo studies. An abundance of evidence indicates that the mesolimbic-dopamine system contributes to the acute

reinforcing effects of opioids. A study by Imenshahidi et al. (2011) highlighted crocins potential in reducing the acquisition and reinstatement of morphine-induced conditioned place preference (CPP). They reported that pretreatment of mice with i.p. injection of crocin (600 mg/kg during four days and 30 min before the morphine administration) blocked the acquisition of morphine CPP. Hosseinzadeh's research team conducted an additional study evaluating the effects of aqueous and ethanolic extracts of stigma and its constituents crocin and safranal on morphine withdrawal syndrome in mice. Results showed that both aqueous and ethanolic extracts and crocin suppressed the syndrome by reducing the number of jumping episodes. They also reported that the aqueous extract, clonidine as well as higher doses of ethanolic extract decreased locomotor parameters in open field tests; however, the inhibitory activity of crocin was much lower. Thus, the effect of crocin on the opioid system seemed to be more specific than aqueous and ethanolic extracts, since crocin ameliorated withdrawal symptoms associated with morphine, without reducing locomotor activity (Hosseinzadeh and Jahanian, 2010).

3.1.4. Effect on cerebral ischemia

3.1.4.1. Basic studies.

3.1.4.1.1. In vitro studies. The cumulative evidence supports the involvement of reactive oxygen species (ROS) in cerebral ischemia-induced neural cell damage. In fact, ROS are responsible for generating nitric oxide (NO) and malondialdehyde (MDA) and reducing activities of superoxide dismutase (SOD) and glutathione peroxidase (GSH). Antioxidants including α-tocopherol, β-carotene, ascorbic acid and more, too numerous to list, are considered promising for neuroprotection due to their potential ROS suppressing abilities (Ochiai et al., 2004; Saleem et al., 2006). The authors reported that treatment of PC-12 cells with crocin inhibited cell membrane lipid peroxidation and restored intracellular SOD activity even more efficacious than α-tocopherol at the same concentration (Ochiai et al., 2004). Further, *in vitro* studies demonstrated that the underlying mechanism through which crocin combats ischemic stress-induced neural cell death is by increasing GSH activities and preventing the activation of c-Jun NH2-terminal kinases (JNK) pathway which is well depicted in Fig. 4 (Ochiai et al., 2004; Soeda et al., 2005).

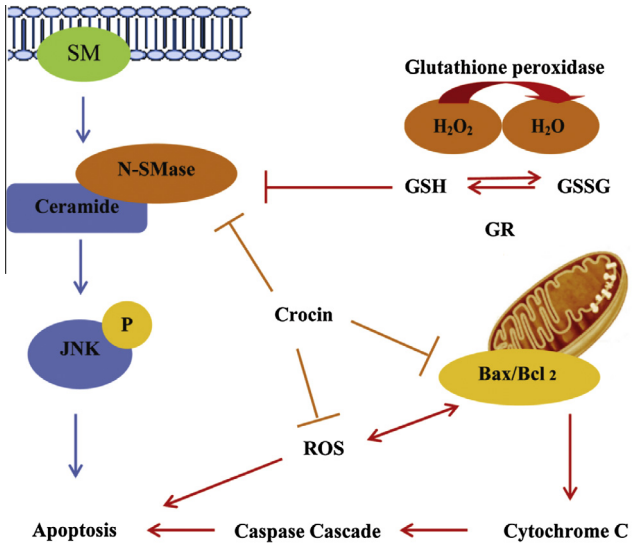

Fig. 4. Overview of the mechanisms involved in apoptosis inhibition by crocin. SM, N-SMase, P, GSH, GSSG, GR respectively refers to: sphingomyelinase, neutral sphingomyelinase, phosphorylated, glutathione, oxidized glutathione, glutathione reductase. Adapted from (Soeda et al., 2007).

3.1.4.1.2. In vivo studies. Pretreatment of mice with crocin significantly suppressed oxidizing reactions and modulated the ultrastructure of microvascular endothelial cells. In addition, crocin inhibited matrix metalloproteinase-9 (MMP-9) expression in mice within 20 min of bilateral common carotid artery occlusion followed by 24 h of reperfusion *in vivo* (Dong et al., 2009; Zheng et al., 2007). Hosseinzadeh et al. (2012) also reported that crocin (25 mg/kg) prevented many detrimental consequences of cerebral ischemia on memory in adult Wistar rats.

3.1.5. Antidepressant and anxiolytic effects

Several lines of evidence indicate that saffron and its components possess antidepressant and anxiolytic effects.

3.1.5.1. Basic studies.

3.1.5.1.1. In vivo studies. The antidepressant effect of saffron stigmas was shown to be significantly higher than placebos when treating major depression (Richelson, 1994). This effect was further investigated in aqueous and ethanolic extracts of *C. sativus* L. stigma and petal by means of forced swimming tests on mice (Karimi et al., 2001). They found this activity was likely mediated by safranal (0.05–0.15 ml/kg) and crocin (50–600 mg/kg) via the uptake inhibition of dopamine, norepinephrine and serotonin (Hosseinzadeh et al., 2004). Kaempferol, a *C. sativus* L. petal constituent, showed a marked reduction in time for the forced swimming test (Hosseinzadeh et al., 2007). Later, by means of behavioral models it was demonstrated that antidepressant-like characteristics of aqueous stigmas extract of *C. sativus* L. were attributed to the presence of crocin 1 and 2 (Wang et al., 2010). In addition to being a potent antidepressant, some studies report that crocin may have anxiolytic properties. Pitsikas et al. (2008) indicated that treatment with crocin (50 mg/kg) could significantly change mice behavior and induce anxiolytic-like effects in rats. Conversely, a study by Hosseinzadeh and Noraei (2009) demonstrated that anxiolytic and hypnotic effects are mainly observed with saffron aqueous extract and safranal and not crocin.

3.1.5.2. Clinical findings. Clinical trials reinforce previous findings for the treatment of mild to moderate depression. In a double-blind, single-center trial conducted by Akhondzadeh and colleagues, thirty adult outpatients with a baseline Hamilton rating scale for depression score of at least 18, were randomly assigned to receive capsules of dried extract of saffron (30 mg/day, three times a day, TDS) and imipramine (100 mg/day TDS) for 6 weeks. Saffron at this dose was found to be as efficacious as imipramine in treating mild to moderate depression (Akhondzadeh et al., 2004). In another study, forty adult outpatients participated in a 6-week double-blind single-center trial. Patients were randomly selected to receive either saffron or fluoxetine capsules in a dose of 30 and 20 mg/day twice daily, respectively. Saffron at this dose was as effective as fluoxetine with no difference in terms of observed side effects (Noorbala et al., 2005). Overall, these trials suggest that further comprehensive studies regarding dose-dependent toxicities and tolerable adverse effects profile of saffron are required to expedite its application as an alternative treatment for depression.

3.1.6. Anticonvulsant activity

3.1.6.1. Basic studies.

3.1.6.1.1. In vivo studies. Traditionally, *C. sativus* L. has been used as an anticonvulsant. This use of *C. sativus* L. aqueous and ethanolic extracts was seen in mice using pentylenetetrazole (PTZ) and the maximal electroshock seizure (MES) tests. In PTZ test, plant extracts failed to produce complete protection against mortality, however, in MES test both extracts decreased the duration of tonic seizures (Hosseinzadeh and Khosravan, 2002). Investigation

indicated that contrary to safranal, crocin seemed to be devoid of anticonvulsant activity in PTZ-induced seizures (Hosseinzadeh and Talebzadeh, 2005). Recently, studying coadministration of crocin and diazepam on penicillin-induced epilepsy demonstrated a GABAA–benzodiazepine receptor mediated antiepileptic activity at brain level (Tamaddonfard et al., 2012). Other studies demonstrated that the potential of safranal to prevent seizure was more likely through GABAA–benzodiazepine receptor complex (Hosseinzadeh and Sadeghnia, 2007b; Hosseinzadeh et al., 2008b; Pathan et al., 2009; Sadeghnia et al., 2008). Additional studies using various models are required to clarify the anticonvulsant capacity of *C. sativus* L. and its ingredient crocin.

3.2. Effect of crocin on the cardiovascular system

Recent studies have demonstrated the potential of crocin in the treatment of atherosclerosis, hyperlipidemia and several other cardiovascular related disorders which are classified as follows.

3.2.1. Effect on atherosclerosis

3.2.1.1. Basic studies.

3.2.1.1.1. In vitro studies. Liu and Qian (2005) studied the role of crocin on cholestane-3β, 5α, 6β-triol-induced apoptosis and the gene expression patterns of cultured endothelial cells; the results indicated that inhibition of apoptosis by crocin is by increasing Bcl2/Bax ratio expression which is a well-known mechanism (see Fig. 4). Further studies by Xu et al. (2007) reinforced previous findings and verified that prevention of apoptosis by crocin had an important role in the inhibition and regression of atherosclerosis. Another study highlighted a dose-dependent cytoprotective effect of crocin (1–10 μM) against hydrogen peroxide-induced endothelial cell injury which certainly contributed to its application in cardiovascular related disorders (Xu et al., 2006). It should be noted, crocetin also showed the potential to inhibit the formation of atherosclerosis in quail (He et al., 2007).

3.2.1.1.2. In vivo studies. In a pioneer study in 1999, crocin increases the blood coagulation time in mice and also significantly inhibits thrombin induced blood platelet aggregation in rabbits (Shiping et al., 1999). A further study by He et al. (2005) demonstrated the potential of crocin in reducing the level of total serum cholesterol, triglyceride (TG), LDL-C and restricting the formation of aortic plaque in experimental atherosclerosis induced by the hyperlipidemic diet in quail; thus, decreasing endothelial cell apoptosis induced by oxidized LDL.

3.2.2. Effect on hyperlipidemia and hypertension

3.2.2.1. Basic studies.

3.2.2.1.1. In vivo studies. Lipid metabolism normally occurs as an impressive balance between synthesis and degradation. Chinese research indicates that crocin extracted from *G. fructus* and *C. sativus* L. significantly decreased serum TG, total LDL and LDL cholesterol levels in experimental hyperlipidemia models; and that this was more likely due to inhibition of pancreatic lipase (Lee et al., 2005; Sheng et al., 2006). Inhibitions of both smooth muscle cells proliferation and p38MAPK activation were also reported (Xu et al., 2005). Another study demonstrated that using lactic acid bacteria, fermented crocin showed more potent antihyperlipidemia activities compared with crocin in corn oil feeding-induced triglyceridemic and Triton WR-1339-induced hyperlipidemic mice (Lee et al., 2006). Though studies are more focused on crocin, crocetin has also been shown to be efficacious in improving hyperlipidemia (Lee et al., 2005). Another investigation compared the hypotensive effect of saffron aqueous extract and its two active ingredients in rats; results revealed that aqueous extract of saffron stigma, safranal and crocin decreased the mean arterial blood pressure of animals in a dose-dependent manner. It seemed that safranal is the major contributor to the hypotensive activity of the aqueous extract (Imenshahidi et al., 2010).

3.2.3. Effect on myocardial injury

3.2.3.1. Basic studies.

3.2.3.1.1. In vivo studies. An *in vivo* rat model of myocardial injury indicated that single intravenous administration of crocin decreased the areas of myocardial injury, serum levels of lactate dehydrogenase (LDH) and creatine kinase (CK) while neither multiple, nor single intragasterical administrations of crocin showed significant results (Du et al., 2005). Orally administered crocetin represents a promising therapeutical target for antimyocardial ischemia. Crocetin significantly reduced the levels of LDH, CK, MDA and increased the level of SOD and the activity of cardiac myocytes in rats; this implies that crocin barely absorbed through the gastrointestinal tract and subsequently showed a very low serum concentration (Zhang et al., 2009). Inconsistent with previous reports, orally administered crocin in rats (20 mg/kg) was shown preventative against isoproterenol induced myocardial infarction which was confirmed by enzymatical, histopathological and ultrastructural parameters (Goyal et al., 2010).

3.3. The study of crocin on sexual function

3.3.1. Basic studies

3.3.1.1. In vivo studies. Plant materials have a long history of use in sexual dysfunction. In traditional medicine, saffron was given as an aphrodisiac. Investigations concerning the aphrodisiacal effect of *C. sativus* L. aqueous extract and its constituents, safranal and crocin, in male rats indicates that crocin (100, 200 and 400 mg/kg) and the extract (160 and 320 mg/kg) increased mounting frequency, intromission frequency, erection frequency and reduced mounting latency, intromission latency and ejaculation latency, while safranal did not result in any pharmacological effects (Hosseinzadeh et al., 2008c).

3.3.2. Clinical findings

There is no clinical evidence regarding the aphrodisiacal effects of crocin and there are inconsistent reports regarding the role of *C. sativus* L. in treating male erectile dysfunction.

In 2009, an open clinical trial was conducted to evaluate the efficacy of saffron on erectile dysfunction. 20 male patients received a tablet containing 200 mg of saffron each morning for 10 days. A significant increase in mean scores for the erectile function, orgasmic function, sexual desire, intercourse and overall satisfaction was observed (Shamsa et al., 2009). Despite some limitations in study design including sample size, optimum saffron dosage and side effects-related excess use, results suggested a beneficial effect with saffron for the treatment of erectile dysfunction. On the other hand, a crossover study comparing efficacy of sildenafil citrate and saffron showed no improvement of erectile dysfunction with saffron. 346 men with erectile dysfunction (mean age 46.6 ± 8.4 years) were randomly selected to receive on-demand sildenafil for 12 weeks followed by 30 mg saffron twice daily for another 12 weeks or vice versa, separated by a 2-week washout period. No significant improvements were observed regarding the international index of erectile function, sexual encounter profile questions and erectile dysfunction inventory of treatment satisfaction scores with saffron administration. Mean per patient 'yes' responses to global efficacy question was 91.2% and 4.2% for sildenafil and saffron, respectively ($P = 0.0001$) (Safarinejad et al., 2010).

3.4. Tumoricidal properties

3.4.1. Basic studies

3.4.1.1. In vitro studies. Studies have shown that the crocin component of saffron has the potential to inhibit growth of different types of tumor cells. In one experiment the half maximal inhibitory concentration (IC_{50}) of crocin in the presence of HL-60 leukemic cells was 2 μM which was less toxic than retinoids (Tarantilis et al., 1994a). Other cell lines including K562, HeLa, and HT-29, had IC_{50} of 3, 0.4, and 1 mM, respectively (Abdullaev, 2002).

Escribano et al. (1996) reported that treating Hela cells with crocin caused reduced cytoplasm, cell shrinkage and other apoptosis like morphological changes in cells while crocetin and safranal played a minor role in the cytotoxic effect of saffron extract. Another study investigating long-term treatment with crocin on colorectal cancer indicated that crocin exerted a potent cytotoxic effect on human and animal adenocarcinoma cells *in vitro* which caused the same morphological changes in cells as was previously reported. Further investigation on three colorectal (HCT-116, SW-480, and HT-29) and two breast cancer cell lines reinforced previous findings and suggested crocin, either extracted from *C. sativus* L., or other species of *crocus*, as the major responsible constituent, irrespective of the degree of glycosylation inhibited growth of cancer cells while not influencing normal cells (Aung et al., 2007; Chryssanthi et al., 2007). Mechanistically it was shown that crocin induced broad changes in the expression profile of genes involved in cell cycle controlling. In fact by down-regulating the expression of Bcl-2 and up-regulating the expression of Bax, crocin increased the number of cells in G0/G1 phase and thus increased the percentage of cell apoptosis (Lv et al., 2008; Xu et al., 2010; Zhao et al., 2008). Apoptosis induction by crocin and G1 cell cycle arrest was further reported when human pancreatic and tongue squamous cancer cell lines were treated with 10 μg/L and 0.4 mM of crocin, respectively (Bakshi et al., 2010; Sun et al., 2011). Hosseinzadeh and his colleagues reported that the ethanolic extracts of saffron stigma and petal possessed antitumor activity with the more potent and less toxic effects of the stigma extract (Hosseinzadeh et al., 2005a).

Interestingly, encapsulation of crocin in liposomal form induced a sub-G1 peak in flow cytometry histogram of HeLa and MCF-7 treated cells, indicating involvement of apoptosis in this toxicity which was more pronounced than crocin alone when comparing IC_{50} values (Crocin liposomal forms IC_{50} values after 48 h: 0.61, 0.64, and 1.2 mM and crocin IC_{50} after 48 h: 1.603 mM in HeLa cells) (Mousavi et al., 2011). Numerous *in vitro* studies highlight the antitumor properties of crocin, while clinical studies in humans are still scarce and in-depth studies are required to define underlying mechanisms contributing to the therapeutic properties of crocin. Comparing the cytotoxic activity of crocin and safranal using potato discs and brine shrimp assays, results indicated that both crocin (0.5–8 mg/ml) and safranal (0.075–8 mg/ml) possessed a remarkable dose-dependent antitumoral property that confirmed previous findings (Behravan et al., 2010).

3.4.1.2. In vivo studies. Crocin can cause a delay in the formation of mouse skin papillomas (Konoshima et al., 1998). Subcutaneous (s.c.) administration of crocin increased rats life span and decreased tumor growth more intensely in female rats without showing any significant toxic effects (Garc-Olmo et al., 1999). Recently, the antitumor activity of PEGylated nanoliposomes containing crocin (50 and 100 mg/kg, intravenously) was shown in BALB/c mice bearing C26 colon carcinoma (Rastgoo et al., 2013).

3.5. Effect on renal function

3.5.1. Basic studies

3.5.1.1. In vivo studies. Naghizadeh et al. (2008) investigated the effect of crocin on cisplatin-induced renal failure. The results indicate that pretreatment of rats with crocin lowers the blood urea, creatinine and urinary glucose and protein concentrations in a dose-dependent manner. In addition, treatment with crocin resulted in a more pronounced elevation of the total thiol and glutathione peroxidase concentrations. Oral administration of crocin-1 enhanced SOD and total antioxidant capacity of the kidney which supported previous findings (Chen et al., 2010a). It is conceivable that the protective effect of crocin on the kidney depends heavily on its antioxidant characteristics. In conclusion, further studies including clinical trials are required to clarify the potential of crocin for kidney protection.

3.6. Hepatoprotective properties

3.6.1. Basic studies

3.6.1.1. In vivo studies. The liver as the major site of xenobiotic metabolism can be injured by exposure to various toxic chemicals, drugs, infection, etc. There are a number of plants originating from traditional medicine, endowed with hepatoprotective properties; which act as radical scavengers, enzyme inhibitors, and some as mitogens. Crocin extracted from G. jasminoides at 0.1% in the diet, protected rats against hepatic damage induced by aflatoxin B1 and dimethylnitrosamine via suppressing serum levels of enzymes such as alkaline phosphatase and LDH. However, higher doses failed to show hepatoprotective effect (Lin and Wang, 1986). In another study, it was shown that i.p. injection of crocin (200 mg/kg) prevents iron-induced liver injury in rats reflected by significant changes in the liver function indices, hyperammonemia and reduced serum urea levels (EL-Maraghy et al., 2009). Though the exact hepatoprotective mechanism is not well defined and it requires further investigation, it seems that this protective effect is performed via oxidative pathways.

3.7. Antioxidant activities

3.7.1. Basic studies

3.7.1.1. In vitro studies. The radical scavenging potential of crocin is involved in its neuroprotective, anti-aging, anti-inflammatory and antitumoral activities. First reported in 1982, Bors et al. (1982) indicated involvement of radicals in bleaching the aqueous solution of crocin. Later, this phenomenon was used as a reward to evaluate antioxidant activity (Bors et al., 1984). Heavily studied, this crocin bleaching inhibition method is used to measure the antioxidant capacity of substances (Bathaie et al., 2011; Bors et al., 1984; Bountagkidou et al., 2012; Hosseinzadeh et al., 2010; Kampa et al., 2002; Tubaro et al., 1996). Crocin extracted from *gardenias* fruit showed antioxidant activity at a lower concentration as evaluated by 2-thiobarbituric acid method (TBA) mainly through reduction in lipid peroxidation (Han et al., 1994). An additional study indicated that the antioxidant property of crocin evaluated by the thiocyanate method was better than that of the 2-thiobarbituric acid (Pham et al., 2000).

It reported that methanolic solution of crocin extracted from *C. sativus* L. possessed high radical scavenging activity (50% and 65% for 500 and 1000 ppm, respectively) (Assimopoulou et al., 2005). A series of studies by Soeda et al. (2007) proposed that a GSH-dependent mechanism is involved in crocin inhibitory effects on oxidative stress-induced cell death. Unlike *in vivo* studies, there was a direct correlation between total crocin content and antioxidant properties *in vitro*; this antioxidant function was strongly affected by the attached sugar moieties in crocin struc-

tures (Chen et al., 2010b, 2008). Hosseinzadeh and colleagues noted that the aqueous and ethanolic extracts of saffron as well as crocin and safranal diminished the extent of MDA generation. They also suggested that the inhibitory potential of crocin (1and 2 mM) and ethanolic extract (500 and 1000 μg/ml) on liver microsomal lipid peroxidation was as efficacious as that of BHT (100 μM) (Hosseinzadeh et al., 2009b).

Ordoudi et al. (2009) indicated that saffron extract antioxidant activity is low in cell-free systems compared with well established scavengers, whereas its ability to reduce intracellular ROS production was as equally efficacious as phenolic antioxidants. The reduction in ROS production was also observed by pretreatment of glucose-induced neurotoxicity in PC12 cells with crocin (10 and 50 μM) (Mousavi et al., 2010). A study by Sebastin Santhosh et al. (2012) proposed the role of crocin in treating the secondary complications of snakebite by ameliorating proinflammatory cytokine levels including IL-1β, TNF-α and IL-6 and decreasing the oxidative stress induced by venom. Crocin is the major contributor to the antioxidant activity of saffron and *gardenia* extracts with several involved mechanisms including nitrite scavenging ability, 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) radical cation inhibition, SOD-like activity, and elongation of lipid peroxidation (Yang et al., 2011).

To the best of our knowledge the antioxidant potential of saffron and crocin underlies many of its pharmacological activities and further research concerning this issue is therefore required.

3.7.1.2. In vivo studies. A 2010 study comparing the antioxidant capacity of orally administered crocin-1 and crocetin extracted from gardenia suggests the similar efficacy in Kunming mice (Chen et al., 2010a). In a recent study it was reported that pretreatment of BeCl2-intoxicated rats with crocin (200 mg/kg i.p.) significantly increased mRNA expression of antioxidant genes including catalase and SOD (El-Beshbishy et al., 2012).

3.8. Effect on ocular disease

3.8.1. Basic studies

3.8.1.1. In vitro studies. Ischemic retinopathy and age-related macular degeneration (AMD) are the two leading causes of blindness. A study investigating the effect of crocin on light-induced cell death in primary retinal cell culture indicated that crocin prevented light-mediated detrimental damage on photoreceptors in a concentration dependent manner (EC50:30 μM) (Laabich et al., 2006).

3.8.2. Clinical findings

Oral administration of saffron (20 mg/day) to patients over a 3-month period improved retinal flicker sensitivity in early AMD (Falsini et al., 2010). To evaluate the potency of crocin in improving ocular blood flow, crocin analogues isolated from *C. sativus* L. were studied for their vasodilation effect in rabbits; by increasing blood flow and subsequent oxygenation of retinal structure crocin enhanced retinal function recovery (Xuan et al., 1999). Though the results were promising, further replications to investigate clinical significance are yet to be evaluated.

3.9. Antinociceptive and anti-inflammatory effects

3.9.1. Basic studies

3.9.1.1. In vitro studies. Several constituents isolated from the fruits of *G. jasminoides Ellis* were tested for anti-inflammatory activity. Among them genipin exerted a significant inhibitory effect on prostaglandin and NO production in lipopolysaccharide-treated RAW 264.7 cells, while crocin failed to do so (Lim et al., 2008). Another study to the contrary, indicated that crocin exhibited a dual inhibitory effect *in vitro* against both cycloxoygenase 1 and 2 enzymes and prostaglandin E2 production (Xu et al., 2009).

3.9.1.2. In vivo studies. In vivo investigations reinforced the previous *in vitro* findings. Crocin inhibited edema in a dose-dependent manner in two animal models including xylene-induced ear edema in mice and carrageenan-induced paw edema in rats. Results showed that crocin modulates inflammatory pathways (Xu et al., 2009). The anti-inflammatory potential of crocin was also seen in neuroinflammatory diseases like multiple sclerosis (Deslauriers et al., 2011). Hosseinzadeh and Younesi (2002) reported that aqueous and ethanolic extracts of saffron stigma and petal possessed antinociceptive as well as anti-inflammatory properties. In a subsequent study which was published in 2010, it indicated that crocin at a dose of 100 mg/kg, increases morphine-induced antinociception, however, naloxan was not able to reverse this effect (Tamaddonfard and Hamzeh-Gooshchi, 2010a). They also reported that crocin alleviated hypertonic saline-induced corneal pain which was not opioid-receptor dependent (Tamaddonfard and Hamzeh-Gooshchi, 2010b). In a recent study, Hosseinzadeh's laboratory looked at an alternative mechanism for crocin antinociceptive property. Results of this investigation demonstrated that i.p. injection of crocin even at a dose of 50 mg/kg failed to attenuate the behavioral symptoms of chronic constriction injury-induced neuropathic pain in rats which was in disagreement with the results of the above mentioned reports (Amin and Hosseinzadeh, 2012). In addition, safranal was also studied for antinociceptive effects in mice. The results indicated that safranal possess antinociceptive effects in formalin and acid acetic tests (Hosseinzadeh and Shariaty, 2007).

Based on the current results more studies are warranted to determine the beneficial effects of crocin for its use in human subjects.

3.10. Effect of crocin on ischemia

3.10.1. Basic studies

3.10.1.1. In vivo studies. Since the generation of ROS is tightly associated with kidney tissue injury following ischemic insult, use of antioxidants to ameliorate kidney diseases therapy appears rational. In a study by Hosseinzadeh et al. (2005b), in a rat model of renal ischemia–reperfusion (IR) injury it was shown that pretreatment with crocin (400 mg/kg) caused a significant reduction in cellular redox status (thiobarbituric acid reactive species) and a subsequent elevation in antioxidant power. This effect was more pronounced with saffron extract than with crocin. The same research group reported safranal potential for preventing IR oxidative damage in rat hippocampus (Hosseinzadeh and Sadeghnia, 2005). They also showed the protective effect of crocin in IR injury in skeletal muscle seen in the close range between 50 and 400 mg/kg. It was shown that following administration of saffron, crocin and safranal 1 h prior reperfusion, the total sulfhydryl (SH) contents and antioxidant capacity increased and the MDA level decreased in the muscle flap. These results suggested the protective role of saffron extract and its constituents against lower limb IR in rats (Hosseinzadeh et al., 2009a).

3.11. Crocin as an antidote against acrylamide and diazinon

3.11.1. Basic studies

3.11.1.1. In vitro studies. Organophosphorus compounds such as diazinon are classified as neurotoxic chemical agents. Some proposed that in addition to acetylcholinesterase inhibitory activity; by generating free radicals they induce oxidative stress. A recent study by Mehri et al. (2012) highlighted the protective role of cro-

cin on acrylamide-induced neurotoxicity. Pretreatment of PC12 cells with crocin (10–50 μM) markedly decreased apoptosis and inhibited ROS generation in a dose-dependent manner.

3.11.1.2. In vivo studies. In the 2010 study both crocin and safranal influenced the occurrence of biomedical indices and enzyme level changes induced by diazinon, significantly decreasing the level of direct 8-iso-prostaglandin F2a and soluble protein-100 b in rats (Hariri et al., 2010). One year later, the same team investigated the effect of crocin and safranal on hematological and genotoxicity indices in diazinon intoxicated rats; results indicated that both ingredients protect against diazinon-induced hematological toxicity with no significant effect on genotoxicity (Hariri et al., 2011).

Taken together, the potential application of crocin as an antidote against neurotoxicity is mainly via suppressing intracellular ROS production and interrupting the cell death cascade.

3.12. Genoprotective properties of crocin

3.12.1. Basic studies

3.12.1.1. In vivo studies. A study by Premkumar et al. (2001) demonstrated the modulatory effect of saffron on the *in vivo* genotoxicity of cisplatin, cyclophosphamide, mitomycin C and urethane. In this study pretreatment of Swiss albino mice using three different doses (20, 40 and 80 mg/kg of body weight) of the aqueous extract of saffron in a 5-day period, remarkably inhibited the genotoxicity of cytotoxic agents in the mouse bone marrow micronucleus test, but had a minor inhibitory effect on glutathione S-transferase activity. Later, it was reported that safranal could repress methyl methanesulfonate (MMS)-induced DNA damage in different mouse organs as measured by the comet assay. However, the exact mechanism involved is still unknown (Hosseinzadeh and Sadeghnia, 2007a). A similar study by the same team reported that in addition to safranal, crocin significantly decreased DNA damage by MMS in a dose-dependent manner (Hosseinzadeh et al., 2008a). These studies highlight the potential of *C. sativus* L stigma, safranal and crocin to inhibit genotoxicity induced by genotoxic agents.

3.13. Miscellaneous effects

In addition to the above listed biological effects of crocin, further investigation has been associated with pharmacological activities that are rather new or rarely studied. For example, Hosseinzadeh and Ghenaati (2006) investigated the antitussive activity of crocin, but no significant pharmacological outcome was observed. In other study, crocin was found to protect against gasteric mucosal damage induced by indomethacin. This phenomenon was mediated by decreasing lipid peroxidation and increasing glutathione levels in non-diabetic and diabetic rats with a similar efficacy as omeprazol (Kianbakht and Mozaffari, 2009). Promising results were obtained regarding the potency of crocin as an immunomodulator in animals; by using various tests, it was revealed that crocin could stimulate cellular as well as humoral immune responses quite effectively (Khajuria et al., 2010). Kianbakht and Hajiaghaee (2011) designed a 6-week trial to test the ability of crocin to counteract alloxan-induced hyperglycemia. They found that crocin significantly reduced fasting blood glucose and hemoglobin A1c and increased blood insulin levels. In another study, Masaki et al. (2012) examined the sleep-promoting activity of crocin by investigating locomotor activity. They found that crocin at a lights-off time increased the total time of non-rapid eye movement.

4. Crocin toxicity

As crocin is considered the main coloring pigment of *C. sativus* L and *G. jasminodes* and saffron containing crocin is widely used as a spice and food colorant for culinary purposes, investigating the potential toxicity of saffron extract and its crocin principle is of crucial importance. In a study published in 2004, rats were administered daily with i.p. doses of aqueous extract of stigma (0.16, 0.32 and 0.48 g/kg) and petal (1.2, 2.4 and 3.6 g/kg) for a 2-week period. Both extracts produced normochromic normocytic anemia with petal extract exerting toxic effects on liver and lung organs (Karimi et al., 2004). It was previously reported that crocin dyes, isolated from *G. jasminodes* caused reversible and dose-dependent black pigmentation of the liver and acute hepatic damage associated with discoloration. A daily dose of 50 mg/kg for 8 days did not affect hepatic function but a higher dosage of 100 mg/kg for a duration of 2 weeks induced hepatic damage and black pigmentation. Feeding rats a diet consisting of 1% crocin dyes for a 4-month period resulted in reversible pigmentation, suggesting that crocin is safe for coloring purposes (Wang et al., 1984). In a more comprehensive study, Hosseinzadeh et al. (2010) investigated biochemical, hematological and pathological criterias in mice and rats after treatment with crocin (up to 3 g, p.o. and i.p. as well as 15–180 mg/kg, i.p.). The results revealed that at pharmacological doses crocin did not cause damage to any major organ in the body.

4.1. Clinical findings

Modaghegh et al. (2008) designed a double-blind, placebo-controlled clinical trial testing of a 1-week course of saffron tablet treatment. Healthy adult volunteers were divided into 3 groups of 10 each (5 males and 5 females). Group 1 received placebos; groups 2 and 3 received 200 and 400 mg saffron tablets. Clinical examination exhibited no remarkable changes in the general health status of volunteers after intervention. However, some hematological and biochemical indices changes were observed in normal ranges. A recent study evaluated a 1-month treatment with 20 mg crocin tablets in healthy volunteers. Except for minor changes of hematological parameters, no major adverse effects were reported (Mohamadpour et al., 2013). In terms of medical application, further long-term and large-scale investigations are required to elucidate the effect of saffron and its constituents on human health.

5. Conclusions

Saffron has a long history of being used by diverse cultures as a seasoning and coloring agent. It has been used extensively as an indigenous medicine throughout the world. A large body of research has been conducted on *C. sativus* and its major constituent's pharmacological characteristics notably for their potential to treat CNS related diseases and various types of cancer. Among the phytochemicals present in saffron, crocin is the most frequently investigated in many *in vitro* and *in vivo* studies. These studies clearly underscore the convergent role of crocin in preventing, treating or alleviating various health related conditions. Most *in vivo* studies were carried out on the effects of crocin against CNS and cardiovascular related diseases; however research projects on other organs including the liver and kidney are not negligible. These findings have not yet been verified by clinical trials on humans. Only one study has dealt with evaluating the safety of crocin tablets in healthy volunteers showing a relatively safe profile at 20 mg/kg per month. However, more comprehensive and in-depth studies involving longer periods of time, varied dose schedules and

a greater number of participants with specific disorders are required to confirm the safety and effects of crocin. Clinical application of saffron tablets continues to highlight the promising role of saffron in the treatment of Alzheimer, depression, sexual dysfunction and age-related macular degeneration. The promising positive effects of saffron were replicated in all disease conditions. These trials were described as double blind, using different dosing schedules ranging from 20 to 200 mg/day and for duration of 10 days to 16 weeks. Saffron extracts at all doses were well tolerated and efficacious in treating various conditions. No study was carried out to compare the effects of saffron and crocin and to clearly specify dosage correlation of the two components. As saffron contains approximately 10% crocin, the proposed dose for this constituent is around 10–20 mg for clinical trials. These effects as a whole describe crocin as a promising natural lead compound and candidate for medicinal applications. However, clinical evidence is still scarce in this regard and more comprehensive studies with special focus on human clinical trials is required.

Conflict of Interest

The authors declare that there are no conflicts of interest.

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