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Review

A comprehensive review on biological activities and toxicology of crocetin

Maryam Hashemi[a], Hossein Hosseinzadeh[b,c,*]

[a] Nanotechnology Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran
[b] Department of Pharmacodynamics and Toxicology, School of Pharmacy, Mashhad University of Medical Sciences, Mashhad, Iran
[c] Pharmaceutical Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran

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ABSTRACT

Natural products with high pharmacological potential and low toxicity have been considered as the novel therapeutic agents. Crocetin is an active constituent of saffron (*Crocus sativus* L.) stigma, which in its free-acid form is insoluble in water and most organic solvents. Crocetin exhibits various health-promoting properties including anti-tumor, neuroprotective effects, anti-diabetics, anti-inflammatory, anti-hyperlipidemia, etc. These therapeutic effects can be achieved with different mechanisms such as improvement of oxygenation in hypoxic tissues, antioxidant effects, inhibition of pro-inflammatory mediators, anti-proliferative activity and stimulation of apoptosis in cancer cells. It is also worth considering that crocetin could be tolerated without major toxicity at therapeutic dosage in experimental models. In the present review, we discuss the biosynthesis, pharmacokinetic properties of crocetin and provide a comprehensive study on the biological activities and toxicity along with the mechanism of actions and clinical trials data of crocetin.

1. Introduction

Natural products have played an important role to develop the novel therapeutic agents with high pharmacological potential and low toxicity (Moshiri et al., 2015).

Saffron, derived from the *Crocus sativus* stigmas has been used in prevention and treatment of various diseases for many centuries. Some health benefits of saffron have been summarized in Fig. 1(Khorasany and Hosseinzadeh, 2016).

The therapeutic activity of saffron is mainly due to its major bioactive derivatives including crocin and crocetin (Fig. 2) (Bathaie et al., 2014; Rameshrad et al., 2018).

Crocin, a diester of the disaccharide gentiobiose, has many health benefits such as antidepressant, antioxidant, anti-cancer and neuroprotective effects (Rajeev K Singla, 2011). After oral administration of crocin, crocetin and its glucuronide conjugates were found in plasma, however, crocin was not detectable. It could be concluded that crocin are hydrolyzed to crocetin before or during intestinal absorption (Asai et al., 2005; Xi et al., 2007a).

Crocetin ($C_{20}H_{24}O_4$; molecular weight 328 g/mol) is the result of crocin glycosides hydrolysis, which in its free-acid form is insoluble in water and most organic solvents, except for pyridine and dimethylsulfoxide. Crocetin in its anionic form is highly water-soluble, therefore, it easily dissolves in dilute aqueous sodium hydroxide or other aqueous alkali solutions pH ≥ 9 (Escribano et al., 1996).

Recently, the most important therapeutic effects of saffron have been attributed to crocetin. This substance could act with different mechanisms such as enhancement the rate of oxygen transport and diffusivity during shock resulted in increased ATP production, inhibition of pro-inflammatory mediators, protection of cells from ROS damage and stimulation of apoptosis in cancer cells. Therefore, crocetin can be useful in different disorders, such as arteriosclerosis, hemorrhages, arthritis, tumors, etc. (Festuccia et al., 2018; Giaccio, 2004; Nam et al., 2010).

This review outlines the biosynthesis and pharmacokinetic properties, the biological activities and toxicity along with the mechanism of action and clinical trials data of crocetin.

2. Search strategy

A comprehensive literature review was performed by searching in Scopus (http://www.scopus.com) and PubMed (http://www.ncbi.nlm.nih.gov/pubmed), to identify all published articles about the biosynthesis, pharmacokinetics, and pharmaceutical applications of crocetin. The search terms included “crocetin”, “Extraction,” “Pharmacokinetic” and “Biological activities” in titles and abstracts. The search was conducted from inception to April 2019.

* Corresponding author. Pharmaceutical Research Center, Pharmaceutical Technology Institute, Mashhad University of Medical Sciences, Mashhad, Iran.
E-mail address: hosseinzadehh@mums.ac.ir (H. Hosseinzadeh).

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Abbreviations

AD	Alzheimer's Disease
ADA	adenosine deaminase
AFB1	aflatoxin B1
AHH	arylhydrocarbon-hydroxylase
ALD	aldehyde dehydrogenase
ALP	alkaline phosphatase
ALT	alanine transaminase
Ang II	angiotensin II
AST	aspartate transaminase
ATRA	all-trans retinoic acid
BBB	the blood-brain barrier
BCEC	porcine brain capillary endothelial cells
BCSFB	blood cerebrospinal fluid barrier
CCD	carotenoid cleavage dioxygenase
Cmax	maximum concentration
COX-2	cyclooxygenase-2
CrtZ	β-carotene hydroxylase
DMSO	dimethyl sulphoxide
EGFR	epidermal growth factor receptor
EPC	endothelial progenitor cell
ER +	estrogen receptor positive
EMT	epithelial-mesenchymal transition
ESR	electron spin resonance
GC	gas chromatography
GGT	gamma-glutamyl transpeptidase
GPX	glutathione peroxidase
GR	glutathione reductase
GSH	glutathione
CSE	hydro-ethanolic saffron extract
GST	glutathione S-tranferase
HPLC	high-performance liquid chromatography
HRMECs	human retinal microvascular endothelial cells
HUVEC	human umbilical vein endothelial cell
i.p.	intraperitoneal injection
LDH	lactate dehydrogenase
IL-1β	interleukin-1β
LPS	Lipopolysaccharide
MCP-1	macrophage chemoattractant protein-1
MDA	maleic dialdehyde
MITF	Microphthalmia-Associated Transcription Factor
MMP	matrix metalloproteinases
MMP	mitochondrial membrane potential
MNNG	1-methyl-3-nitro-1-nitrosoguanidine
MPO	lung myeloperoxidase
MWM	Morris water maze
NE	norepinephrine
NMDA	N-methyl-D-aspartate
NMU	N-nitroso-N-methylurea
Nrf2	nuclear factor erythroid 2-related factor 2
OKD	oxidative stress detector
OVA	ovalbumin
PKC	protein kinase C
PLGA	poly (lactic-co-glycolic acid)
PMN	polymorphonuclear
PQ	paraquat
RPE	retinal pigment epithelial
ROS	reactive oxygen species
SCI	spinal cord injury
SHRSPs	Stroke-prone spontaneously hypertensive rat
SNI	spared nerve injury
SOD	superoxide dismutase
SRF	serum response factor
SSc	systemic scleroderma
STZ	streptozotocin
TBARS	the thiobarbituric acid reactive substance
TIPE2	alpha-induced protein 8-like 2
TLC	thin-layer chromatography
TNF	tumor necrosis factor
UPLC–MS/MS	ultra-performance liquid chromatography tandem mass spectroscopy
UV-A	ultraviolet-A
VEGFR-2	vascular endothelial growth factor receptor-2
VSMCs	vascular smooth muscle cells
XXO	xanthine/xanthine oxidase

3. Synthesis, extraction and detection

Two major sources of crocetin are the fruit of *Gardenia jasminoides Ellis* (*cape jasmine*) and stigma of saffron (Carmona et al., 2006).

Crocetin could be extracted from its sources by different methods. Lautenschläger prepared Trans-crocetin using an enzymatic deglycosylation of EtOH–water of saffron extract by Röhm Enzyme® and Rohament CL® enzymes (Lautenschlager et al., 2014).

Livzon Pharmaceutical Group Inc has been developed a technique to produce the crocetin from *G. jasminoides var. radicans Makino*. Briefly, gardenia yellow pigments were extracted by macroporous resin, subjected to alkaline hydrolysis to obtain crude crocetin, and finally purified by recrystallization (Chu et al., 2018). In another study, crocetin was extracted from gardenia fruits with 50% aqueous methanol solution, concentrated in vacuum and applied onto an Amberlite XAD-7 resin column. Then, the obtained pigments were saponified with 10% sodium hydroxide aqueous solution at 65 °C for 3.5 h. After acidification of the solution with phosphoric acid, the yielded precipitate was washed with water twice and then with methanol. Finally, crocetin was crystallized from dimethylformamide, washed with methanol and dried in vacuum (Asai et al., 2005). Crocetin could also prepared by this saponification method from the *C. sativus* style extract (Chryssanthi et al., 2007). In another method, crocetin was prepared from crocin (extracted from saffron) by acidic hydrolyzation to remove saccharides. For this purpose, HCl 33% was added to crocin, sparged with N_2, and kept in 50 °C for 1 h. The crocetin precipitated after adding the water to this solution and centrifuge (Tashakori-Sabzevar et al., 2013). In a recent study, crocetin prepared in an existing β-carotene producing *Saccharomyces cerevisiae* strain through three steps catalyzed using three key enzymes including β-carotene hydroxylase (CrtZ), carotenoid cleavage dioxygenase (CCD) and aldehyde dehydrogenase (ALD)(Chai et al., 2017).

Different techniques have been developed for the analysis of crocetin including UV-visible spectrophotometry, high-performance liquid chromatography (HPLC), ultra-performance liquid chromatography tandem mass spectroscopy (UPLC–MS/MS), thin-layer chromatography (TLC), and gas chromatography (GC) (Karkoula et al., 2018; Mohammadpour et al., 2013; Sanchez et al., 2008; Sujata et al., 1992; Zhang et al., 2017b).

HPLC is a sensitive method, which is commonly used for detection of crocetin. Since crocin converts to crocetin in gastrointestinal tract after oral administration, different strategies have been used to separate the crocetin from plasma such as solid phase extraction or direct precipitation method for HPLC analyze (Chryssanthi et al., 2011b; Mohammadpour et al., 2013). UV–Visible spectrophotometry can also be used for rapid determination of crocetin and crocetin esters (Hafezi Ghahestani et al., 2017; Sanchez et al., 2008; Soltani et al., 2017). The common extraction and detection methods for crocetin are summarized in Table 1.

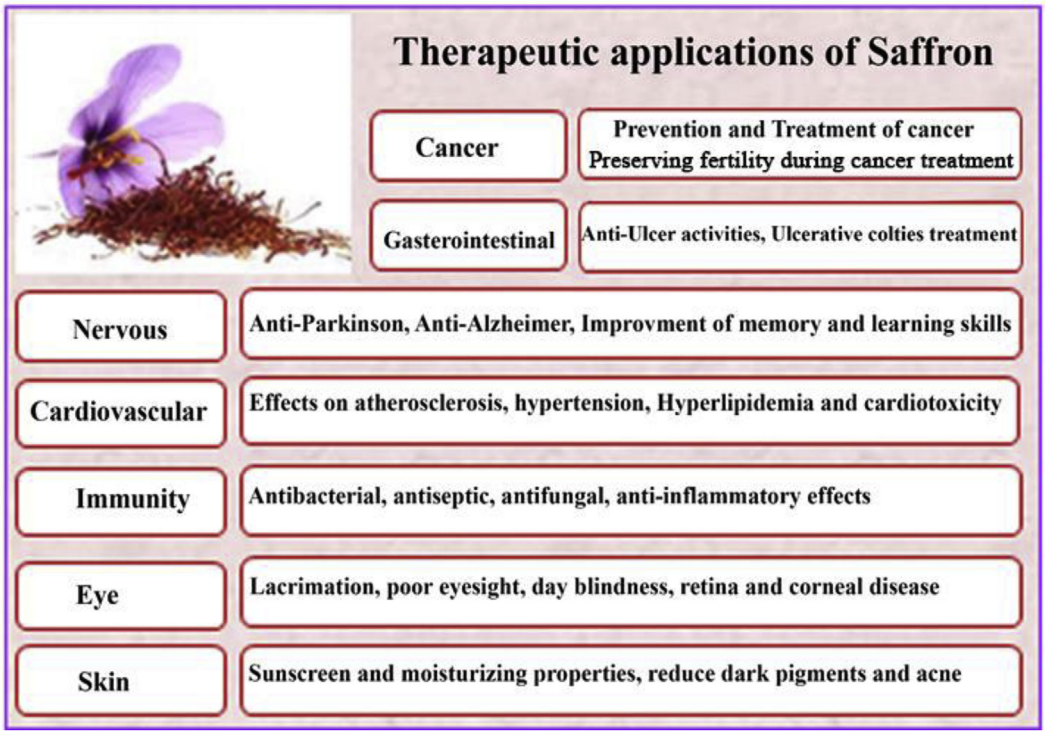
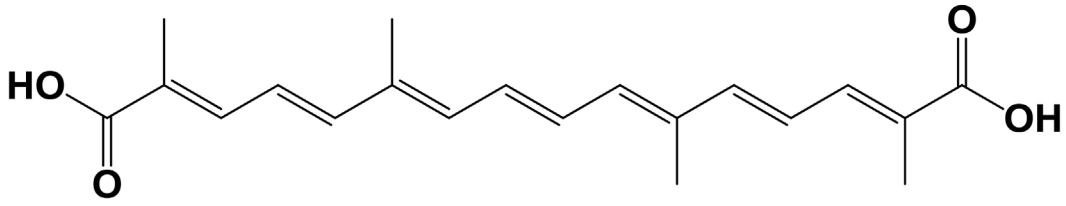


Fig. 1. Some therapeutic applications of saffron.

Crocetin



Crocin

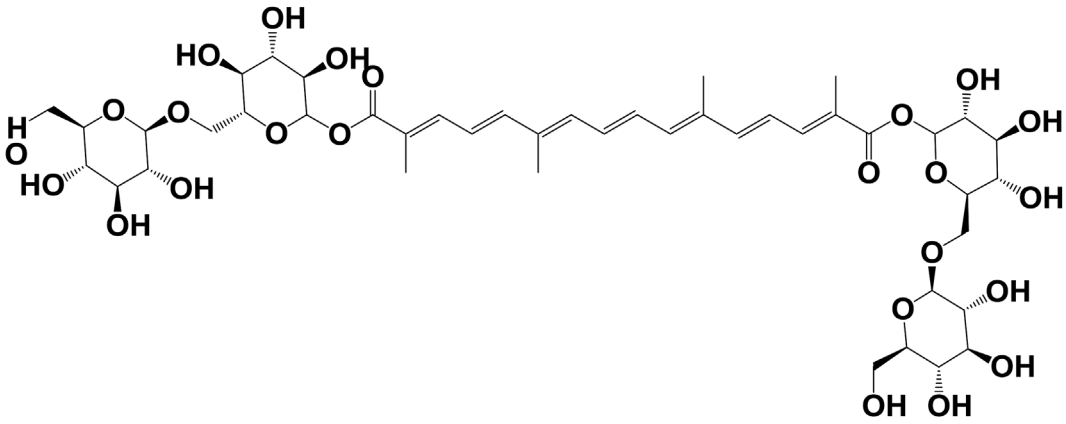


Fig. 2. Molecular structures of crocetin and its diglycosyl ester (crocin).

4. Pharmacokinetic profile

There are few reporters about the pharmacokinetic of crocetin (Lautenschlager et al., 2015; Mizuma et al., 2009; Umigai et al., 2011). It was shown that the oral administration of crocetin limited its bioavailability due to slowly dissolve in gut fluid (17). Nano-formulation of crocetin may be one appropriate approach to improve its solubility, stability, and pharmacokinetic properties (Jia, 2005; Merisko-Liversidge and Liversidge, 2008). Puglia et al. showed that solid lipid nanoparticles (NPs) containing crocin and crocetin had improved stability, antioxidant and cytotoxic activity on human melanoma A375 and malignant Schwann sNF96.2 cells with different mechanisms

Table 1
Methods for extraction and detection of crocetin.

Extraction Methods and Source	References	Detection Methods	References
Enzymatic deglycosylation, Saffron Using resin column, alkaline hydrolysis and crystallization, Gardenia fruits, *G. jasminoides var. radicans Makino*, *C. sativus*	(Lautenschlager et al., 2014) (Asai et al., 2005), (Chu et al., 2018), (Chryssanthi et al., 2007)	UV-visible spectrophotometry HPLC TLC GC	(Sanchez et al., 2008), (Hafezi Ghahestani et al., 2017), (Soltani et al., 2017) (Mohammadpour et al., 2013), (Sujata et al., 1992), (Chryssanthi et al., 2011b) (Sujata et al., 1992)
Acidic hydrolyzation of crocetin, Saffron	(Tashakori-Sabzevar et al., 2013)	UPLC–MS/MS	(Sujata et al., 1992) (Zhang et al., 2017b)
Microbial production, *Saccharomyces cerevisiae* strain	(Chai et al., 2017)		

compared to free forms of these two substances (Puglia et al., 2019). The antioxidant properties of crocetin-loaded lipid NPs were evaluated against cyclosporine A-mediated toxicity in Human Embryonic Kidney (HEK-293) cells. This formulation exhibited more protective effects compared to native crocetin by more enhancement of free radical scavenging, and more inhibition potential of lipid peroxidation (Pradhan et al., 2019). In another study, encapsulation of crocetin into poly (lactic-co-glycolic acid) (PLGA) NPs enhanced its cytotoxicity on breast cancer cells (Hafezi Ghahestani et al., 2017).

In this part of review article, we will discuss about absorption, distribution, metabolism, and excretion of crocetin in *in vitro, in vivo* and clinical studies.

4.1. Absorption

4.1.1. In vitro studies

In research by Lautenschläger et al., *in vitro* studies were carried out to investigate the absorption of crocin-1 and trans-crocetin from intestinal and blood brain barrier. Crocin-1, even at high concentrations (1000 μM) does not penetrate from Caco-2 monolayers cells but trans-crocetin could permeate in a concentration-independent manner (10–114 μM) through the intestinal barrier during 2 h by passive transcellular diffusion mechanism. When verapamil, as an inhibitor of p-glycoprotein, was added to the transport buffer, a significant higher permeation of trans-crocetin was observed which may result in the role of trans-crocetin as a substrate for efflux pumps of the ABC-transporter family. Trans-crocetin (27 μM) passed through blood cerebrospinal fluid barrier (BCSFB) with a slow but constant rate during 29 h period. Verapamil did not have any effect on the permission of crocetin through this barrier (Lautenschlager et al., 2015).

4.1.2. Clinical studies

In the study by Umigai et al., a single dose of crocetin at three doses (7.5, 15 and 22.5 mg) was given orally to healthy adult human volunteers over one-week interval. At different times, blood samples were collected and the plasma concentration of crocetin was determined by HPLC. The results showed crocetin could rapidly absorb and detect during the first hour of administration. An enhancement in the plasma concentration of crocetin was observed up to around 4 h. Then, they decreased gradually to the limit of quantification by 24 h. The mean maximum concentration (Cmax) and the estimates of the pharmacokinetic parameters (AUC0–24) of crocetin were about from 100.9 to 279.7 ng/ml and 556.5–1720.8 ng h/ml, respectively, after 4.0–4.8 h. No serious adverse effects were observed up to 22.5 mg dose of crocetin. On the other hand, it was observed that crocetin could to be absorbed by passive transcellular diffusion more rapidly than the other carotenoids such as β-carotene, lutein and lycopene (Umigai et al., 2011).

4.2. Distribution

4.2.1. In vitro studies

About the binding of crocetin to human and bovine serum albumin, it was shown that the free form of crocetin may be bound to albumin in blood plasma through occupying fatty acid binding site (Miller et al., 1982). However, the weak interaction between crocetin and albumin resulted in rapid distribution of crocetin into tissues of body (Kanakis et al., 2007). On the other hand, in the study by Jafarisani et al., different spectroscopic methods and molecular docking investigated the binding of crocin and crocetin to human serum albumin. It was shown the mechanisms of interaction of these compounds with albumin are different and it may be due to the differences in the structure and hydrophobicity of these ligands (Jafarisani et al., 2018).

4.2.2. In vivo studies

Christodoulou et al. evaluated the pharmacokinetic profile of crocetin (parent, total and metabolite) after single dose (i.v. and oral) administration of saffron aqueous extract (SFE) (60 mg/kg containing all-trans-crocin (27.8 ± 0.1% w/w) to C56/Bl6J mice. Serum and tissue levels of crocetin were measured by HPLC-PDA method and the results was subjected to compartmental and non-compartmental PK analysis. The higher serum levels of crocetin detected after oral administration compared to I.V. administration of the equal dose of SFE may be due to the hydrolysis of crocin in gastrointestinal tract. One-compartment model with first-order absorption was found for both crocetin and crocetin metabolite after oral administration. However, crocetin (unconjugated, parent) and crocetin metabolite (conjugated) showed one-compartment model and a first-order input kinetic parameter (ka), respectively after I.V. administration (Christodoulou et al., 2019).

4.3. Metabolism and excretion

It has been reported that crocetin is metabolized to the ester type glucuronides in the intestinal mucosa or in the liver after oral administration. This form of crocetin showed more stability in plasma and may be consider as bioactive molecules or as a carrier for delivery of crocetin to the target tissues. About the excretion of crocetin, Umigai et al. showed that crocetin was eliminated from human plasma with a half-life ($T_{1/2}$) of 6.1–7.5 h (7.5, 15 and 22.5 mg in one week interval) (Umigai et al., 2011). In another study by Zhang et al., $T_{1/2}$ was calculated from 2.5 to 2.9 h detected by ultra-performance liquid chromatography tandem mass spectroscopy (UPLC–MS/MS) after oral administration of crocetin in rat (29.3, 58.6, 117.2 mg/kg) (Zhang et al., 2017b).

5. Toxicity effects of crocetin

Regarding to the wide therapeutic applications of crocetin, the evaluation of its possible toxic effects is necessary. There is no data about the LD_{50} value of crocetin. However, the LD_{50} values of saffron

Table 2
Some examples of Anti-tumor and cytotoxicity effects of crocetin.

Type of tumor	Cell lines or animal models	Concentration/Dose,	Mechanism of action	Ref.
Breast	MDA-MB 231 cells	1 and 10 μM	Downregulation of MMPs expression	Chryssanthi et al. (2011a)
	MCF-7 cells	50 μM	Modulation of the expression of ATG1 and Beclin-1	Zhang and Li (2017)
	Wistar rats	100 mg/kg (oral),	Decreased tumor size, latency period, and tumor number	Sajjadi and Bathaie (2017)
Lung	A549 cells	IC50 about 0.41 mM	Inhibited proliferation and enhanced apoptosis	Kim et al. (2014)
	A549 and VA13 cells	l-100 μg/ml	Inhibition of nucleic acid and protein synthesis	Abdullaev (1994)
	Mice	50 mg/kg (i.p),	Decrease glycoproteins and polyamines levels, Scavenge free radicals	Magesh et al. (2009)
	Mice	20 mg/kg (i.p),		Magesh et al. (2006)
Cervical and Ovarian	HeLa and SKOV3 cells	IC50 from 100 to 120 μM	Induction of p53 as the regulation the G1 checkpoint	Zhong et al. (2011)
	Mice bearing cervical tumor	40 mg/kg (oral)	Downregulation of the proinflammatory cytokine	Chen et al. (2015)
Gastric	BGC-823 cells	IC50 about 200 μM	Reduction of MMP, caspase 3 activation and cytochrome *c* translocation into the cytosol	He et al. (2014)
	Rat	50, 75, and 100 mg/kg	Changes in serum antioxidant activity and lactate dehydrogenase	Bathaie et al. (2013)
Colon	SW480 cells	0.2, 0.4, 0.8 mM	Induction cell cycle arrest through P21induction	Li et al. (2012)
	HCT116 (p53 + / +), HCT116 (p53 − / −), HT29 (p53mt) cells	100 μM	Cytotoxic effect by p53-dependent and p53-independent manner	Ray et al. (2016)
Liver	Hep G2	IC50 about 0.6 μM	Increasing of Nrf2 and decreasing of LDHA levels	Kim et al. (2014)
Pancreatic	MIAPaCa-2, BxPC3, Capan-1, and ASPC-1	200 μM	Changes in cell cycle proteins, Cdc-2, Cdc-25C, Cyclin-B1, and EGFR	Dhar et al. (2009)
	Nude mice	4 mg/kg (oral)	Induction of apoptosis as well as inhibition of proliferation	Dhar et al. (2009)

stigma extracts containing crocetin have been evaluated in some studies. Conducted research projects have been shown that the LC_{50} values for saffron and its constituents against normal cells can be very higher than therapeutic dose (Milajerdi et al., 2016).

Some side effects including nausea, vomiting, diarrhea and bleeding have been observed by oral administration of saffron with doses between 1.2 and 2 g in humans (Schmidt et al., 2007). Administration of high doses (200 and 400 mg/day) of saffron for 7 days resulted in some no clinically important changes in hematological and biochemical parameters in healthy adult volunteers (Modaghegh et al., 2008).

In study by Martin et al., the teratogenic potential of crocetin was assayed in frog (Xenopus) embryos and compared with all-trans retinoic acid (ATRA) as a teratogen agent. The results showed that high concentrations of crocetin (200 μm) needed to induce teratogenic effects in frog embryo. On the other hand, crocetin has much less teratogenic effects than ATRA in all parameters examined. Therefore, crocetin can be considered as a safe alternative to treat ATRA-sensitive cancers (Martin et al., 2002).

The protective effect of crocetin on genotoxicity induced by benzo (a) pyrene and neoplastic transformation was evaluated in C3H10T1/2 cells (mouse mesenchymal). Pretreatment of cells with crocetin (at non-toxic dose of 0.01–0.10 mM) increased glutathione (GSH)-S-tranferase activity resulting in the reduction of the formation of a B (a)P-DNA adduct (Chang et al., 1996).

Ozaki et al. also studied genotoxicity effects of crocetin inV79 Chinese hamster cells. Crocetin in gardenia yellow was found not to be mutagenic and not to have DNA-damaging or tetraploids-increasing properties (Ozaki et al., 2002). In another study, it has been demonstrated that crocetin at doses of 5–20 μg/ml had selective cytotoxic effects against human rhabdomyosarcoma cells and less toxic effects on normal cells (African green monkey kidney (Vero) cells) compared with cisplatin (Jagadeeswaran et al., 2000).

Wang et al., evaluated the toxicity, pharmacokinetics and inhibitory effect of crocetin on development of proliferative vitreoretinopathy (PVR) using the rabbit eye as a model system. No retinal toxicity was observed 2 weeks after the injection of 0.4 μmol crocetin. The half-life of 4.231 h was obtained for intravitreal injection of crocetin (0.4 μmol) using a non-compartmental model. This result indicated the inhibitory potential of crocetin on the progression of PVR (Wang et al., 2019).

The safety of crocetin was evaluated in healthy adult volunteers by Yamashita et al. In this clinical trial, crocetin was administrated at dose of 37.5 mg/day for four weeks. No clinically significant changes in blood biochemistry and hematology and also no adverse changes in any volunteers were observed by excessive intake of crocetin (Takara, 2018).

6. Biological activities

Crocetin has various pharmacological effects such as anti-tumor, neuroprotective, cardioprotective, hepatoprotective, antidepressant, antiangiogenesis, healing burn, and improving asthma, Alzheimer, diabetes mellitus and colitis diseases. In the following, the studies about biological activities of crocetin will be discussed in details.

6.1. Anti-tumor and cytotoxicity effects against cancer cells

Saffron and its derivatives particularly crocin and crocetin showed significant anti-tumor activity. However, it was indicated that crocetin has approximately 5–18-fold higher cytotoxicity with more induction in cellular reactive oxygen species (ROS) than crocin in different human cancer cell (Kim et al., 2014). There are several hypotheses about the molecular mechanisms of anticancer activity of crocetin such as the inhibition of DNA, RNA, protein synthesis, and RNA polymerase II activity in cancer cells, resulted in prevention of proliferation and increment of apoptosis. Crocetin could also interact with the structure of histone H1 and H1-DNA. The antioxidant effects of crocetin may be

considered as other anti-cancer mechanisms (Festuccia et al., 2018; Gutheil et al., 2012; Milajerdi et al., 2016; Moradzadeh et al., 2018). Table 2 summarizes the effects and mechanisms of action of crocetin against several cancer types.

6.1.1. Breast cancer

6.1.1.1. In vitro studies. It has been demonstrated that saffron extract can be considered as a potential chemotherapeutic agent against both estrogen receptors positive (ER+) and ER-breast cancer cells (Chryssanthi et al., 2007; Mousavi et al., 2009).

In study by Chryssanthi et al., it was shown that crocetin at concentration of 1 and 10 μM could significantly inhibit both proliferation and invasion of the highly invasive MDA-MB-231 cells via down-regulation of matrix metalloproteinases (MMPs) expression (Chryssanthi et al., 2011a). In another study, crocetin was encapsulated into PLGA NPs to improve its solubility and anticancer efficiency. The cytotoxicity of crocetin and PLGA-crocetin NPs were evaluated on MCF-7 cells. The results indicate that loading of crocetin in PLGA leads to significantly reduction in the IC_{50} (84.73 ± 12.14 μM) value compared to crocetin alone (589.65 ± 5.72 μM) (Hafezi Ghahestani et al., 2017).

The encapsulation of crocetin into alkylated-PAMAM G4 and PPI G4 also improved its solubility and antitumor cytotoxicity effects against MCF-7 cell lines. The modified PAMAM and PPI could change the IC_{50} of crocetin from 598.65 to 2.176 and 5.947 μM, respectively (Soltani et al., 2017).

Crocetin was also used with other anticancer agents to improve their efficiency and reduce side effects in breast cancer treatment. The effect of PLGA NPs containing doxorubicin (DOX) and crocetin was investigated on MCF-7 cell lines. Co-formulation could significantly decrease the IC_{50} (0.82 μM) value in comparison with DOX alone (7.81 μM) (Alebooye Langroodi et al., 2016). Zhang et al. showed that crocetin significantly increased the suppressive effects of fluorouracil on MCF-7 cell growth by shift autophagic cell survival to autophagic cell death may be due to modulation of the expression of ATG1 and Beclin-1 (related proteins in autophagy) (Zhang and Li, 2017).

6.1.1.2. In vivo studies. The preventive effects of crocetin and crocin were investigated against N-nitroso-N-methylurea (NMU)-induced breast cancer in female Wistar rats at both the initiation and promotion stages. The oral dose was 100 mg/kg once every three days that was administrated 20 days before, or one week after, the first NMU injection, in order to prevention effect at the initiation or promotion stages, respectively, for 120 days after NMU administration. The results showed that both compounds had significant protective effects; however, crocetin was more efficient than crocin. On the other hand, the prevention at the initiation stage was more effective than at the promotion stage (Sajjadi and Bathaie, 2017).

6.1.2. Lung cancer

6.1.2.1. In vitro studies. To explore the effect of crocetin on lung cancer, A549 (lung adenocarcinoma) cell line was treated with crocetin. The inhibited proliferation and enhanced apoptosis was observed in a time- and concentration-dependent manner by inducing G1 arrest, p53-dependent and independent induction of cyclin-dependent kinase inhibitor p21WAF1/Cip1. On the other hand, crocetin could improve the anticancer efficiency of vincristine (Zhong et al., 2011). It was also observed that the viability of A549 cell lines decreased in the present of crocetin (IC_{50} value about 0.41 mmol/L) (Kim et al., 2014). In another experiment, the effect of crocetin on colony formation and cellular DNA, RNA and protein synthesis was investigated in A549 and VA13 (SV-40 transformed fetal lung fibroblast) cells. Crocetin showed a dose-dependent inhibitory effect (at doses ranging from l-100 μg/ml) on nucleic acid and protein synthesis in these cells (Abdullaev, 1994).

6.1.2.2. In vivo studies. In the study by Magesh et al., the effect of crocetin was investigated in benzo (a) pyrene-induced lung carcinoma mice. The results showed that crocetin (50 mg/kg body weight, i.p. injection 3 days/week) reduced proliferating cells by 45% and 68%, 8 and18 weeks after treatment, respectively. The protective effect of crocetin against lung cancer may be due to its inhibitory effect on the synthesis of polyamine and glycoprotein, which have high levels in lung cancer cells (Magesh et al., 2009).

In another *in vivo* study, administration of crocetin (20 mg/kg body weight, i.p. injection, 4 weeks before (pre-initiation) and from 12th week after Benzo (a) pyrene induction) showed antitumor activities in lung cancer-bearing mice. Antioxidant and some marker enzymes were assayed in lung of control and experimental animals. The results indicated that crocetin could scavenge free radicals by inhibiting lipid peroxidation and increase of the activity of antioxidant enzymes including GST, GSH-Px, catalase, and superoxide dismutase. Crocetin also decreased marker enzymes such as arylhydrocarbon-hydroxylase (AHH), lactate dehydrogenase (LDH), gamma-glutamyl transpeptidase (GGT), adenosine deaminase (ADA) and 5′-nucleotidase related to carcinogen following administration of benzo[*a*]pyrene (B[*a*]P) in lung tissues (Magesh et al., 2006).

6.1.3. Cervical and ovarian cancer

6.1.3.1. In vitro studies. Cervical cancer cell line (HeLa) and ovarian cancer cell line (SKOV3) were treated with crocetin (60–240 μmol/L) alone or in combination with vincristine. The results showed a significant inhibition in the proliferation of treated cells in a concentration-dependent manner. The IC_{50} value of crocetin was from 100 to 120 μmol/L. In these cancer cells, crocetin (60 μmol/L) significantly increased the cytotoxicity induced by vincristine (1 μmol/L). In addition, this synergistic effect was also observed in the vincristine-resistant breast cancer cell line MCF-7/VCR. Crocetin could induce p53 as the regulation the G1 checkpoint by activating transcription of genes that influence cell-cycle progression, including cyclin-dependent kinase inhibitor p21WAF1/Cip1 (Zhong et al., 2011).

Kim et al. showed that crocetin induced cytotoxicity in HeLa cells via the induction of cellular ROS, activation of nuclear factor erythroid 2-related factor 2 (Nrf2) and reduction of the protein expression of lactate dehydrogenase A (LDHA). In this study, the reduction in SKOV3 cell viability was observed in the present of crocetin (IC_{50} value about 0.19 mmol/L) (Kim et al., 2014).

6.1.3.2. In vivo studies. In a study by Chen, the effect of orally administration of crocetin on methylcholanthrene- (MCA) induced uterine cervical cancer in mice was investigated. Inflammatory mediators including maleic dialdehyde (MDA), polymorphonuclear cells (PMN), interleukin-1β (IL-1β), and tumor necrosis factor-α (TNF-α) were assayed. Further, the expression of cyclooxygenase-2 (COX-2) in HeLa cells was also evaluated. The results showed crocetin (40 mg/kg, twice daily for 35 days) has significant anti-inflammatory activities by suppressing the level of these inflammatory factors. Therefore, crocetin may act as a chemopreventive and an anti-inflammatory agent against cervical cancer (Chen et al., 2015).

6.1.4. Gastric adenocarcinoma and colon cancer

6.1.4.1. In vitro studies. In study by HE et al., BGC823 human gastric cancer (GC) cells were treated by crocetin. The IC_{50} value was obtained about 200 μM. The mechanism of crocetin against GC was attributed to reduction of the mitochondrial membrane potential (MMP), caspase-3 activation and cytochrome *c* translocation into the cytosol from the mitochondria (He et al., 2014).

Bathaie et al. studied the effect of crocetin (100–300 μM) on gastric adenocarcinoma (AGS) cells. The results showed the cell proliferation inhibition and apoptosis induction in crocetin–treated AGS cells by suppression of Bcl-2 and up-regulation of Bax expression (Bathaie et al., 2013).

About the effect of crocetin on colon cancer cells, SW480 cells were

Table 3
Some examples of neuroprotective effects of crocetin.

Brain injury or type of disease	Cell line or animal type	Concentration/Dose	Mechanism	Ref.
Chronic microglial activation	Microglial cells	5–20 μM	Inhibition of pro-inflammatory mediators and NF-κB activation, reduced NO release	Nam et al. (2010)
Neurotoxicity	HT22 hippocampal cells	5 μM	Decreased CuONPs-induced apoptosis by modulation of Bax and Bcl-2 mRNA, Reduction of activities of antioxidant/detoxification enzymes	Niska et al. (2015)
Alzheimer's Disease	Mouse hippocampal-derived Ht22 cells	1 and 5 μM	Reduction in reactive oxygen species formation, increased mitochondrial membrane potential and activation of extracellular signal-regulated kinase 1/2 phosphorylation.	Kong et al. (2014)
High-oxidative stress in the brain	Stroke-prone spontaneously hypertensive rat (SHRSPs)	100 mg/kg oral	Inhibition of hydroxyl radical generation	Yoshino et al. (2011)
Cerebral trauma	Rat	50 mg/kg by gastric perfusion	Inhibition of apoptosis and promotion of angiogenesis	Bie et al. (2011)
Neuropathic pain	Mouse	(5–50 mg/kg) infused into the subarachnoid space	Reduction of IL-1β and superoxide dismutase (SOD)	Wang et al. (2017c)
Spinal cord injury	Rat	40 mg/kg, intraperitoneal injection	Improvement of serotoninergic (5-HT) fiber growth	Wang et al. (2017b)
Parkinsonism	Rat	25 μg/kg, i.p.	Enhancement of the level, or activity of the antioxidant enzymes	Ahmad et al. (2005)
Cerebral ischemia with reducing memory	Rat	8 mg/kg per day, i.p.	Memory enhancing by the protection effect of cerebrocortical and hippocampus neurons against ischemia	Tashakori-Sabzevar et al. (2013)
Depression	Mice	(12.5, 25 and 50 mg/kg), Oral	Maybe due to antioxidant and anti-inflammatory properties	Amin et al. (2015)

treated with crocetin (0.2, 0.4, 0.8 mmol/L) for 48 h. A significant inhibition in the proliferation was observed in a concentration and time-dependent manner by enhancing apoptosis, decreasing DNA repair capacity and induction cell cycle arrest through p53-independent mechanisms accompanied by P21induction (Li et al., 2012). About the mechanism of crocetin on the proliferation inhibition of colon cancer cells, Zhuang et al. also indicated that crocetin treatment caused a significant reduction in the expression of genes involved in inflammation including HMGB1, IL-6 and IL-8 in the colon cancer cells (Zhuang et al., 2018).

It was also shown that in wild type, mutated p53-expressing or p53-null human colon cancer cells (HCT116 (p53+/+), HCT116 (p53−/−), HT29 (p53mt), crocetin (100 μM) induced p53-mediated cell death in p53-expressing cancer cells through BAX and PIDD-caspase-2-t-BID pathway. However, crocetin induced p53-impaired cancer cell death by p73-mediated FAS-FADD-caspase-8 activation and BID cleavage (Ray et al., 2016).

Kim et al. demonstrated that crocetin increased cell death in HCT-116 colorectal cancer cells as well as in SKOV3 and HeLa cells (Kim et al., 2014).

6.1.4.2. In vivo studies. In *in vivo* study by Bathaie et al., crocetin (50, 75, and 100 mg/kg, per day by i.p. injection for 50 days) showed antitumor efficiency in dose-depended manner by changes in the activity of antioxidant agents (Bcl-2/Bax ratio) and lactate dehydrogenase in 1-methyl-3-nitro-1-nitrosoguanidine (MNNG)-induced gastric cancer in rats serum (Bathaie et al., 2013).

6.1.5. Liver and pancreatic cancer

6.1.5.1. In vitro studies. In a study by Kim, crocetin could enhance the cell death in HepG2 liver cancer cells (IC_{50} about 0.6 μM) by increasing of Nrf2 and decreasing of LDHA levels (22). In another study, the effect of crocetin on the human pancreatic adenocarcinoma cell lines, MIAPaCa-2, BxPC3, Capan-1, and ASPC-1, was investigated. In *in vitro* study, crocetin at concentration of 200 μmol/L significantly inhibited the proliferation all studied cell lines with more inhibition effect on ASPC-1 cells. They also showed that crocetin induced apoptosis via changes in cell cycle proteins, Cdc-2, Cdc-25C, Cyclin-B1, and epidermal growth factor receptor (EGFR) (Dhar et al., 2009).

6.1.5.2. In vivo studies. In *in vivo* study, MIA-PaCa-2 cells were introduced in the athymic nude-mice. This mouse was treated with orally administration of crocetin (4 mg/kg/day, for 30 days). A significant reduction in the growth of tumor was observed due to the induction of apoptosis as well as inhibition of proliferation (Dhar et al., 2009).

6.1.6. Other cancers

In other studies, the therapeutic effects of crocetin on leukemia (HL-60 cells), prostate (PC3 and 22rv1cells), esophageal (KYSE-150 cells) cancers have been demonstrated mainly through the inhibition of cell proliferation, migration and induction of apoptosis (Chen et al., 2015; Festuccia et al., 2014; Tarantilis et al., 1994). In esophageal cancer, it was shown that crocetin in combination with cisplatin (200 μmol/L of crocetin and 2 μg/mL of cisplatin) had synergistic effects on KYSE-150 cells by up-regulating the p53/p21 pathway (Li et al., 2017). It was also indicated that crocetin significantly inhibited the proliferation and induced apoptosis of KYSE-150 cells by different mechanisms including the activation of PI3K/AKT, p38, and upregulated the p53/p21 level resulted in disruption of MMP, increased amount of Bax and cleaved caspase-3, and decreased levels of Bcl-2 (Li et al., 2019).

In vitro and *in vivo* studies about the effect of crocetin on skin cancer were shown that crocetin had cytotoxic effects on mouse fibroblast NIH/3T3 cells and also could delay skin tumor formation and reduce tumor size in mice (Gainer et al., 1976; Hsu et al., 1999; Mathews-Roth, 1982; Wang et al., 1995).

6.2. Nervous system

Neurodegeneration is the major reason of many nervous system diseases, such as Alzheimer, Parkinson's disease, neuropathic pain, spinal cord injury, epilepsy and head trauma.

In different studies, the neuromodulatory effect of crocetin in various experimental models of brain disorders has been evaluated. This beneficial effect has been mainly attributed to the strong antioxidant properties of crocetin. On the other hand, C20-dicarboxylic acid trans-crocetin is the only active metabolite of crocin which could cross the blood-brain barrier (BBB) after saffron administration (Lautenschlager et al., 2015). Table 3 summarizes the effects and mechanisms of action of crocetin in several nervous disorders.

6.2.1. In vitro studies

It was shown that microglia cells could help in restoring CNS homeostasis under pathological conditions, but the chronic microglial activation causes damage to nerve cells through the release of various pro-inflammatory and neurotoxic factors (Graeber and Streit, 2010; Sugama et al., 2009). In a study by Nam et al., the effect of crocin or crocetin on suppression of microglial activation was evaluated. Primary microglial cells, prepared from the cerebral cortices of one-day-old rat pups, were pretreated with crocin or crocetin (5–20 μM) before the addition of lipopolysaccharide (LPS) (10 μg/ml). The results showed that both crocin and crocetin were effective in the inhibition of pro-inflammatory mediators including LPS-induced nitric oxide (NO), interleukin-1β (IL-1β), TNF-α, and ROS from microglial cells.

These compounds also effectively reduced LPS-elicited NF-κB activation at concentration of 20 and 40 μM. In addition, crocin reduced NO release from stimulated microglia cells with interferon-γ and amyloid-β. In organotypic hippocampal slice cultures, the effect of LPS on hippocampal cell death was blocked in the present of crocin or crocetin (Nam et al., 2010).

In another study, the induced-neurotoxicity mechanisms of CuONPs and neuroprotective effects of crocetin were evaluated. HT22 hippocampal cells was treated with CuONPs or crocetin (5 μM), 1 h prior to the exposure of cells to CuONPs (10, 25 μg/ml). The increased apoptosis in Cuo-treated cells was associated with up-regulation of pro-apoptotic Bax and down-regulation of anti-apoptotic Bcl-2 genes. Interestingly, crocetin at 5 μM could decrease CuONPs-induced apoptosis in HT22 cells by modulation of Bax and Bcl-2 mRNA.

It was also reported that other protective mechanisms of crocetin in CuONPs-induced damage in hippocampal cells were the reduction of CuONPs-induced effects on the expression and activity of antioxidant/detoxification enzymes such as glutathione peroxidase (GPx), superoxide dismutase (SOD), GST and GSH levels, and ROS production. It was demonstrated that CuONPs decreased the activities of these enzymes in HT22 cell line (Niska et al., 2015).

Since oxidative stress plays an essential role in the progression of Alzheimer's Disease (AD), then the neuroprotective effects of crocetin as an anti-oxidative agent was evaluated in Aβ1-42 induced toxicity in mouse hippocampal-derived Ht22 cells. The results indicated that pre-incubated of cells with crocetin (1 and 5 μM) followed by treatment with Aβ1-42 (0.5 μM) resulted in a significant increase in cell viability, reduction in reactive oxygen species formation, and increased mitochondrial membrane potential. Pre-treatment with crocetin (5 μM) also activated extracellular signal-regulated kinase 1/2 phosphorylation. Therefore, crocetin can be considered as a potential therapeutic candidate for the treatment of AD (Kong et al., 2014). The effects of crocetin on Aβ aggregation as a hallmark molecular process found in AD, was also studied. It was demonstrated that crocetin inhibited fibril formation of Aβ, destabilized pre-formed Aβ fibrils, caused stabilization of Aβ oligomers and prevented the conversion of Aβ oligomers into Aβ fibrils (Ahn et al., 2011). In another study, the effect of trans-crocetin on Aβ42 degradation was investigated in monocytes from AD patients. Crocetin at concentration of 5 μM could increase Aβ42 degradation in AD monocytes by the upregulation of the lysosomal protease cathepsin B as an Aβ42 degrading enzyme (Tiribuzi et al., 2017).

It has been suggested that the effect of saffron on CNS might at least partly be attributed to crocetin. To investigate the mechanism of hydro-ethanolic saffron extract (CSE) and trans-crocetin on synaptic transmission, postsynaptic potentials (PSPs) were induced by focal electrical stimulation in pyramidal cells from rat cingulate cortex. It was indicated that an excess neuron excitation by activating glutamate receptors leads to neuronal dysfunction or even cell death. It was also demonstrated that CSE at 100 μg/ml could inhibit glutamatergic synaptic transmission by interacting with glutamate receptors (NMDA and kainate). Trans-crocetin at 10 μM decreased NMDA (20 μM)-induced membrane depolarization, but did not inhibit the isolated non-NMDA component of PSPs. Therefore, trans-crocetin is involved in the antagonistic effect of CSE on NMDA but not on kainate receptors (Berger et al., 2011).

6.2.2. In vivo studies

The antioxidant effects of crocetin on reactive oxygen species such as hydroxyl radical were evaluated *in vitro* and *ex-vivo* using UV irradiation and by electron spin resonance (ESR) spin trapping. The stroke-prone spontaneously hypertensive rat (SHRSPs) was used as an experimental model with high-oxidative stress in the brain. The results indicated that crocetin with concentrations in the range of 125–1000 μM for *in vitro* study and 100 mg/kg orally 90 min prior to *ex-vivo* study significantly inhibited hydroxyl radical generation compared with the control. After oral administration, crocetin was also detected using HPLC at high levels in the plasma and the brain of stroke-prone spontaneously hypertensive rats (Yoshino et al., 2011).

In another study, the mechanism of protective effects of crocetin in brain injury was contributed to inhibition of apoptosis at early stages of the injury and its ability to promote angiogenesis at the sub-acute stage. In this study, in addition to TUNEL staining and electron microscopy, the expression levels of Bcl-2 protein, vascular endothelial growth factor receptor-2 (VEGFR-2) and serum response factor (SRF) were used in rat with cerebral trauma for detection of apoptosis and angiogenesis. The expression levels of these factors were higher in the crocetin therapy group (50 mg/kg administrated by gastric perfusion, once every day for 15 days) in comparison to the other groups (Bie et al., 2011).

The effect of crocetin in a mouse model with neuropathic pain induced by spared nerve injury (SNI) was evaluated by Wang et al. Crocetin (5–50 mg/kg) was infused into the subarachnoid space for up to 12 days. It was found that a high dose of crocetin reduced pro-inflammatory cytokines TNF-α and IL-1β and also restored the enzymatic activity of superoxide dismutase (SOD) which was reduced in the sciatic nerve and the spinal cord of SNI mice. Therefore, crocetin can effectively apply in the treatment against neuropathic pain (Wang et al., 2017c).

In another study, the spinal cord injury (SCI) rat's model were treated with crocetin (40 mg/kg, intraperitoneal injection). An improvement in locomotion and sensorimotor functions was observed in treatment group with the Basso, Beattie, and Bresnahan (BBB) and contact plantar placement (CPP) assay. On the other hand, crocetin decreased the level of TNF-α and IL-1β at day 3, but returned to the initial levels at day 5. There were no changes in the trend of either IL-6 or IL-8. These results suggested that crocetin might not change the inflammatory responses to the injury process in the SCI rat model. However, crocetin improved serotoninergic (5-HT) fiber growth. In primary cultures of hippocampal neurons, crocetin at concentration of 10 μmol/L could increase neurite growth with preference for the longest process likely to be axons (Wang et al., 2017b).

The neuromodulatory effects of crocetin were also evaluated by pre-treated of rats with crocetin (25, 50 and 75 μg/kg body weight, intraperitoneally (i.p.)) for 7 days before 6-hydroxydopamine (6-OHDA)-induced Parkinsonism in rat model. The results showed that in the

crocetin-treated groups, an enhanced level, or activity of the antioxidant enzymes including GPx, GST, glutathione reductase (GR), catalase, and superoxide dismutase (SOD), depletion of the thiobarbituric acid reactive substance (TBARS) level as an index of lipid peroxidation, and a marked elevation in the content of dopamine and its metabolites was observed. It can be concluded that crocetin can afford neuroprotection by inhibiting the cascade of events that leads to neurodegeneration (Ahmad et al., 2005).

The synergistic neuroprotective effects of low dose of the extract of *Nardosatchys jatamansi* (N), crocetin (C) and selenium (Se) (NCSe), as antioxidant agents, (N, 200 mg/kg + C, 25 μg/kg + Se, 0.05 mg/kg body weight) investigated in streptozotocin (STZ)-induced rats resulted in cognitive impairment due to decreased cholinergic activities induced by oxidative stress. Pretreatment of the animals with NCSe improved the sustained oxidative stress by up-regulation of the activities of antioxidant enzymes (GPx, GR, GST, SOD, and CAT) and reduced the level of TBARS. This combination therapy reversed the impaired learning and memory in rats by two separate behavioral paradigms, in passive avoidance test and Morris water maze (MWM) navigation tasks (Khan et al., 2012).

Tashakori et al. evaluated the memory enhancing effect of crocetin in a cerebral ischemia model in rat. Crocetin (2, 4 and 8 mg/kg per day) was administered via i.p. rout. The spatial learning and memory function were assayed using MWM test. The histopathological changes in cerebral cortex and hippocampus were also investigated. The results showed that crocetin at dose 8 mg/kg could reduce escape latency time and increase the percentage of time spent and traveled distance in target quadrant. No difference was observed between groups in swimming speed. In addition, crocetin (8 mg/kg) could effectively protect cerebrocortical and hippocampus neurons against ischemia (Tashakori-Sabzevar et al., 2013).

Amin et al. compared the antidepressant effects of crocin and crocetin extracted from saffron following acute and sub-acute administration. In behavioral tests, it was shown that acute treatment with crocin (40 mg/kg) and crocetin (20 and 40 mg/kg) produced an antidepressant-like effect in forced swim (FST) without affecting the baseline locomotion in mice. Sub-acute oral administration of crocin (only at the highest dose, 100 mg/kg) and crocetin (12.5, 25 and 50 mg/kg) significantly attenuated immobility time in FST and TST tests. As crocetin was effective at lower dose compare to crocin, it may be concluded that crocetin has more antidepressant effect than crocin (Amin et al., 2015).

6.3. Cardiovascular system

It has been demonstrated that crocetin has a potential intensity in prevention and treatment of cardiovascular disorders such as hypertension, thrombosis formation, and myocardial infraction (MI). Table 4 summarizes the effects and mechanisms of action of crocetin in some cardiovascular disorders.

6.3.1. In vitro studies

Shen et al. investigated the protective effect of crocetin against cytotoxicity produced by exposure of primary cultured rat cardiac myocytes to norepinephrine (NE). The cells were pretreated with crocetin (1.0 mM) for 30 min followed by exposure to NE (1.0 mM).

The results showed that crocetin significantly reduced intracellular accumulation of ROS, Ca^{2+}, and production of lipid peroxidation resulting from NE. Crocetin also enhanced SOD activity and GSH level in the cells exposed to NE. On the other hand, crocetin significantly attenuated NE-induced apoptotic cells. In conclusion, crocetin can be considered as a potential drug to control and prevent NE-induced cardiovascular disorders (Shen et al., 2009).

In another study, the different amide derivatives of crocetin were synthetized to neutralize the carboxyl group and increase liposolubility as well as permeability of crocetin. The cardioprotective activities of

Table 4
Some examples from *in vitro* and *in vivo* effects of crocetin on cardiovascular system disorders

Type of cardiovascular disease	Cell line or animal type	Concentration/Dose	Mechanism	Ref.
Cytotoxicity by exposure to NE	Primary cultured rat cardiac myocytes	0.01,0.1 and 1 μM	Enhancement of SOD activity and GSH level, decrease lipid peroxidation and Ca(2+) in cells, and apoptosis death ratio	Shen et al. (2009)
Hypertension	Aortic rings from hypertensive (SHR) rats induced by ACH	1.2×10^{-5} M	Improvement of endothelium-dependent ACH relaxations via endothelial nitric oxide	Mancini et al. (2014)
Vascular inflammation	Human umbilical vein endothelial cell (HUVEC)	1,5 and 10 ng/ml	Inhibition of MCP-1 and IL- 8 expression and secretion through suppressing NF-κB p65 signaling transduction	Song et al. (2016)
Hypertension and Cerebral Thrombogenesis	Stroke-prone spontaneously hypertensive rats (SHRSPs)	25 and 50 mg/kg/day	Antihypertensive and antithrombotic effects by antioxidant activity	Higashino et al. (2014)
Cardiac hypertrophy	Rat	50 and 100 mg/Kg	Reduction in the cardiac indexes and the content of hydroxyproline in heart, increase in the activity of Na+, K + -ATPase, Ca2+, Mg2+ -ATPase and inhibited MMPs activity	Shen and Qian (2004)
Myocardial infraction (MI)	Rat	50, 100 and 200 mg/kg/day, p.o.	Reduction of oxidative stress and inflammatory cytokines and thereby decreasing the apoptosis	Zhang et al. (2017a)

these derivatives were evaluated in the model of hypoxia-induced injury in H9c2 cardiomyocyte cell line. The modification of crocetin by ethylamine and diethylamine resulted in more potent cardioprotective activity than crocetin at a concentration of 0.2 μM. On the other hand, these synthetized compounds were more efficient compared to crocetin in the reduction of LDH release, preserving mitochondrial viabilities and reducing oxidative stress-induced depolarization of MMP (Gao et al., 2017).

In some studies, the potential effect of crocetin on hypertension has been evaluated. Hypertension is associated with endothelial dysfunction, which is accompanied by a decrease in vasorelaxation. In a study by Mancini et al., aortic rings obtained from normotensive (Wistar) and spontaneously hypertensive rats (SHR) induced by acetylcholine (ACH), were incubated with or without crocetin (1.2×10^{-5} M) or saffron extract and L-NAME (NG-nitro-L-arginine methyl ester, 10^{-5} M) or indomethacin (10^{-5} M) as NO synthase (NOS) inhibitor. The results indicated that crocetin but not saffron extract increased the ACH relaxations in aorta of hypertensive (strongly) and normotensive rats (weakly). The addition of crocetin to L -NAME suppressed the relaxant response in SHR but not in Wistar aorta. Crocetin with indomethacin did not change the indomethacin response in either SHR or Wistar aorta. Therefore, crocetin can induce vasorelaxation through endothelial nitric oxide and the cyclooxygenase pathways which is not involved in this effect (Mancini et al., 2014).

In another report, the effects of crocetin and crocin on endothelium-dependent and -independent regulation of smooth muscle contractility in genetic hypertension were investigated. Crocetin (1.2×10^{-5} M) showed more prorelaxing actions through endothelial cells than saffron extract while crocins have procontractile actions by smooth muscle cell mechanisms (Llorens et al., 2015). The therapeutic effect of crocetin in vascular inflammation was examined using LPS-induced inflammatory response in human umbilical vein endothelial cell (HUVEC). It was shown that crocetin caused lower cytotoxicity and LDH leakage under inflammatory stress. On the other hand, crocetin (1, 5 and 10 ng/ml) in dose depended manner suppressed pro-inflammatory chemokine monocyte chemoattractant protein-1 (MCP-1) and interleukin-8 (IL-8) expressions through suppressing NF-κB p65 signaling transduction resulted in the inhibition immune cells adhesion and infiltration to inflamed endothelium, which is an important step in inflammatory vascular injury (Song et al., 2016).

About the mechanism by which crocetin inhibits angiotensin II (Ang II)-induced vascular smooth muscle cells (VSMCs) proliferation, these cells were pretreated with or without crocetin (0.01, 0.1, 1 μM) for 24 h followed by stimulation with Ang II (100 nM). It was shown that crocetin inhibited cell cycle G1/S transition in VSMCs by suppression of $p27^{kip1}$ which overexpressed in VSMCs (resulted in G_1arrest and inhibition of cell growth) and the inhibition of cyclin D1 (one of the main D-type cyclins in VSMCs which synthesizes in the cell cycle progression). Therefore, crocetin with this antiproliferative mechanisms can be used in vascular proliferative diseases (Zhou et al., 2010a).

In similar research, the growth-inhibitory action of crocetin in VSMCs attributed to the inhibition of protein kinase C (PKC) activity which increased in the membrane fraction of VSMCs following stimulation with Ang II (Zhou et al., 2010b).

In another report, it was suggested that the effects of crocetin on angiotensin II-induced ERK1/2 activation in VSMCs may be mediated via Ca^{2+}-dependent pathway (Zhou et al., 2007).

The effects of crocetin (1 and 3 μM) on vascular endothelial growth factor (VEGF)-induced angiogenesis was assayed by tube formation, proliferation, and migration of human umbilical vein endothelial cells (HUVECs) and/or human retinal microvascular endothelial cells (HRMECs) as model in *in vitro* systems. It was demonstrated that crocetin inhibited angiogenesis by suppression of cell migration, through prohibition of the p38 pathways and protection of VE-cadherin expression (Umigai et al., 2012).

6.3.2. In vivo studies

The antihypertensive and antithrombotic effects of crocetin were evaluated in stroke-prone spontaneously hypertensive rats (SHRSPs). In this study, the rats received crocetin (25 and 50 mg/kg/day) for 3 weeks. The results indicated that systolic blood pressures could be reduced by crocetin. On the other hand, thrombus generation, assessed by the He–Ne laser technique, was significantly delayed at both concentrations of crocetin compared to control group. Crocetin also significantly decreased urinary 8-Hydroxy-2′-deoxyguanosine (8-OHdG) and increased NO metabolites (NO2/NO3) levels. In rat with Acetylcholine-induced vasodilation, endothelial function was significantly improved by crocetin. According to these results, crocetin mediate a protective role against cardiovascular diseases through its antioxidant activity (Higashino et al., 2014).

In another study, the mechanism of crocetin on thrombosis formation and platelet activity was systematically investigated. In this research, rats were treated with 25 or 50 mg/kg crocetin, and 30 mg/kg aspirin for two days, twice a day. Then, blood was collected from the abdominal aorta and different parameters such as platelet aggregation, platelet adhesion to collagen, intracellular Ca^{2+} mobilization in platelet and electrical stimulation-induced carotid arterial thrombosis were assayed. The results showed that crocetin reduced ADP and collagen-induced platelet aggregation via the inhibition of Ca^{2+} release from an internal store and extracellular Ca^{2+} influx (Yang et al., 2008).

In research about the influence of crocetin on cardiac hypertrophy induced by overloading pressure in rats, it was indicated that crocetin (50 and 100 mg/Kg, for 30 d by i.g.) reduced the cardiac indexes and the content of hydroxyproline in heart, increased the activity of Na^+, K^+-ATPase, Ca^{2+}, Mg^{2+} -ATPase and inhibited MMPs activity (Shen and Qian, 2004). In another report, it was shown that crocetin (1–10 μM) blocked cardiac hypertrophy induced by angiogensin II (Ang II; 1 μM), *in vitro*. In addition, crocetin (50 mg/kg, orally three times a day for 1 week.) prevented and reversed cardiac hypertrophy induced by blocking all of the proposed ROS-dependent signaling pathways. Crocetin also showed protective effects against cardiac hypertrophy, inflammation and fibrosis as well as the progression of heart failure (Cai et al., 2009).

The cardioprotective effect of crocetin was also evaluated in rats with myocardial infraction (MI) produced by administering isoproterenol (90 mg/kg/day, i.p.). In treated groups, animals received crocetin (50, 100 and 200 mg/kg/day, p.o.) for 15 days. About the effect of crocetin on oxidative stress parameters, it was observed that crocetin could increase GSH, CAT, CK-MB, and LDH and decrease the level of MDA and activity of SOD in the tissue homogenate of treated group more than MI group. It was also found that treatment with crocetin reduced the level of inflammatory cytokines such as IL1β, IL6, and TNFα in the heart tissue of MI mice. On the other hand, the process of apoptosis was attenuated in crocetin treated group by reduction of the level of caspase-3, Bax and Nrf-2 and enhancement of Bcl-2 as anti-apoptotic protein in the myocardial tissues of MI rats. Crocetin administration significantly reduced the altered cellular architecture of heart tissue. In conclusion, it may be suggested that the crocetin can be considered as a possible protective agent in myocardial infarction by reducing oxidative stress and inflammatory cytokines and thereby decreasing the apoptosis in myocardial cells (Zhang et al., 2017a).

A similar study by Wang et al. supported previous findings and suggested that crocetin (50 mg/kg/day, i.g.) can provide protection against myocardial ischemia reperfusion injury (MIRI) in rats by inhibiting ROS production, blocking inflammation, and decreasing myocardium apoptosis (Wang et al., 2014).

The antithrombotic effect of crocetin on an endotoxin-induced disseminated intravascular coagulation (DIC) model in rabbits was evaluated. The results indicated that administration of crocetin (3 mg/kg), 30 min before the beginning of endotoxin infusion, improved DIC related haemostatic indexes such as platelet blood counts, blood plasma fibrinogen and protein C concentration. In addition, crocetin could

improve DIC associated disease and fibrin deposition in the glomeruli (Tsantarliotou et al., 2013).

In another study, the influence of crocetin on blood pressure restoration and inflammatory cascades was evaluated in heart after hemorrhagic shock using anesthetized rats. The results indicated that treatment with crocetin (50 mg/kg) through the duodenal catheter improved the mean arterial pressure (MAP) and attenuated the heart injury, followed by hemorrhage shock and resuscitation. The mechanisms of these beneficial effects of crocetin on cardiac injury include the restoring antioxidant/oxidant via total superoxide dismutase activity and inhibition the superoxide anion and/or free radicals, preventing inflammatory markers such as NF-κB, TNF-α and IL-6 and inhibition of iNOS activity and induction of NO (Yan et al., 2010).

6.4. Hepatoprotective properties

In different studies, the effect of crocetin as hepatoprotective agent has been demonstrated. The main mechanisms of the protective effects of crocetin in liver damage are mediated by antioxidant and anti-apoptotic effects. Table 5 summarizes some studies about the hepatoprotective of crocetin.

6.4.1. In vivo studies

In a study by Chen et al., CCl4-induced liver damage in mice pretreated with geniposide (400 mg/kg b.w.), crocins (400 mg/kg b.w.), crocin-1 (400 mg/kg b.w.) and crocetin (140 mg/kg b.w.) extracted from *G. jasminoides* Ellis. 18 h after the oral administration on the sixth day, all mice except those in the normal control group were given simultaneously a CCl4-peanut oil mixture (1:1, v/v intraperitoneally, 2 ml/kg b.w.). In all treated groups, especially crocin and crocetin, a significant decrease of serum alanine transaminase (ALT), aspartate transaminase (AST) and alkaline phosphatase (ALP) levels was observed. In mice liver, the activities of antioxidant enzymes (CAT and SOD), as well as the levels of cytosol of hepatic GSH with an important role in maintaining the body's antioxidant defense mechanism by conjugation with free radicals, were evaluated. Treatment with geniposide, crocins, crocin-1 and crocetin induced the restoration of antioxidant enzymes activity and GSH level in CCl4-induced liver injury mice. In histopathological examination, the results showed that these components reduced the deformability, irregular arrangement, and rupture of hepatocyte in CCl4-treated mice (Chen et al., 2016).

Wang et al. demonstrated the protective effects of crocetin on aflatoxin B1 (AFB1) hepatotoxicity in rats. Crocetin (2 or 6 mg/kg, daily for three consecutive days) reduced the activities of serum AST, ALT, ALP and GGT (Wang et al., 1991a). This group also showed that the suppression of crocetin on AFB1 hepatotoxicity might be due to the defense mechanisms of hepatic tissues such as the elevation of GSH content and the activities GST and SH-Px as well as reduction of hepatic AFB1-DNA adducts formation (Wang et al., 1991b).

In another research, the hepatoprotective mechanism of crocetin was evaluated with xanthine/xanthine oxidase (XXO) or paraquat (PQ) system inducing oxidative damage in rat primary hepatocytes. It was shown that crocetin (10, 20 μM) could decrease the formation of malondialdehyde (MDA) as an index of lipid peroxidation induced by ROS. The effect of crocetin on DNA repair synthesis was determined by the method unscheduled DNA synthesis (UDS). The results revealed that crocetin inhibited genotoxicity in rat hepatocytes induced by ROS. The data also showed that crocetin inhibited the superoxide anions and/or free radicals (Tseng et al., 1995). It was reported that hemorrhagic shock-induced followed by resuscitation can create an extensive hepatic apoptosis in animals. In study by Yang et al., the effect of crocetin administration on hepatic cellular apoptosis was evaluated in rats subjected to hemorrhagic shock. The results indicated that administration of crocetin (2 mg/kg) during resuscitation resulted in less extensive of hepatic apoptosis by reductions in levels of postshock cytosolic cytochrome *c* and activated caspase 3, associated with an increases in the levels of protective Bcl-2 protein and increased survival relative compared to controls (Yang et al., 2011). A similar study by Dhar et al. supported previous findings and also suggested that crocetin increased tissue ATP post-hemorrhage resulted in reducing apoptosis (Dhar et al., 2005).

6.5. Effect of crocetin on hyperlipidemia

The loss of balance between synthesis and degradation of lipids resulted in hyperlipidemia leading to some serious diseases, such as arteriosclerosis, hypertension, obesity, diabetes, functional depression of some organs and etc. (Goldstein et al., 1973). The antihyperlipidemic effect of crocetin has been demonstrated in some studies. In Table 6, some studies about the effect of crocetin on hyperlipidemia are presented.

6.5.1. In vivo studies

In a research by Lee et al., the effect of crocin isolated from *Gardeniae Fructus* (GF) and its metabolite was assayed. In hyperlipidemic mouse model, crocin and crocetin at doses of 50 mg/kg/day were orally administered for 5 weeks and then serum total cholesterol, triglyceride and LDL levels were determined. Crocin and crocetin decreased serum lipids levels in mice. The results suggested that the hypolipidemic activity of crocin and crocetin may be due to the inhibition of pancreatic lipase (Lee et al., 2005). The inhibitory effect of crocetin isolated from *G. jasminoids* Ellis was also investigated in atherosclerosis model established by feeding hyperlipidemic diet to quail. Crocetin (25, 50, 100 mg/kg/day) was administered by oral gavage for 9 weeks. The results showed that crocetin could reduce the levels of serum lipids and suppress the formation of aortic plaque. Crocetin also inhibited serum malondialdehyde content and increased the level of nitric oxide in serum. The results suggested that antihyperlipidemic effects along with the antioxidative properties of crocetin might be useful in the inhibition of the formation of atherosclerosis (He et al., 2007). Inconsistent with previous reports, it was shown that adding of crocetin to high lipid diet (HLD) resulted in markedly improved atherosclerosis, associated with a significantly decreased VCAM-1 expression, however, plasma lipids level (TC, LDL-C and HDL-C) remained comparable to that of HLD group. In addition, immunohistochemical analysis showed crocetin reduced the activation of nuclear factor kappa B (NF-κB), a redox

Table 5
Some examples from *in vivo* effects of crocetin as hepatoprotective agent.

Animal type with induced liver damage	Concentration/Dose	Mechanism	Ref.
CCl4-induced liver damage in mice	140 mg/kg b.w.	Restoration of antioxidant enzymes activity and GSH level	Chen et al. (2016)
Aflatoxin B1 (AFB1) hepatotoxicity in the rat	2 or 6 mg/kg, daily	Elevation of GSH content and the activities GST and GSH-Px as well as reduction of hepatic AFB1-DNA adducts formation	Wang et al. (1991b)
XXO or PQ system inducing oxidative damage in rat primary hepatocytes	10, 20 microM	Inhibition of genotoxicity in rat hepatocytes induced by ROS and inhibition of the superoxide anion and/or free radical	Tseng et al. (1995)
Rats subjected to hemorrhagic shock	2 mg/kg	Reductions in levels of postshock cytosolic cytochrome *c* and activated caspase 3, associated with an increases in the levels of protective Bcl-2 protein	Yang et al. (2011)

Table 6
Some studies from *in vivo* positive effects of crocetin on hyperlipidemia.

Animal type	Concentration/Dose	Mechanism	Ref.
Mouse	50 mg/kg/day, Oral	Inhibition of pancreatic lipase	Lee et al. (2005)
Male quails	25, 50, 100 mg/kg/day, Oral	Antioxidative properties	He et al. (2007)
Thoracic aorta isolated from hypercholesterolemic rabbit	15, 30 mg/kg, Oral	Improvement of endothelium-dependent relaxation (RIDR) of thoracic aorta by increasing the vessel eNOS activity, leading to elevation of NO production	Tang et al. (2006)

sensitive transcription factor essential for VCAM-1 expression. This study indicated that the antioxidants activity of crocetin attenuated atherosclerosis (Zheng et al., 2005).

Because endothelial dysfunction strongly contributes to the initiation and progression of atherosclerosis, in a study by Tang et al., the ability of crocetin to improve this dysfunction in thoracic aorta isolated from hypercholesterolemic rabbit was investigated. It was shown that oral administration of crocetin (15, 30 mg/kg) could improve endothelium-dependent relaxation (RIDR) of thoracic aorta by increasing the vessel eNOS activity, leading to elevation of NO production (Tang et al., 2006).

6.6. Effect of crocetin on diabetes

In diabetes, the high level of glucose can cause different disorders in the function of some organs. It different research projects, the effect of crocetin on diabetes has been evaluated. Table 7 summarizes some *in vitro* and *in vivo* studies about the mechanisms action of crocetin in diabetes.

6.6.1. In vitro studies

Meng et al. evaluated the inhibition effect of crocetin on high glucose-induced apoptosis in $HUVEC_s$. It was revealed that crocetin (0.1 μM, 1.0 μM) prevented high glucose-induced apoptosis by the increase of activation of p-Akt, following the up-regulation of eNOS and NO production. These results suggested that crocetin might be useful in preventing diabetes-associated cardiovascular complications (Meng and Cui, 2008).

In another study, the effect of crocetin on insulin resistance induced by palmitate in 3T3-L1 adipocytes was investigated. The cells were treated with palmitate (300 μM) or/and crocetin (1 or 10 μM). The exposure of palmitate treatment led to increase the activity of protein kinase Cθ (PKCθ) and subsequently induce the activation of c-Jun NH2-terminal kinase (JNK) and inhibitor κB kinase β (IKKβ). The activation of these two serine kinases resulted in increase in insulin receptor substrate-1 (IRS-1) serine307 phosphorylation that is responsible for reductions of IRS-1 function and glucose metabolism. Interestingly, pretreatment with crocetin almost reversed all of these abnormalities by the inhibitory effect on PKCθ activation in a dose-dependent manner (Yang et al., 2010). In *in vitro* study by Xi et al., the protective effect of crocetin was investigated in rat adipocytes against the impaired insulin-stimulated glucose uptake and disordered expression of TNF-α induced by palmitate. The antioxidant effects of crocetin at concentration 5 or 50 μM including inhibition of intracellular ROS production by inactivation of NADPH oxidase may explain the ability of this compound to prevent insulin insensitivity in this cell model (Xi et al., 2007b).

The influence of crocetin on the migration of VSMCs induced by advanced glycosylation end products (AGEs) was also studied by Xiang et al. The results demonstrated that pre-treatment with crocetin (1.0 μM) inhibited AGEs-induced VSMCs migration by suppression of RAGE (AGEs receptor) expression leading to reduce of the levels of inflammatory factors (TNF-α and IL-6) and decrease activity of MMP-2/9. These findings may be useful in the clinical application of crocetin in diabetic vascular-injury (Xiang et al., 2017).

6.6.2. In vivo studies

It was shown that hyperglycemia stimulates endothelial progenitor cell (EPC) apoptosis by increasing ROS production resulted in the prevention of this cell migration to injury sites and subsequent vascular abnormality (Yu et al., 2016). The ability of crocetin on restoring diabetic EPC was evaluated by Cao et al. In this research, experimental diabetes was induced in C57BL/6 mice by injection of STZ. Then, EPCs derived were extracted from bone marrow in tibia and femur of mice. The results showed that crocetin (5 μM) improved EPC cell proliferation and colony formation. Simultaneously, LDH release, cell apoptosis, and caspase-3 activity were also limited following crocetin treatment. It was demonstrated that crocetin restored EPC injury via antioxidant activity and activation of PI3K/AKT-eNOS pathway (Cao et al., 2017).

To improve insulin resistance, much effort has been focused on the reduction of the accumulation of detrimental lipids in muscle and liver, by decreasing the availability of circulating lipids (non-esterified fatty acid, NEFA and triglyceride, TG) (Chavez and Summers, 2003). In one research, the regulation of lipid metabolism by crocetin was evaluated to clarify the insulin-sensitizing mechanism of crocetin. Rats given a high-fat diet were treated with crocetin in a daily dose of 50 mg/kg for 6 weeks. Crocetin administration could improve insulin action by increase in the glucose infusion rate, necessary to maintain euglycaemia under hyperinsulinemia conditions. On the other hand, crocetin modulated the peroxisome proliferator-activated receptor-α (PPARα) related genes, involved in lipid metabolism resulted in reduced lipid availability to muscle, enhanced ability of the liver to take up and oxidize NEFA and TG leading to the redistribution of tissue-specific NEFA clearance and depressed TG availability and consequently improving sensitivity to insulin (Sheng et al., 2008). In a study by

Table 7
Some studies from *in vitro* and *in vivo* effects of crocetin on diabetes.

Cell line or Animal type	Concentration/Dose	Mechanism	Ref.
HUVECs	0.1 μM, 1.0 μM	prevention high glucose-induced apoptosis by the increase of activation of p-Akt, following the up-regulation of eNOS and NO production	Meng and Cui (2008)
Insulin resistance induced by palmitate in rat adipocytes	5 or 50 μM	Inhibition of intracellular ROS production by inactivation of NADPH oxidase	Xi et al. (2007b)
EPCs extracted induced diabetic mice	5 μM	Improvement of dysfunction of EPCs mainly by restoring the PI3K/AKT-eNOS and suppressing ROS pathway	Cao et al. (2017)
Rat with high-fat-diet	50 mg/kg, Oral	Improvement of insulin action in induced insulin resistance rat with high fat diet by decreasing lipid accumulation in muscle and liver, and consequently improving sensitivity to insulin	Sheng et al. (2008)

Mahdavifar et al., the combination of crocetin, glycine and N-acetyl cysteine (MB-92) was applied in diabetic-atherosclerotic rat. The results indicated that this formulation was more effective than treatment alone on the improvement of FBS, lipid profile and atherosclerotic index, reduction of oxidation and inflammatory markers, inhibition of the formation of different glycation products and induction of glyoxalase system (I and II) in the diabetic-atherosclerotic rats (Mahdavifard et al., 2016).

6.7. Effect of crocetin on skin disorders

Since skin aging and inflammation is characterized by oxidative damage, therefore, antioxidant agents can be useful in treatment of skin disorders. Due to antioxidant properties of crocetin, the effect of this natural compound in some skin damages has been evaluated.

6.7.1. In vitro studies

Hashemi-Shahri et al. evaluated the inhibitory effect of crocetin (0–32 μM) on melanogenesis in B16 melanoma cells. Crocetin reduced the amount of melanin and inhibited the mushroom tyrosinase activity with no significant cytotoxic effect on cells. In this research, the mechanism of antimelanogenesis effects of crocetin was also investigated. It was shown that protein levels of tyrosinase, microphthalmia-associated transcription factor (MITF) and cellular reactive oxygen species (ROS) content were reduced in the presence of crocetin (Hashemi-Shahri et al., 2018).

6.7.2. In vivo studies

The protective effects of crocetin against ultraviolet-A (UV-A)-induced skin damage was investigated in normal human skin fibroblast cells (NB1-RGB) and in oxidative stress detector (OKD) mice. Crocetin could protect NB1-RGB cells against cell death and reduce the production of ROS after exposure to UV-A irradiation (10 J/cm2). Crocetin treatment also suppressed the expression of cleaved caspase-3, which is a well-known indicator of apoptosis progression. The administration of crocetin (100 mg/kg, p.o.) to the OKD mice, decreased oxidative stress and downregulated the nuclear factor erythroid 2 (NFE2)-related factor 2 (Nrf2) as an emerging regulator of cellular resistance to oxidants, in the mouse skin. Crocetin administration also decreased lipid peroxidation in the skin. According to these results, it can be concluded that the protective effects of crocetin against UV-A induced skin damage are based on reducing reactive oxygen species production and cell apoptosis (Ohba et al., 2016).

Burn injury can induce inflammatory reactions in many organs through gastrointestinal system. Therefore, the preservation of intestinal mucosal barrier has important clinical implications for the outcome of burn victims (Gosain and Gamelli, 2005). It was also demonstrated that oxidative stress and inflammation has essential roles in burn-induced intestinal injury. The protective effect of crocetin, as a potential antioxidant agent, against burn-induced intestinal injury was investigated. The results showed that crocetin (100 and 200 mg/kg) could increase content of antioxidant enzymes and reduced intestinal oxidative damage in burn models. Crocetin also reduced inflammatory factors such as TNF- α and Int-6 concentrations as well as restored the mucosal barrier and integrity of intestinal tissues. Therefore, crocetin treatment may protect against burn-induced small intestinal injury, possibly by inhibiting burn-induced oxidative stress and inflammatory responses (Zhou et al., 2015).

6.8. Crocetin and lung disorders

Inflammation plays an important role in lung disorders. Therefore, crocetin with anti-inflammatory properties may act as an effective agent in this field. In the following, some studies about the effect of crocetin on asthma and lung injury have been presented.

6.8.1. In vivo studies

Ding et al. evaluated the effects of crocetin on the severity of an ovalbumin (OVA)-induced allergic asthma in mice. After OVA challenge, 100 μl crocetin (100 μM) in DMSO was given by intranasal administration for one week. Then, $CD4^+CD25^+$ Treg cells, as an essential inflammation suppressor in asthma, was isolated by flow cytometry. Then, the role of two immunoregulatory proteins, forkhead box transcription factor (Foxp3) and TNF-alpha-induced protein 8-like 2 (TIPE2) in crocetin-treated OVA-asthma in mice was evaluated by suppression of Foxp3 and TIPE2 expression through lentivirus-mediated delivery of shRNA. It was shown that, crocetin could inhibit severity of asthma by enhancement of the number of Treg cells through increase of Foxp3 and TIPE2 expression in OVA-treated mouse (Ding et al., 2015). The effect of crocetin on acute lung injury induced by lipopolysaccharide (LPS) was studied in mouse model. Pretreatment with crocetin at dosages of 50 and 100 mg/kg protected animals against LPS-induced acute lung injury. This protective effect of crocetin was mediated by increasing SOD activity, and decreasing lung myeloperoxidase (MPO) activity. Furthermore, treatment with crocetin significantly decreased pro-inflammatory cytokines such as macrophage chemoattractant protein-1 (MCP-1), TNF-α, and IL-6 in the acute lung injury model. In addition, crocetin at different dosages reduced phospho-IκB expression and NF-κB activity in LPS-induced lung tissue alteration (Yang et al., 2012).

6.9. Crocetin and fertilization

Increased oxidative stress plays an important role in impairing embryo development. The controlling of oxidative stress during *in vitro* culture (IVC) can be achieved by adding antioxidants to the culture medium. Crocetin with antioxidant properties may protect the embryo from oxidative stress and improve the development and quality of blastocyst.

6.9.1. In vitro studies

Sapanidou et al. studied the protective role of crocetin in bovine spermatozoa against oxidative stress during *in vitro* fertilization. For this reason, frozen/thawed bovine spermatozoa and the IVF medium were treated with crocetin. It was shown that crocetin (2.5 μm) by regulating ROS concentration and lipid peroxidation resulted in better maintenance of motility parameters, viability, and acrosomal integrity, with a very small number of cells with DNA fragmentation. On the other hand, the presence of crocetin in the fertilization medium also significantly increased the acrosome-reacted spermatozoa and blastocyst production (Sapanidou et al., 2016).

In another study, the effect of adding crocetin to bovine culture medium on *in vitro* blastocyst development and quality was studied. In bovine culture medium, crocetin increased blastocyst formation rate and reduced apoptosis levels which helped embryo output and quality (Zullo et al., 2016). In a research by Dos Santos et al., it was demonstrated that adding of crocetin (1 μM) to the culture media of bovine embryos resulted in increased blastocyst rates, decreased intracellular levels of ROS along with upregulation of genes related to lipid metabolism and adaptive response to stress. Therefore, crocetin supplementation can be considered as a protective agent for embryos from oxidative stress (Dos Santos et al., 2019).

6.9.2. In vivo studies

In study by Di Emidio, the protective effects of crocetin and the synthetic compound AS101 on mouse ovary against cyclophosphamide (CPM), the most ovotoxic anticancer drug, were evaluated in female CD1 mice. The animals received crocetin (100 mg/kg, orally) and AS101 (10 μg per mouse, i.p.). After 15 days, they received a single i.p. injection of 100 μL of CPM (100 mg/kg). It was found that the number of primordial follicles of 1 mice receiving crocetin plus CPM increased compared to groups receiving CPM alone and similar to AS101. The

results indicated that crocetin and AS101 could protect the ovary against CPM by modulating SIRT1, an important sensor of oxidative stress (OS), which is an important mechanism behind ovarian toxicity by CPM. On the other hand, these compounds could increase mitochondrial markers (SOD2 and PGC1-α) in mice revealing the mitochondrial protection. Therefore, crocetin could be considered as a potential agent for preserving fertility in cancer patients (Di Emidio et al., 2017).

6.10. Crocetin and retinal damages

6.10.1. In vitro studies

Crocetin at a concentration of 3 μM showed inhibitory effects up to 50–60% against tunicamycin- and H_2O_2-induced retinal cell death (Retinal ganglion cell (RGC-5)). It also increased caspase-3 and -9 activities (Yamauchi et al., 2011). In a study by Wang et al., it was shown that crocetin had the inhibitory effect on the proliferation, migration, and TGF-β2-mediated epithelial-mesenchymal transition (EMT) of the retinal pigment epithelial (RPE) cells. EMT process has an important role in the development of proliferative vitreoretinopathy (PVR) (Wang et al., 2017a). In another study, it was indicated that crocetin (100–400 μM) could suppress the proliferation and migration of the human RPE cell line ARPE-19 through the modulation of Bcl-2 family regulators. Therefore, crocetin may be useful in the management of patients with PVR (Zhang et al., 2019).

6.10.2. In vivo studies

The effects of crocetin (20 mg/kg, p.o. 1 h before the ischemia and then twice a day for the 4 days) on ischemia/reperfusion (I/R)-induced retinal damage were evaluated. The results showed that crocetin could prevent ischemia-induced retinal damage through its inhibition of oxidative stress by different mechanisms such as decreased the numbers of TUNEL-positive cells and 8-OHdG-positive cells, and the phosphorylation levels of p38, JNK, NF-κB, and c-Jun present in the retina after I/R (Ishizuka et al., 2013).

In another study, the effects of oral administration of crocetin at doses of 100 mg/kg were assayed on damage induced by N-methyl-D-aspartate (NMDA) in the murine retina before and after intravitreal injection of NMDA. Crocetin could inhibit cell apoptosis in the ganglion cell layer in part through inhibition of the caspase pathway (Ohno et al., 2012).

In similar research, retinal damage in mice was induced by exposure to white light at 8000lx for 3 h after dark adaptation. Crocetin at dosage of 100 mg/kg, p.o. significantly inhibited photoreceptor degeneration and retinal dysfunction. It also halved the expression of TUNEL-positive cells (Yamauchi et al., 2011). Wang et al., evaluated the toxicity, pharmacokinetics and inhibition potential of intraocular crocetin in proliferative vitreoretinopathy (PVR) in a rabbit model. No retinal toxicity was observed 2 weeks after the injection of 0.4 μmol crocetin. The half-life of 4.231 h was obtained for intravitreal injection of crocetin (0.4 μmol) using a non-compartmental model. Treatment with crocetin significantly inhibited the progression of PVR (induced with an intravitreal injection of ARPE-19 cells in rabbit eyes) along with expression of α-SMA (mesenchymal markers), collagen fibers and Ki67 (proliferative cell markers) (Wang et al., 2019).

6.11. Other therapeutic applications

In study by Wang et al., the protective effect of crocetin was studied on hemorrhagic shock-induced acute renal failure in rats. Crocetin was given at a dose of 50 mg/kg 40 min after hemorrhage. It was demonstrated that crocetin could attenuate renal dysfunction in hemorrhagic shock by different mechanisms including restoring T-SOD activity and suppressing the superoxide anion and/or free radicals, inhibiting of the activation of NF- κB and preventing the production of TNF-α, IL-6 and inhibiting of the activity of iNOS and NO production (Wang et al., 2012).

The possible antifibrotic effect of crocetin was investigated in fibroblasts isolated from patients with systemic scleroderma (SSc) and in bleomycin-induced dermal and lung sclerotic mice (Song et al., 2013). Crocetin (0.1, 1 or 10 μM) inhibited the proliferation of SSc and normal fibroblasts in dose dependent manner. Crocetin at a concentration of 1 μM resulted in the most significant inhibitory effect on the expression of COL1A1, COL3A1, MMP-1 mRNA levels and α-SMA expression while crocetin increased TIMP-1 mRNA levels in SSc and normal fibroblasts (Song et al., 2013).

The upregulated COL1A1 and COL3A1 genes in SSc fibroblasts play important roles in the pathogenesis of SSc. MMP-1 promotes the degradation of both type I and type III collagen, and MMP-1 activity is inhibited by TIMP-1(Woessner, 1991). Crocetin (50 mg/kg/d) was injected intraperitoneally for 14 days into SSc mice. Crocetin significantly reversed skin thickening, lung fibrosis and COL1A1 mRNA levels in the skin and lungs of bleomycin-induced sclerotic mice, especially within the early phase (1–3 weeks). Simultaneously, crocetin decreased plasma ET-1 levels and ET-1 mRNA levels (an important endogenous peptide hormone that potently promotes vasculopathy, inflammation and fibrosis) in the skin and lungs of bleomycin-induced sclerotic mice, especially within the early phase (1–3 weeks) (Song et al., 2013). The effect of crocetin on polycystic ovary syndrome (PCOS) induced by prenatally exposure of mice to dihydrotestosterone (DHT) was evaluated by Hu et al. Crocetin (40 mg/kg, daily for 4 weeks by oral gavage) could improve the levels of GnRH, FSH, LH, progesterone (P4), estradiol (E2) and testosterone (T). On the other hand, crocetin increased the kisspeptin level in anteroventral periventricular nucleus and reduced it in arcuate nucleus (Hu et al., 2018).

The other pharmacological properties of crocetin include the prevention of myopia progression (Mori et al., 2019), reduction of drug resistance through modulation of MRP transporters (Neyshaburinezhad et al., 2018) and induction of cell differentiation of rat bone marrow-derived mesenchymal stem cells (Kalalinia et al., 2018).

7. Clinical trails

7.1. Effect of crocetin on sleep

A clinical study was performed to examine the effect of crocetin supplementation on sleep in healthy adult males, 25–59 years with sleep complaints. Crocetin from *G. jasminoides* Ellis and dextrin were mixed and filled into hard gelatin capsules. This capsule contained 7.5 mg of crocetin. For 6 weeks, each subject took one capsule daily in each 2-week intake period, which was separated by a 2-week washout period. The actigraph analysis results showed that crocetin without side effects, reduced the number of awakening episodes in comparison with placebo group resulted in the efficiency of crocetin in the maintenance of sleep. On the other hand, crocetin could improve the quality of sleep. About the mechanism of these effects, it was shown that using of *G. jasminoides* Ellis as the traditional herbal medicine had sedative and antianxiety effects. (Kuratsune et al., 2010). In recent study by Umigai et al., the effect of crocetin on the quality of sleep was randomly investigated in healthy adult participants with mild sleep complaints. Crocetin at dosage of 7.5 mg per day was administrated by two crossover 14-day intake periods separated by a 14-day wash-out period. Effect of crocetin on objective and subjective (mean OSA-MA scores) sleep parameters were measured. The objective sleep parameters include delta power, sleep latency, rapid eye movement (REM) sleep latency, sleep efficiency, total sleep time, and wake after sleep onset (WASO). The results indicated that crocetin could significantly increase delta power and subjective scores for sleepiness on rising and feeling refreshed compared with placebo. There were no significant differences in the other sleep parameters. However, in this study it was shown that crocetin can be considered as a potential agent for improvement of sleep quality but the effect of crocetin on serious sleep disturbance and

the dosage of crocetin require more investigations (Umigai et al., 2018).

7.2. Effect of crocetin on physical fatigue

In a clinical trial, the effect of daily oral administration of crocetin was evaluated in physical fatigue in human subjects. Crocetin (15 mg), ascorbic acid (3,000 mg), or placebo were administrated orally to healthy volunteers for 8 days. Workloads tests was used as a fatigue-inducing physical task. The change in maximum velocity (MV) from the 30- to the 210-min test was significantly higher in men who received crocetin compared to other groups. This effect of crocetin was specific to males. These results suggest that daily administration of crocetin may attenuate physical fatigue in men (Mizuma et al., 2009).

8. Conclusions and future perspectives

Saffron (*C. sativus* stigmas) has been traditionally used in prevention and treatment of different disorders for many centuries. The therapeutic activity of saffron is mainly due to its major bioactive derivatives including crocin and crocetin. Crocin could not absorb through the gastrointestinal tract and hydrolyze to crocetin before or during intestinal absorption. Crocetin is insoluble in water, however, in its anionic form it is highly water-soluble; therefore, it easily dissolves in aqueous alkali solutions pH ≥ 9. This solubility property may be the reason of rapid absorption of crocetin after oral administration. However, new studies are needed to understand more about the pharmacological and toxicological profile of crocetin. In addition, developing nanoparticle platforms to deliver crocetin may be one appropriate approach to improve its solubility, stability, and pharmacokinetic properties.

Most *in vivo* studies were carried out on the effects of crocetin against cancer, nervous and cardiovascular diseases, liver damage, hyperlipidemia, diabetes, and skin disorders. These therapeutic effects can be achieved with different mechanisms in different diseases but mainly including antioxidant effects, inhibition of pro-inflammatory mediators and prevention of proliferation or stimulation of apoptosis in cancer cells.

Crocetin can also be considered as a potential therapeutic agent for medicinal applications in human. However, the clinical usage of crocetin is very limited. The safety of crocetin was also evaluated in healthy adult volunteers. Crocetin, at dose of 37.5 mg/day for four weeks (excessive intake of crocetin), has been shown no adverse effects in any volunteers.

Further clinical trials are needed in order to clarify the therapeutic activities of crocetin. On the other hands, more safety studies should be performed to detect the possible toxic effects of crocetin in long-term administration.

Conflicts of interest

The authors declare that there are no conflicts of interest.

Transparency document

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