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Pharmacological Properties of Crocetin and Crocin (Digentiobiosyl Ester of Crocetin) from Saffron

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Functional plant foods and medicinal herbs provide a wide variety of natural products for new drug research and development. Crocetin and crocin (digentiobiosyl ester of crocetin) are the major bioactive ingredients of saffron which is used as a costly spice, food colorant and traditional herbal medicine. These particular carotenoids have gained much research attention for their extensive pharmacological activities. Following oral administration, crocetin is rapidly absorbed into the blood circulation and widely distributed into the extra-vascular tissues of the body, whereas the water-soluble compound crocin is hardly absorbed through the gastrointestinal tract. Crocetin and crocin have been shown to be effective in the prevention and/or treatment of several diseases such as atherosclerosis, myocardial ischemia, hemorrhagic shock, cancer and cerebral injury. The compounds exert their biological and pharmacological effects largely through their strong antioxidant activity. However, there seems to be substantial variation in the effectiveness of both phytochemicals when used in different diseases. The aim of this review is to discuss the pharmacokinetic and medicinal properties of crocetin and crocin based on related literature and our research results.

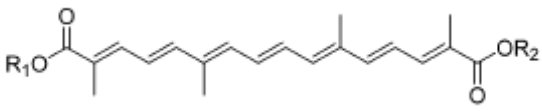
Keywords: crocetin, crocin, saffron, *Crocus sativus*, pharmacokinetics, medicinal properties.

Saffron (*Crocus sativus* Linne), the most expensive spice in the world, has been cultivated in the region from Greece to Persia for at least 35 centuries. Nowadays, it is still used as a food coloring, flavoring agent and as an herbal medicine in various parts of the world, but the large-scale production of saffron is limited to a few countries. A primary problem with saffron cultivation and production is that the plant grows in desert regions but needs sufficient water to thrive. However, irrigation in many of these areas is difficult and costly, and severe draughts cause substantial crop losses [1]. Due to being triploid, saffron is sterile and its propagation is possible only via corms. Consequently, the cultivation and harvest of the ancient plant still need human help, which further elevates its cost [2]. Commercial saffron is produced at the rate of 50 tons per year which costs about 50 million dollars worldwide [3].

The medicinal properties attributed to saffron are extensive. Saffron was described in the Chinese compendium *Bencao Gangmu* in 1596, mentioning that it was used to benefit the blood (vitalizes blood, stops bleeding) and to calm fright. Modern pharmacological studies have demonstrated that saffron extracts are useful in the prevention and treatment of numerous diseases such as hypertension, coronary heart diseases, angina, cancer, fatty liver, and nephritis [4]. In Tibet, saffron is often an ingredient in medicinal incenses and is considered a tonic for the cardiovascular and the nervous system.

It is well known that the bioactive components contained in edible and medicinal plants may provide

desirable health benefits and potential medicinal uses. Research has focused on identifying the active phytochemicals found in such plants and the mechanisms by which they function to provide the medicinal properties. The composition of saffron has also been analyzed widely. Besides some popular plant components including sugars, water, proteins, amino acids, cellulose, fats, minerals and other chemicals, saffron contains at least the following active ingredients: (1) carotenoids responsible for saffron's color including crocetin, crocins and other carotenes, (2) an essential oil including safranal, and (3) bitter substances including picrocrocin. Although saffron contains conventional carotenoids such as α- and β-carotene, lycopin and zeaxanthin, its staining capability is mostly caused by crocetin and crocin, a diester of crocetin with gentiobiose. Crocin is also called crocin-1 in order to distinguish it from crocin analogs.



crocetin: $R_1 = R_2 = H$
crocin: $R_1 = R_2$ = gentiobiosyl

As two major pharmacologically active components in saffron, crocetin and crocin have attracted great research interest and encouraging experimental results have been obtained. However, saffron is an herb most people are unlikely to utilize either for medicinal or research purpose largely because the material is extraordinarily expensive. Therefore, it is meaningful and necessary to search for and utilize potential substitutes for saffron. Fortunately, crocetin and crocin are also found in the fruit of *Gardenia jasminoides* Ellis. This widely used traditional Chinese medicine has been successfully employed in our laboratory to isolate crocetin and crocin with high purities (>98%). This article outlines the pharmacokinetic and medicinal properties of crocin and crocetin and provides updated information related to these promising natural products for medicinal purposes.

Pharmacokinetic properties of crocetin and crocin

Studies have shown that crocin is hardly absorbed after being orally given to animals [5, 6] and healthy volunteers [unpublished data]. Although orally administered crocin is known to be metabolized into crocetin in the body [6, 7], the accurate metabolic rate of crocin and whether it is converted into other active metabolites are still unclear. Actually, the excretory rate of intact crocin is relatively high and the detected plasma crocetin concentration is at a low level after crocin is orally administered to rats [5]. Contrariwise, crocetin is rapidly absorbed and substantially distributed into extra-vascular tissues following oral administration [6, 8]. The absorbed crocetin is present as an intact form and as monoglucuronide and diglucuronide conjugates in plasma [6]. By using compartment analysis, it is shown that the pharmacokinetics of crocetin after oral administration is consistent with a two-compartment model. Crocetin is mainly distributed into liver, heart, lung, kidney, spleen and fat in the body. The pharmacokinetic parameters of orally administered crocetin are listed in Table 1 [8]. Based on these findings, crocetin is likely to act as an important active metabolite of crocin in the body. Nonetheless, whether crocetin or other unknown metabolite(s) exerts major pharmacological effects observed after oral dose(s) of crocin is elusive. Because it is uncertain whether crocetin can replace crocin in some therapeutic fields, it is still meaningful to seek suitable crocin absorption-stimulating agents when oral administration is considered as a satisfactory route.

The pharmacokinetics of crocin following intravenous administration is best described by a two-compartment model based on compartment analysis method [9, 10]. The plasma half-life of crocin ranges from 1.7 h to 2.4 h, and the V_d value is 0.25-0.51 L/kg, indicating a limited distribution of crocin in the body. Crocin is mainly distributed into the heart, lung, kidney and spleen after intravenous injection, while it is undetectable in the brain, fat and testicle [9]. The distributive characteristics of crocin are probably associated with its high water solubility.

Table 1: The pharmacokinetic parameters of crocetin after oral administration in rats [8].

Pharmacokinetic parameters	Dose (mg/kg)		
	20	40	80
C_{max} (μg/ml)	4.49±1.24	9.13±1.66	11.23±2.63
t_{max} (min)	52.1±9.7	57.0±8.0	59.0±8.2
$t_{1/2}$ (min)	54.6±7.0	66.3±9.2	70.0±8.0
AUC (μg/ml·min)	680±35	1244±115	1723±103
Cl (ml/min/kg)	36.85±1.84	20.1±0.49	14.52±0.46
V_d (L/kg)	2.89±0.38	1.92±0.42	1.46±0.54

In some *in vivo* studies, crocin and crocetin are also administered by other routes such as intraperitoneal and subcutaneous injection. Related pharmacokinetic properties are unclear due to the absence of pertinent reports.

Noticeably, as highly reactive substances, the plasma half-lives of orally administered crocetin (Table 1) and intravenously administered crocin are both relatively short, suggesting a rapid elimination of these compounds. Although the pharmacokinetic data of crocetin after intravenous injection is currently lacking, it is known that the plasma half-life of the *trans* isomer of crocetin, *trans*-sodium crocetinate (TSC), given through intravenous administration, is quite short (about 15 min) [11]. The instability of these agents is likely to handicap their application, especially in acute situations. As for short-acting agents, repeated administration is undoubtedly an effective method, but evidently this approach is quite unsatisfactory.

Our research has focused on the beneficial effects of crocetin and crocin on the cardiovascular system. Since oral administration is a convenient and preferred route of administration, especially for chronic diseases, crocetin has become the more important experimental material instead of crocin. More importantly, the possible waste of plant resources may be avoided.

Potential medicinal uses of crocetin and crocin

During the last decades, crocetin and crocin have been shown to be effective in the prevention and/or treatment of atherosclerosis, myocardial ischemia, hemorrhagic shock, cancer, cerebral diseases, *etc*. These medicinal properties are mainly associated with the powerful antioxidant activity of these compounds. A summary of the experimental target, mode of application and dose of crocetin and crocin involved in the core studies is listed in Table 2. ***Atherosclerosis:*** After the preventive effect of crocetin on the formation of atherosclerosis was reported in 1975 [12], no reports followed in this field for a long period. However, the anti-atherosclerotic effect of crocetin is recognized as a prominent biological function in our laboratory. Crocetin exhibits a potent lipid-regulating effect; it significantly reduces plasma total cholesterol, triglyceride, and low-density lipoprotein cholesterol (LDL-C) and elevates high-density lipoprotein cholesterol (HDL-C) in high-fat fed animals, together with an improvement of the anti-atherosclerotic index and the imbalance between increased malondialdehyde (MDA) and reduced superoxide dismutase (SOD) [13].

It is well known that oxidative stress, especially the oxidation of LDL-C, plays a critical role in the development of atherosclerosis. Thus, antioxidants are potential anti-atherogenic agents, of which probucol is representative. Compared with the favorable impact of crocetin on the plasma lipids profile, probucol decreases plasma HDL-C as well as total cholesterol and LDL-C [14]. It is accepted that inhibition of oxidative deterioration of LDL-C mainly accounts for the anti-atherogenic property of probucol. Similarly, crocetin also reduces the formation of oxidized LDL-C due to its strong radical scavenger properties. Furthermore, our laboratory has recently reported that even low-dose crocetin can markedly ameliorate atherosclerosis, coupled with a significant reduction in vascular cell adhesion molecule-1 expression and NF-κB activation in the aorta of high fat-fed rabbits [15].

Interestingly, oral doses of crocin also effectively manage the development of experimental atherosclerosis. Crocin reduces the levels of plasma total cholesterol, triglyceride and LDL-C, and inhibits the formation of aortic plaque in high fat-fed animals [16]. The hypolipidemic activity of crocin has been proposed to be mediated by the inhibition of smooth muscle cells proliferation and p38MAPK activation based on *in vitro* observations [17]. However, the use of the cell culture system should be reconsidered in this study, because crocin cannot reach aortic smooth muscle cells *in vivo* following oral administration. It has been recently reported that crocin shows significant hypolipidemic activity, probably due to the inhibition of pancreatic lipase activity, and its metabolite crocetin can improve hyperlipidemia [7]. This finding may shed light on the mechanism(s) by which the oral administration of crocin exerts its lipid-regulating effects. We should attach importance to at least two aspects: (1) the effects of crocin in the intestinal tract where it is largely present in its intact form, and (2) the active metabolites and metabolic rate of orally administered crocin.

Myocardial ischemia: Much evidence implicates an important role of reactive oxygen species in ischemia/reperfusion, and thus antioxidant therapy

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Table 2: Brief information on the experimental targets, modes of application and doses of crocetin and crocin involved in the core studies.

Disease	Agent	Experimental target	Mode of application	Effective dose	References
Atherosclerosis	Crocetin	Animals	Chronic administration (p.o.)	15-100 mg/kg/d	13, 15
	Crocin	Animals	Chronic administration (p.o.)	25-100 mg/kg/d	16
Myocardial ischemia	Crocetin	Animals	Preventive administration (p.o.)	25-100 mg/kg/d	19, 20
		Cultured myocardial cells	Pre-incubation	0.1-50 μM	21-24
Hemorrhagic shock	Crocetin/ TSC	Animals	Acute treatment (i.v.)	2 mg/kg(crocetin); 0.05-0.418 mg/kg (TSC)	11, 32, 33, 40
Cancer	Crocetin	Animals (AFB1-induced hepatotoxity)	Preventive administration (p.o.)	2-6 mg/kg/d	48
		Animals (TPA-induced skin tumors)	Preventive administration(topical application)	0.2-3.0 μmol	52, 54
		HL-60 cells	Incubation	2 μM(IC_{50})	46
		BP-treated C3H10T1/2 cells	Pre-incubation	0.01-0.10 mM	51
		TPA-treated NIH/3T3 cells	Pre-incubation	0.03-0.12 mM	53
	Crocin	DHD/K12-PROb-inoculated animals	Chronic administration (s.c.)	400 mg/kg/wk	62
		HT-29;DHD/K12-PROb;Hela; HL-60 cells	Incubation	0.4 mM, 1.0 mM, 3mM, 2 μM (IC_{50}), respectively	46, 59, 62
Cerebral disorders	Crocetin	Animals	Preventive administration (i.p.)	25-75μg/kg/d	72
	Crocin	Animals	Preventive administration (intracerebroventricular injection)	51.2 nmol	64
		Hippocampal slices	Pre-incubation	10-30 μM	65
		PC-12 cells	Pre-incubation	10 μM	69

has been proposed as a new pharmacological approach to such an injury [18]. Saffron has been used as an anti-anginal traditional Chinese medicine for a long time. As one of the major pharmacologically active ingredients of saffron, crocetin has also been shown able to protect against myocardial ischemia based on related experimental data obtained in our laboratory.

In a series of animal experiments, pretreatment with crocetin effectively combats pituitrin-, isoproterenal- and coronary artery ligation-induced acute myocardial injury in rats. After intravenous injection of pituitrin, crocetin significantly reduces the elevation of ST segment, the change of T wave, and the incidence of arrhythmia [unpublished data]. In isoproterenal-treated rats, crocetin markedly decreases the formation of MDA and the release of lactic dehydrogenase (LDH) and creatine kinase, and increases the activities of Na^+-K^+-ATPase, Ca^{2+}-Mg^{2+}-ATPase and glutathione peroxidase (GSH-PX) in injured myocardial tissue [19]. Similar results are obtained when myocardial infarction is induced by ligation of the coronary artery. In addition, crocetin increases ATP and ADP concentrations in heart homogenate, indicating an improvement in energy metabolism in injured hearts [20].

Observations *in vitro* have also demonstrated this pharmacological property of crocetin. Crocetin

increases coronary blood flow, heart rate and the amplitude of contraction of the isolated guinea pigs' hearts subjected to ischemia/reperfusion injury [unpublished data]. In the studies using cell culture systems, pre-incubation with crocetin reduces the oxidative injury of low glucose/hypoxia-, noradrenaline- and H_2O_2-treated cultured myocardial cells. Disorders in LDH leakage, intracellular MDA production, intracellular SOD activities, cell apoptosis and energy metabolism are all improved by crocetin pretreatment [21-24].

These data suggest that crocetin may prevent ischemia/reperfusion injury via its antioxidant activity. However, the effectiveness of crocetin as an acute treatment after the occurrence of myocardial ischemia is unknown at present.

Hemorrhagic shock: There is a substantial reduction in oxygen consumption during hemorrhagic shock, which is tightly associated with mortality. It is proposed that the oxygen consumption depends on oxygen diffusion from the red cells to the mitochondria; thus enhancing this rate may increase oxygen intake by tissues during hypovolemia. This has been shown to be possible by using crocetin. A large quantity of the research with crocetin, as well as with a similar compound, *trans*-sodium crocetinate (TSC), has focused on the treatment of hemorrhagic shock. It should be pointed out here that crocetin mainly exists in its *trans* form naturally.

Crocetin enhances oxygen diffusivity through plasma and provided a net increase in oxygen to the capillary endothelial cell in animals [25, 26]. It has been shown that crocetin improves tissue oxygenation in different experimental models *in vivo* [27-30]. The beneficial effect of crocetin on red cell deformability and mitochondrial respiration rates is also observed [30].

Crocetin and glutamine (a substrate and precursor of ATP) are recognized as two distinct promising pharmacologic agents that may be used as adjunct remedies for fluid and blood resuscitation. Both agents significantly improve post-shock restoration of cellular energy stores and reduce cell apoptosis. It is suggested that prevention of mitochondrial damage may be involved in the mechanism(s) of this action [31, 32]. These studies imply the similar effects of crocetin and glutamine when given during resuscitation, but contain no information about the difference between their action times.

However, the oxygen diffusivity-enhancing property of crocetin occurs over a relatively narrow concentration range. TSC also enhances oxygen consumption and survival rate in animals after hemorrhage. Intravenous administration of TSC is able to improve hemodynamic status, restore arterial blood pressure, lessen tachycardia, reduce plasma lactate level, and prolong survival in hemorrhagic rats [33, 34]. In rats subjected to combined traumatic brain injury and hemorrhagic shock, TSC treatment can cause a rapid but transient improvement in hemodynamics and oxygen kinetics, a long-term impact on metabolic profile, and a significant reduction in mortality [11].

As routine fluid resuscitation therapy for hemorrhagic shock, timely infusion of large volumes of electrolyte solution are required. TSC may offer a novel resuscitation method in which delayed infusion is allowed, and this may reduce the required infusion volume and result in a greater survival rate [35]. The following reports support the efficacy of this mode of therapy. The therapeutic effects of TSC and 100% oxygen are similar when applied in the same hemorrhagic model, but oxygen therapy is only effective when given immediately after hemorrhage while TSC acts differently. When injected 20 min posterior to the end of hemorrhage in rats bled of 60% of their total blood volume and then repeated four times, TSC maintains the ability to restore blood pressure and other biological parameters. This treatment also prevents an increase in hepatic transaminases after hemorrhage, showing a protective action on liver damage secondary to trauma. When an additional bleeding is induced 10 min later, the majority of TSC-treated animals still survive without subsequent fluid infusion [36].

Besides the oxygen diffusivity-enhancing property, studies on other mechanism(s) involved in the beneficial effects of crocetin/TSC in the treatment of hemorrhagic shock are relatively lacking. It has been found that TSC can lower TNF-α level in the liver and spleen and IL-10 level in the spleen in hemorrhagic rats [37]. The impact on the release of the inflammatory cytokines may also be involved in the modes of TSC's positive action in hemorrhagic shock, because these cytokines are implicated in mortality and tissue damage. Accordingly, the effect of crocetin on cytokines in other diseases may be a fascinating research filed.

On the other hand, due to the high reactivity of TSC, its action is correspondingly brief after acute

administration. Noticeably, several studies have observed that the beneficial impact of TSC in hypoxemic status is rapid but transient and that periodically repeated administration of the agent is necessary to maintain its efficacy during hemorrhagic shock [11, 36, 37]. Although repeated doses can conquer the shortcoming, it is rather inconvenient for application and the suitable administration interval and dose are hard to determine.

Some authors have reported negative results for the oxygen diffusivity-enhancing property of crocetin and TSC [38-41]. These observations imply that either plasma oxygen diffusivity is unaffected by the agents in these situations or plasma diffusion of oxygen is not rate limiting in the employed animal models. Further, whether or not the doses of the agents are adequate is also undetermined. Despite the existence of some defects, these papers question the efficacy of crocetin/TSC in some acute situations.

Taken together, crocetin/TSC is a promising treatment for hemorrhagic shock and accompanied disorders with the advantages of rapid and definite action. The mechanism(s) involved is mainly related to the increased oxygen diffusion. However, the short action of crocetin/TSC is undoubtedly an obstacle to acute treatment of hemorrhagic shock. In addition to further assessment of the value of repeated doses of the agents, novel drug delivery systems need to be developed to prolong action time after acute administration.

Cancer: The possibility that saffron and its ingredients may inhibit the occurrence of cancer has received considerable attention because chemoprevention is regarded as a promising strategy for cancer prevention [42]. Much evidence indicates that crocetin and crocin have anti-carcinogenic and anti-tumor activities.

Crocetin was reported early on to decrease tumor numbers and retard tumor onset when tested on animal tumors [43]. Later, the inhibitory effect of crocetin on the growth and development of rat C-6 glioma cells were observed *in vitro* and *in vivo* successively [44, 45]. It has also shown that crocetin is capable of inhibiting cell proliferation and enhancing differentiation of HL-60 cells [46].

Chemoprevention studies in rats have shown crocetin to be effective against chemical carcinogen-induced toxicity or carcinogenesis. Crocetin reduces aflatoxin B1 (AFB1) toxicity and AFB1-DNA adduct formation in C3H10T1/2 cells and in rat liver with consistent elevation in glutathione (GSH) levels and the activities of GSH S-transferase (GST) and GSH-peroxidase (GSH-Px) [47-49]. Besides, crocetin is beneficial in preventing oxidative damage in rat hepatocytes [50]. Similarly, crocetin inhibits the benzo(a)pyrene (BP)-induced genotoxic effect and neoplastic transformation in C3H1OT1/2 cells, which is proposed to be due to a mechanism that increases the activity of GST and decreases the formation of a BP-DNA adduct [51]. Crocetin is also a potent inhibitor of tumor promotion induced by 12-O-tetradecanoylphorbol-13-acetate (TPA) in mouse skin. The agent represses TPA-induced epidermal hyperplasia and expressions of oncoproteins including c-Jun, c-Fos and c-Myc in the suprabasal layer of epidermis and the muscle layer of dermis. The authors suggest that crocetin suppressed TPA-induced skin carcinogenesis, probably through its antioxidant property, leading in turn to a reduction in TPA-induced expressions of oncoproteins [52]. *In vitro*, crocetin significantly inhibits TPA-induced translocation of protein kinase C (PKC) from the cytosolic fraction to the particulate fraction, phosphorylation of cellular proteins, and c-jun and c-fos gene expression in mouse fibroblast NIH/3T3 cells [53]. In addition, local application of crocetin markedly restricts TPA-induced promotion of skin tumors, and hyperplasia and edema initiated in mouse ear with benzo[a]pyrene (BP). Simultaneously, the induction of epidermal ornithine decarboxylase activity and the generation of hydrogen peroxide and myeloperoxidase caused by TPA are suppressed by crocetin [54]. 4-(Methylnitrosamino)-1-(3-pyridyl)-butanone (NNK) is a tobacco-specific nitrosamino possessing carcinogenic potential. Crocetin has been found to reduce consumed NNK in the body indicated by an increase in the formation of its major metabolite via carbonyl reduction by cytochrome P450 enzymes [55].

In vitro, crocetin causes a dose-dependent inhibition of nucleic acid and protein synthesis in malignant human cells including HeLa (cervical epitheloid carcinoma), A549 (lung adenocarcinoma) and VA13 (SV-40 transformed fetal lung fibroblast) cells. In this study, DNA and RNA synthesis in isolated nuclei and the activity of RNA polymerase II are also suppressed by crocetin [56]. The agent also inhibits the growth of the malignant cells and causes evident damage of human rhabdomyosarcoma (RD) cells [57].

In addition to the potential application in chemoprevention, crocetin is expected to alleviate the toxicity caused by cancer chemotherapy. Crocetin may ameliorate cyclophosphamide (CX)-induced bladder toxicity without altering its antitumor activity. This property of crocetin is presumed to be associated with its antioxidative activity which allows the agent to trap and scavenge free radicals during the detoxification process [58].

Nevertheless, negative results related to anti-carcinogenic and anti-tumor activities of crocetin have also been shown in some studies. Interestingly, although crocetin was reported to exhibit a cytotoxic activity on HeLa cells, it was shown later that crocetin had no cytotoxic effect. In the later study, crocin was demonstrated to inhibit the growth of this type of tumor cells [59]. Crocetin slightly slows the development of skin tumors induced by application of dimethylbenz[a]anthracene and croton oil to hairless mice. No definite effect of this agent is observed on preventing the development of tumors induced by UV-B radiation [60]. Besides, crocetin has no inhibitory effect on the formation of transformed rat tracheal epithelial cell colonies induced by BP [61].

The literature also reports that crocin exhibits suppresses cancer. Long-term subcutaneous injection of high-dose crocin inhibits tumor growth and enhances the life span of female rats with colon cancer without obvious toxic effects. *In vitro* assays indicate a potent cytotoxic effect of crocin on adenocarcinoma cells [62]. It is reported that crocin is more potent than other crocetin esters in the inhibition of early antigen expression. Crocin also inhibits early tumor antigen expression of adenovirus-infected cells but is ineffective for the reversal of multidrug resistance of mouse lymphoma cells [63].

These results indicate that these agents possess potential as cancer chemopreventive agents against tumor promotion. Compared with vitamin A, their toxicity may be much lower, and, thus, better patient tolerance is expected. However, the types of cancer against which the use of crocetin and crocin are effective should be determined because of the presented negative effects. Moreover, it is known that the findings derived from *in vitro* studies are not consequentially achieved *in vivo*. In particular, the high concentrations of the agents used *in vitro* may be difficult to obtain *in vivo*. On account of the relative lack of related observations *in vivo*, the value of crocetin and crocin in the field of chemoprevention is still to be verified by stricter *in vivo* experiments.

The exact mechanisms of anti-carcinogenic effects of crocetin and crocin are not fully understood. Due to its lipid solubility, crocetin may serve as a membrane-associated strong antioxidant to protect cells from the injuries of free radical chain reactions [2]. Other proposed mechanisms possibly associated with their antioxidant properties include the inhibitory action of the agents on oncoproteins expression, cellular enzymes, PKC translocation, DNA and RNA synthesis, and phosphorylation of cellular proteins. In addition, the intervention of the agents in the metabolism of chemical carcinogens is also an interesting research topic.

Cerebral disorders: Saffron extract or its constituents, crocetin and crocin, may be useful as a preventive treatment for neurodegenerative disorders of the central nervous system.

Neurobiological studies have demonstrated that crocin positively affects learning and memory in animals. Crocin can antagonize the suppressive effect of ethanol on *in vivo* and *in vitro* hippocampal long-term potentiation (a form of activity-dependent synaptic plasticity that may underlie learning and memory). Interestingly, the authors suggest that gentiobioses attached to the unsaturated fatty acid chain are important for this biological activity of crocin [64, 65]. It has been shown that crocin specifically antagonizes the inhibitory effect of ethanol on N-methyl-d-aspartate receptor-mediated responses in hippocampal neurons [66]. Another study implies that crocin, instead of crocetin, is responsible for the antagonizing effect of saffron extract on ethanol-induced impairments of learning behaviors and inhibition of long-term potentiation in the hippocampus in mice [67].

In vitro, crocin inhibits neuronal cell death induced by both internal and external apoptotic stimuli. It has been shown that crocin inhibits apoptosis of neuronally differentiated pheochromocytoma (PC-12) cells by opposing the effects of TNF-α and daunorubicin [68]. Treatment with crocin manages serum/glucose deprivation-induced abnormalities including peroxidation of the cell membrane lipids, decreased intercellular SOD activity and GSH level, ceramide production, and the activation of c-jun

kinase (JNK) and caspase-8 pathway in PC-12 cells. Moreover, some of these antioxidant effects of crocin are more effective than those of α-tocopherol at the same concentration [69, 70].

In rats subjected to craniotomy, intravenous infusion of TSC significantly increases brain tissue oxygen delivery and ameliorates hypoxic/ischemic insults in neurological disorders [71]. A recently published study has revealed that crocetin prevents 6-hydroxydopamine-induced Parkinsonism and has therapeutic potential in combating severe neurological disorder and neuronal death in rats [72].

Based on these reports, crocin and crocetin/TSC possess the ability as potent antioxidants to oppose oxidative injury in neurons. The question remains whether the agents can reach their effective concentrations in the brain, which is dependent on the route of administration. For crocin, intracerebroventricular administration may be necessary to achieve the neuro-protective effect of crocin *in vivo*.

Ocular diseases: As a traditional medicine, saffron has been demonstrated to be useful in the treatment of ocular diseases [2]. Crocetin and crocin, as the active ingredients of saffron, may help prevent or potentially alleviate the problems in ocular microcirculation.

It is well known that the development of ischemic retinopathy and age-related macular degeneration, two major ocular diseases that cause blindness, is partially due to a reduction in local blood flow. Consequently, enhancement of blood flow in the retina or choroid is a practical approach to improve the oxygen and nutrient supply of retinal structures. Crocin significantly increases the blood flow in the retina and choroids, and expedites retinal function recovery. However, crocin is less effective than the monosaccharide analogs of crocin [73]. This is probably associated with the different pharmacokinetic properties of crocin analogues. Another study indicates that crocetin and crocin (i.p.) both significantly enhance the blood flow at the site of retinal ischemia induced by ligation of the central retinal artery in rat eyes [74]. Crocetin has also been shown capable of preventing experimental elevation of aqueous flare in pigmented rabbits [75].

Other diseases: More recently, our laboratory has demonstrated that crocetin may improve insulin resistance induced by low-dose dexmethasone [76] and high fructose consumption in rats [unpublished data]. This pharmacological action may partially explain the advantageous impact of crocetin on the cardiovascular system, and stimulate the discovery of more beneficial effects of this compound, because insulin resistance is a common feature of atherogenic diseases and disposes the affected individuals to develop various diseases such as hypertension, dyslipidemia, cardiovascular problems and type-2 diabetes mellitus [77].

Conclusions: Crocetin and crocin are valuable naturally occurring carotenoids with extensive pharmacological effects. Much research has concentrated on their biological and pharmacological properties, but comprehensive pharmacokinetic properties of both agents, especially crocin, deserve further research because it is the most important ingredient and is a standard control of saffron. To study the mechanism(s) involved in the preventive effects of orally administered crocin on dyslipidemia and atherosclerosis, its influence on the absorption of consumed lipids in the intestinal tract and the role of its metabolite crocetin should be clarified.

Based on the known data, the most promising use of crocetin and crocin are for protecting against dyslipidemia and atherosclerosis. Although considerable evidence has suggested that both phytochemicals possess anti-carcinogenic and anti-tumor activities, *in vivo* studies are relatively inadequate. This emphasizes the need for detailed *in vivo* examination of multiple tissues in long-term intervention trials in the field of cancer chemoprevention. Besides, negative observations have also been put forward. Consequently, their use as chemopreventive agents await affirmation. Crocetin is beneficial in the prevention of myocardial ischemia, but its efficacy as acute treatment after the onset of myocardial ischemia is unclear. It is noteworthy that the high reactivity of the substances may limit their application, especially for acute conditions including hemorrhagic shock. Repeated administration of crocetin/TSC may be a necessary step to maintain the efficacy during resuscitation. However, administering repeated doses is not a preferred method, so new drug delivery systems for TSC should be developed. If the agents are used for cerebral disorders, the administration route should be considered in order to obtain their effective concentrations in the brain. However, all these experiments only have been performed on animals

and *in vitro*, so human studies are needed to define the efficacy of the agents in various therapeutic fields.

The primary biochemical basis for the modes of action of crocetin and crocin has been established as their potent antioxidant activities. Based on the fact that oxidative stress plays important roles in many diseases, it can be speculated that more beneficial effects of these agents may be detected with further studies. More research is required for understanding the precise action mechanisms of the agents, especially at the molecular level. The developing knowledge on crocetin and crocin is expected to infuse new ideas in the field of medicinal natural product research in the future.

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