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Review

Saffron: a natural product with potential pharmaceutical applications

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Abstract

Objectives Recently, a great deal of interest has been developed to isolate and investigate novel bioactive components from natural resources with health beneficial effects. Saffron is the dried stigma of Crocus sativus L. and has been used for centuries in traditional medicine mainly for its healing properties, as well as for the treatment of various pathological conditions. Objectives of the present review are to unravel its therapeutic properties and investigate the potential applications of saffron in contemporary therapy of a wide spectrum of diseases and summarize previous and current evidence regarding the biological/pharmacological activities of saffron and its active ingredients and their possible therapeutic uses.

Key findings Recent phytochemistry and pharmacological experiments have indicated that crocin and safranal, the major active ingredients of saffron, exert important actions, such as antioxidant, anti-tumor, anti-diabetic, anti-inflammatory and anti-atherosclerotic. Unfortunately, the vast majority of those data derive from in vitro studies, whereas a limited number of in vivo experiments support the aforementioned effects. In addition to studies with mechanistic implications, very few clinical trials provide preliminary evidence of saffron potentiality to alleviate depression and increase cognitive function in patients with Alzheimer's disease.

Summary The history and structural features of saffron constituents are given in the first part of the review, followed by a comprehensive and critical presentation of the published preclinical and clinical studies and review papers on the pharmacology and possible therapeutic uses of saffron and its main active components crocin and safranal.

Introduction

Saffron is the dried stigma of *Crocus sativus* L., fam. Iridaceae. It is a perennial bulb found mainly in Europe and Asia and is widely cultivated in Iran, Mediterranean countries (especially in the area of Kozani in Greece) and India.[1,2] The plant's stigmata have been used for centuries in traditional medicine for the treatment of various illnesses, but also have a lot of industrial applications, for example as a dye or a colouring agent, preservative and antioxidant.[3] Saffron is known as the most expensive spice in the world and has been named as 'Red Gold' in Iran. The world's total annual saffron production is estimated at 205 tons, and over 80% of this harvest originated from Iran.[4] Within Europe, Spain is generally believed and seems to be a significant source of cultivated *C. sativus* L., based on an annual export of approximately 60 tons. However, because the collection of saffron is a highly expensive and time-consuming procedure, the bulk of saffron re-exported from Spain is in fact of Iranian and Moroccan origin.[5] Consequently, the largest European saffron producer is currently Greece, with 4.5 tons per year, a number that by far surpasses the estimated Spanish production.

Nowadays, saffron is mainly used for its antioxidant properties and can be found in various food supplements. Saffron tastes bitter and contributes to a luminous yellow–orange colouring to foods. Because of the unusual taste and

colouring, saffron is widely used in Persian, Arab, Central Asian, European, Indian, Moroccan and Cornish cuisines. Confectionaries and liquors also often include saffron.[6] Regarding saffron's pharmacological properties and possible therapeutic uses, several reviews and minireview papers have been published concerning the biological activity of saffron and its constituents.[1,3,7–14] In parallel, the possible uses of saffron and its active constituents lead to an increasing number of research studies, some of which have drawn some very useful and intriguing results of active ingredients of saffron, especially against malignancies, cardiovascular and Alzheimer's disease.[15–22] In this minireview, we aim to summarize and critically address previous and current evidence regarding the biological/pharmacological activities of saffron and its active ingredients and their possible therapeutic uses. The history and structural features of saffron constituents are given in the first part of the review, followed by a comprehensive and critical presentation of the published preclinical and clinical studies and review papers on the pharmacology and potential therapeutic applications of saffron and its main active components.

History

According to ancient Greek mythology, Krokos (Crocus) was a friend of god Hermes. One day, as they were playing, Hermes killed Krokos by mistake by hitting his head. Three blood drops from his head fell on the top of the flower of the plant and the stigmata were created. Since then, the plant obtained the name Krokos (Crocus).[23]

Taking into consideration the literature found and studied, there are three typical uses for saffron. In Mesopotamia, it was widely used for curative purposes. Phoenicians used saffron as a precious dying material and as a trade material with the Assyrian King, Ashur-nasir-pal. Finally, in ancient Rome, it was imported from Cilicia and was used as a treatment and dye, as well as in perfumes and ointments.[8]

Generally, the history of drug uses and especially those deriving from plants is very similar. Drugs were used for specific healing purposes and, later on, for treating many types of diseases, taking into consideration that the diseases at those times were very specific and widely spread. In fact, the efficacy of drugs for a specific illness resulted in the spread of their use to other disorders in the early phase of medical practice, when medicine was essentially a magic ritual. Thereafter, many drugs were also used as a food dressing, which meant that substances suitable for conservation treatments could prevent diseases as well. Also, many drugs were exploited for common uses, such as cloth dye and beauty treatments.[8] The healing properties ascribed to saffron in ancient times are found in *Materia Medica*, by Pedanio Dioscorides, a Greek medical practitioner of the first century A.D. In the Middle Ages and in the following centuries, the commerce of saffron was subjected to very drastic rules because of its rarity and difficult way of cultivating and collecting. It was even the cause of the so-called 'Saffron War' in 1374. In that period, Basle (Switzerland) was the centre of the trade in saffron, which principally came from Italy. The saffron blossom even became part of Basle's coat of arms.

Dezani, in his treatise regarding pharmacognosy,[6,24] explains that in Nuremberg, between 1449 and 1495, three persons were condemned to the pyre for the crime of adulterating saffron. A woman, who was their accomplice, was spared from the pyre out of compassion.

The *Dizionario di Commercio*, written by the Savary brothers, states that 'saffron is a foodstuff used in the kitchen; it is used by painters to make miniatures; it gives a wonderful colour to dyes. Physicians use it with great advantage to treat many diseases.' However, the 1895 Genoa edition of Villavecchia's *Dizionario di Merciologia* reports, 'Saffron is commonly used to dye foods yellow; once, it was used in medicine too; nowadays it is only used in pharmacy to produce laudanum'.[25] Saffron has also been used as a fabric dye, particularly in China and India, and in perfumery.[3]

Modern medicine has rediscovered saffron indicating several therapeutic effects and pharmaceutical applications. As far as discussed in the following sections, saffron seems to exert anticarcinogenic mutation-preventing, immunomodulating and antioxidant properties.[26–29] Recent studies have shown the beneficial effects of saffron in depression, premenstrual syndrome and Alzheimer's disease.[30–33]

Active constituents and chemical properties

The basic composition of saffron is 14–16% water, 11–13% nitrogenous matters, 12–15% sugars, 41–44% extract soluble, 0.6–0.9% volatile oil, 4–5% fibres and 4–6% total ashes. Saffron contains two important vitamins: riboflavin and thiamine and also small quantities of β-carotene. Riboflavin content range from 56 to 138 μg/g and is the highest to be found in any food. Thiamine concentration levels range from 0.7 to 4 μg/g, which are average values found in vegetables.[34] Also, in the petroleum ether extract from the bulbs, the essential fatty acids, linoleic and linolenic are found. Sterols are identified (campesterol, stigmasterol and β-sitosteral) as well as ursolic, oleanolic, palmitic, palmitoleic and oleic acids.[35] Vitamins and essential fatty acid contents are very poor when compared with the quantity of saffron that must be ingested. Consequently, saffron is not to be used for the extraction of such useful substances.

To explain the chemical and pharmacological properties of saffron, the obtained data from the analysis of red stigmatic lobes of the *C. sativus* flower should be used. According to these data, the plant contains three main metabolites: (1) picrocrocins, which are the main substances responsible for saffron's bitter taste; (2) safranal, which is the volatile oil responsible for the characteristic saffron aroma;[36–38] (3) crocins, which are saffron-coloured compounds (unusual water-soluble carotenoids due to their high glycosyl contents).

'Picrocrocin' ($C_6H_{26}O_{7)}$ is a bitter-tasting substance discovered by Kajser;[39] it is present in the plant's stigmata in the amount of 4%. It is a glycoside that due to acids and alkali cracks into a molecule of glucose and into an aglycon named 4-hydroxy-b-cyclocitral. The aglycon loses a molecule of water and easily turns into the volatile compound known as safranal or dheydro-b-cyclocitral. This can be easily observed in its structural formula.[40]

'Safranal', which is 70% of the volatile fraction,[41] is mainly responsible for the aroma of saffron. There is no smell on the fresh stigmata. The distinctive smell appears during the drying and storage stage of saffron because picrocrocin is resistant to demolition. Safranal is a cyclical terpenic aldehyde (2,6,6-trimethyl-1,3-cyclohexadien-1-carboxaldehyde) with a brute formula,$C_{10}H_{14}O$ (m.w. = 150; e.p. 70 ^{O}C/1 mm). Its name comes from Kuhn and Winterstein,[42] who were the first researchers to obtain it from picrocrocin hydrolyzation (hydrolysis).

'Crocin', is the most important glycoside carotenoid that gives saffron its characteristic colour. More precisely, it is a crocetin digentiobiose ester ($C_{20}H_{24}O_4$), with a beta-shaped glycosidic bond able to be hydrolyzed by emulsine (β-glucosidase). In saffron, there can be found more types of esters such as monogentiobioside ester and monoglucoside ester[43] and a stereoisomer of crocin, *13-cis-crocin*.[44]

'Crocetin', belongs to the large family of natural dyes known as carotenoids, but it lacks the provitamin function. The majority of constituents of this class are hydrocarbons, the general formula of which is $C_{40}H_{56}$, or oxygenated derivatives. However, there is a small group of carotenoids having carboxylic and acid groups that cannot be included in the general chemical structure and definition. Crocetin (the aglycon of crocin), 8,8-diapo-8,8-carotenoic acid, belongs to this small group. It is characterized by a diterpenic and symmetrical structure with seven double bonds and four methyl groups. Its elementary composition is $C_{20}H_{24}O_4$ and its molecular weight is 328.4. It crystallizes into red needles with a melting point of 285°C, whereas in solution, it has a yellow colour. It is slightly soluble in basic aqueous solution (20 μM at pH 8), but it is very soluble in organic bases, such as, pyridine. If the concentration overcomes its solubility in aqueous media, a yellow precipitate is formed. Crocetin is mainly referred for its antioxidant properties (because of its chemical structure) and is the most up-to-date saffron constituent under continuous study, as the metabolite of crocin.

In Figure 1, the chemical structures of the main saffron components are shown. Table 1 also includes all possible components found in small or large amounts in the plant.

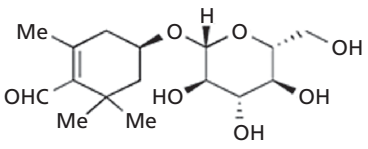
Pharmacological Properties and Potential Therapeutic Applications

Saffron and its constituents are known for a quite large number of possible uses and actions. The pharmacological properties of saffron components, like safranal, crocin and crocetin, are due to their chemical structure. The most important pharmaco-active properties of saffron were reported in studies based on in-vitro experiments that appeared in the Chemical Abstracts between 1925 and 1999. However, those old reports were observational and their clinical relevance remains questionable. For instance, saffron has been proposed as one of the modifiers of the gastrointestinal chemical function. Through this action it may stimulate appetite and prevent gastrointestinal atonia.[45] Saffron has also shown to act therapeutically on the female genitals. Regarding its major components, safranal may be useful in treating respiratory, mostly chronic bronchitis. Because of its extensive distribution to the lungs, safranal sedates coughing, by acting as an anaesthetic on the vagal nerves of the alveoli.[8] Crocin has been proposed for painful dysmenorrhoea relief because it may decrease uterine contractions. Picrocrocin seems to have a sedative effect on spasms and lumbar pains.[46] However, the most remarkable effects of saffron have been attributed to crocetin, because it is a substance able to increase the speed of oxygen transport and diffusivity, both *in vivo* and *in vitro*. This ability to transport oxygen makes crocetin useful therapeutic candidate in various situations, such as atherosclerosis, alveolar hypoxia, haemorrhages, fermentation and cell reproduction, arthritis, tumours, etc.[47] Table 2 summarizes recent advances on the pharmacological and possible therapeutic applications of saffron and its constituents, while in the following sections their effects on various pathological conditions are discussed in details. Compared with old studies, the most recent researches have tried to provide more mechanistic explanations of the saffron's therapeutic effects.

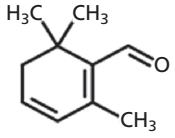
Effect of saffron on psychological disorders and central nervous system (CNS)

Antidepressant and anxiolytic properties

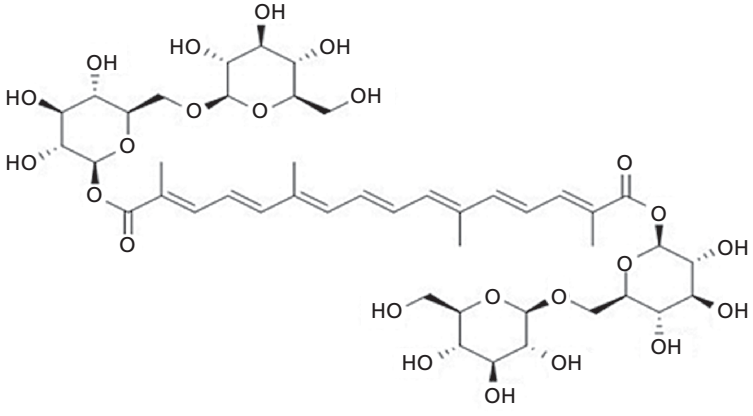
Aqueous and ethanolic extracts of saffron stigmas were found to have antidepressant effect in mice, mainly



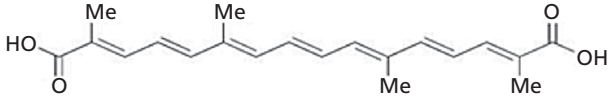
picrocrocin



safranal



crocin



crocetin

Figure 1 Main saffron components.

attributed to safranal and crocin acting by inhibition of dopamine, norepinephrine and serotonine uptake.[68,69] These results were further confirmed by Wang *et al.*[70] who have shown, using behavioural models, that the antidepressant effect of aqueous saffron extract is attribute to crocin 1 and 2. Similarly, in animal model, *C. sativus* L. stigmas are found to yield anxiolytic and hypnotic effects. More specifically, Pitsikas *et al.*[71] have shown that crocin at a dose of 50 mg/kg has positive effect on mice behaviour while possessing anxiolytic effect in rats. Hosseinzadeh and Noraei[53] demonstrated that specifically safranal can bind to some benzodiazepines subtypes (BZ1, BZ2, BZ3) and ameliorate insomnia phenomena in mice. However, safranal slightly influenced muscle relaxation or motor imbalance of those mice.

In clinical studies, the use of saffron extract at doses of 20–30 mg/day twice daily for the treatment of mild to moderate depression has been compared with chemically synthesized molecules, such as fluoxetine (20 mg/day twice daily)[31] and imipramine (100 mg/day three times daily).[30,48,49] The comparative evaluation revealed that saffron was equally effective as chemically synthesized drugs, in mild or moderate depression (antidepressant action) and epilepsy (anticonvulsant action) without causing their side effects. However, clinical trials regarding dose-related toxicity and adverse effects of saffron extract use as alternative in the treatment of depression have not been conducted.

Morphine withdrawal syndrome

Animal studies have shown that some saffron extracts and its constituents crocin and safranal considerably suppress the so-called 'withdrawal syndrome' in morphine-treated

Table 1 Important components of *Crocus sativus* L. stigmata[2]

Family (chemical structure)	Name	Other names	Further information
Carotenoids	Crocetin	α-crocetin or trans-crocetin isomer or crocetin I crocetin II or 13-cis-crocetin isomer β-crocetin or monomethyl ester of crocetin	
	Methyl-crocetin	γ-crocetin or dimethyl ester of crocetin crocin-1 or α-crocin or digentiobioside crocetin	
	Dimethyl-crocetin	crocin-2 or tricrocin or gentioglucoside crocetin crocin-3 or gentiobioside crocetin	
	Crocins	crocin-4 or glucoside crocetin crocin-5 or dicrocin or diglucoside crocetin	The major active constituent of saffron and unique water-soluble carotenoid in nature
	α-carotene β-carotene lycopene zeaxanthin mangicrocin xanthone-carotenoid glycosidic conjugate		
Monoterpene aldehydes	Picrocrocin		In fresh saffron, these substances exist as stable components
	Safranal	deglycosylated derivative of picrocrocin or 2,6,6-trimethyl-1,3-cyclohexadien-1-carboxaldehyde or dehydro-*β*-cyclocitral	
	Isomer of safranal	2,6,6-trimethyl-2-cyclohexen-1,4-dione	
	Isomer of safranal	2,6,6-trimethyl-1,4-cyclohexadiene-1-carboxaldehyde	
Monoterpenoids	Crocusatin D Crocusatin F	Crocusatin 4b	
	Crocusatin G Crocusatin H Crocusatin E		Optically active, colourless oil
	Crocusatin I	Crocusatin 4a	
Isophorones	Isophorone	3,5,5-trimethyl-2-cyclohexen-1-one	For saffron origin differentiation
	Isomer of isophorone	3,5,5-trimethyl-3-cyclohexen-1-one	
Flavonoids		kaempferol-3-sophoroside-7-glucoside kaempferol-3,7,4′-triglucoside kaempferol tetrahexoside kaempferol-3-dihexoside	

mice.[50,51] More specifically, Imenshahidi *et al*. reported that pretreatment of mice with intraperitoneal injection of crocin at a dose of 600 mg/kg for 4 days and 30 min before morphine administration, blocked the acquisition of morphine conditioned place preference (CPP).[51] Similarly, Hosseinzadeh and Jahanian have shown that both aqueous and higher doses of ethanolic saffron extracts suppressed morphine withdrawal syndrome in mice by reducing the number of jumping episodes. Interestingly, they also reported that the inhibitory effect of crocin alone on locomotor parameters was significantly lower than that of the aqueous extract, indicating a more specific effect of crocin on opioid system with reduced adverse effect rate.[50]

Learning and memory effects: impact on Alzheimer's and Parkinson's diseases

Concerning spatial cognitive abilities after chronic cerebral hypoperfusion, the administration of saffron extract or crocin solution significantly improved memory skills in rats compared with controls.[52] Several studies have been published by Abe's Research Group indicating the favourable effect of saffron extract, crocin and crocetin on memory and learning skills, in ethanol-induced learning behaviour impairments in swine.[72–75] These experimental results suggest the beneficial effect of oral administration of saffron and its ingredients (especially crocin) on neurodegenerative

Table 2 Major studies realized including saffron and its constituents for the treatment of different diseases

Diseases	Type of study	Intervention	Results
Depression	Clinical study: 6-week, double-blind, single-centre, placebo-controlled, randomized trial.[30]	Patients with major depression (Hamilton Rating Scale score ≥ 18), free of psychotropic medications and aged 18–55 years old.	*Crocus sativus* (capsule 30 mg) produced a significantly better outcome than the placebo. No differences in side effects.
	Clinical study: 6-week, double-blind, single-centre, randomized trial.[48]	30 adult patients with mild to moderate depression (Hamilton Rating Scale score ≥ 18), randomly assigned to receive capsule of saffron 30 mg/day or capsule of imipramine 100 mg/day.	Equivalent efficacy and no significant differences in side effects.
	Clinical study: 8-week, double-blind, randomized trial.[49]	40 adult patients with mild to moderate depression (Hamilton Rating Scale: 18–25) were randomized to *C. sativus* 15-mg bid versus fluoxetine 10-mg bid.	Equivalent efficacy and no significant differences in side effects.
Withdrawal syndrome	Experimental animal model: Razi male mice.[50]	Each mouse received 10 doses of morphine within 4 days. After morphine cessation and naloxone administration they were divided to receive one of the following: normal saline, clonidine (0.3 mg/kg), variety of doses of aqueous extracts of saffron (80, 160, 320 mg/kg), variety of doses of ethanolic extracts of saffron (200, 400, 800 mg/kg, respectively).	The aqueous and ethanolic extracts of saffron attenuated the severity of precipitated morphine withdrawal.
	Experimental animal model: mice.[51]	Male NMRI mice (25–30 g) were housed in plastic cages in an animal room maintained at 21 ± 2°C on a 12-h dark cycle. Animals had free access to water and food except during behavioural tests. Each mouse was used only once, and each treatment group consisted of seven animals. Animals were injected intraperitoneally with crocin (200, 400, 600 mg/kg) and/or morphine sulphate (40 mg/kg), dissolved in physiological saline (NaCl 0.9%), in a volume of 0.1 ml/10 g. Control group were injected with physiological saline	Subcutaneous administration of morphine (40 mg/kg for 4 days) produced place preference. Intraperitoneal administration of crocin (600 mg/kg for 4 days) 30 min before the morphine administration decreased the acquisition of morphine CPP. In other groups of animals, following extinction of a place preference induced by morphine (40 mg/kg), single administration of morphine (10 mg/kg) reinstated the place reference. Crocin (400 and 600 mg/kg) 30 min before this priming dose of morphine blocked morphine-induced reinstatement of place preference. These results showed that crocin can reduce the acquisition and reinstatement of morphine-induced conditioned place preference
Spatial memory	Experimental animal model: male Wistar rats.[52]	Surgically induced cerebral ischaemia and administration of different doses of either: (1) aqueous solution of crocin, (2) hydroalcohol extract of saffron intraperitoneally (i.p.), (3) saline. Evaluation of spatial memory performances using a water maze with a hidden platform.	Treatment with saffron extract or crocin solution significantly improved memory skills compared to the control group.
Anxiety and insomnia	Experimental animal model: Razi male mice.[53]	Assignment into 13 groups to receive: (1) normal saline, (2) paraffin, (3) diazepam (3 mg/kg), (4–7) saffron aqueous extracts at different doses (56, 80, 320 and 560 mg/kg, respectively), (8–10) crocin at different doses (50, 200 and 600 mg/kg), (11–13), 11–13) safranal at different doses (0.05, 0.15, 0.35 ml/kg, respectively).	The aqueous extract of saffron and especially safranal, but not crocin, yielded anxiolytic and hypnotic effects. The anxiolytic effect of safranal was dose dependent.

Table 2 (*Continued*)

Diseases	Type of study	Intervention	Results
Alzheimer's disease	Clinical study: 16-week, double-blind study, randomized, placebo-controlled study.[54]	40 patients with mild to moderate Alzheimer's disease randomized to receive: capsule saffron (15 mg twice per day) or (two capsules placebo per day).	Saffron produced a significantly better outcome on cognitive function than placebo, while the side effects were similar.
	Clinical study: 22-week, prospective, double-blind, randomized study.[33]	44 patients with mild to moderate Alzheimer's disease randomly assigned to receive capsules saffron (15-mg bid) or donepezil (5-mg bid).	Saffron extract as effective as donezepril in Alzheimer's disease treatment and well tolerated.
Parkinson's disease	Experimental animal model: male Wistar rats.[55]	Evaluation of the pretreatment effect of crocetin in three groups: (1) 6-hydroxydopamine (OHDA)-induced neurotoxicity [antioxidant enzymes (GSH) and thiobarbituric acid reactive substance (TBARS)], (2) OHDA-induced neurotoxicity [dopamine (DA) and its metabolites], (3) histopathological changes on substantia nigra.	Neuroprotective role of crocetin in a 6-OHDA model of rat assessed by biochemical, behavioural and histopathological findings. Crocetin can afford neuroprotection by inhibiting neurodegeneration.
Hyperglycaemia – Glucose uptake/ metabolism	Experimental animal model: streptozotocin-induced diabetic rats.[56]	Crocin administered intraperitoneally at doses of 15, 30 and 60 mg/kg for 6 weeks. The levels of thiobarbituric acid reactive substance (TBARS) and total thiol (SH) groups were measured in the liver and kidney at the end.	The high dose (60 mg/kg) significantly reduced the blood glucose level, as well the total thiol concentrations in the liver and kidney of diabetic animals. Crocin (30 and 60 mg/kg) lowered lipid peroxidation levels in these organs.
	Experimental animal model: neonatal Wistar rats (2–5 days old).[57]	Randomization into five groups for 5 months: three groups received STZ (90 mg/kg body weight i.p.), whereas two of them were treated with crocin (50, 100 mg/kg body weight, respectively). Two other groups served as controls without STZ treatment, while one of them was treated with 100 mg/kg crocin.	Both doses of crocin significantly decreased the levels of serum glucose, advanced glycation end-products, triglycerides, total cholesterol, and LDL and increased the HDL in the diabetic rats.
	Experimental in-vitro model: cell culture lines.[58]	Elucidation of the mechanism of the hypoglycaemic actions of saffron constituents through investigation of the signalling pathways associated with glucose metabolism in C_2C_{12} skeletal muscle cells	Saffron strongly enhanced glucose uptake and the phosphorylation of AMPK/ACC and MAPKs, but not PI 3-kinase /Akt. Co-treatment of saffron and insulin further improved the insulin sensitivity via both insulin-independent (AMPK/ACC and MAPKs) and insulin-dependent (PI 3-kinase/Akt and mTOR) pathways.
Atherosclerosis, myocardial ischaemia, cardioprotection	Experimental animal model: male quails.[59]	6 groups: control (normally fed), model (high-fat diet), crocin (high-fat diet plus crocin in three different doses: 25, 50, 100 mg/kg), Zhibituo (lovastatin treated) for 9 weeks.	Crocin reduced the level of serum lipids and inhibit the formation of aortic plaque. Proposing mechanisms: attenuation of ox-LDL actions leading to less foam cells formation and endothelial cells apoptosis.
	Experimental animal model: Male Wistar albino rats.[60]	Randomly assigned to receive either safranal at different doses (0.1–0.5 ml/kg/day, i.p.) or saline for 14 days. On the 15th day, the left anterior descending coronary artery was ligated for 45 min, followed by 60-min reperfusion	Safranal-induced decreased infarct size, and improved left ventricular functions and overall haemodynamic status of the myocardium. Akt/GSK-3b/eNOS phosphorylation, increased NO bioavailability and suppressed of IKKb/ NF-jB pathway.
	Experimental animal model: ApoE C57BL/6background transgenic mice.[61]	4 groups: control (water for injection administration), saffron administration low dose (30 mg/kg), medium dose (60 mg/kg) and high dose (90 mg/kg) for 4 weeks. In the end, the aorta and heart were removed after euthanasia under deep anaesthesia.	Saffron extract administration induced plaque stability amelioration and better glycaemic control in a dose-dependent manner.

Table 2 (*Continued*)

Diseases	Type of study	Intervention	Results
	Experimental animal model: male Wistar albino rats.[17]	Rats divided into 8 groups: Group 1 (vehicle-control): Rats were administered saline orally (3 ml/kg per day) using intragastric tube for 21 days and on the 20th and 21st day received 0.3-ml saline, s.c. at an interval of 24 h. Groups 2–4 (crocin per se): Animals were treated orally with crocin (5, 10 and 20 mg/kg/day) for a period of 21 days, and on the 20th and 21st day, 0.3-ml saline was administered, s.c. at an interval of 24 h. Group 5 (ISO-control): Rats were administered saline orally (3 ml/kg/day) for 21 days along with ISO (85 mg/kg,s.c., at 24 h interval) on 20th and 21st day. Groups 6–8 (crocin + ISO): Animals were treated with crocin (5, 10 and 20 mg/kg/day) orally for a period of 21 days along with ISO (85 mg/kg,s.c., at 24h interval) on 20th and 21st day The cardiotoxicity was induced by subcutaneous (s.c.) administration of isoproterenol (85 mg/kg) to rats daily for two consecutive days at 24 h interval.	The present results endorse the hypothesis that crocin has cardioprotective potential. Crocin pretreatment improved cardiac functions. Those effects can be attributed to its ability of maintaining redox status, which is disturbed by ISO challenge, via restoration of endogenous antioxidants, controlling lipid peroxide formation and preserving activities of CK-MB, LDH enzymes. Preservation of histoarchitecture of myocyte by crocin pretreatment reconfirms these effects.
Anticancer effects	Experimental animal model: Swiss albino mice.[62]	*Crocus sativus* extract (200 mg/kg) against intraperitoneally transplanted sarcoma-l 80 (S-l BO), Ehrlich ascites carcinoma [EAC] and Dalton's lymphoma ascites (DLA) tumours in mice	The extract increased the life span of S-180, EAC, DLA tumour bearing mice to 111.0%, 83.5% and 112.5%, respectively. It was also cytotoxic to P38B, S-180, EAC and DLA tumour cells *in vitro*.
	Experimental animal model: BD-IX rats.[15]	Groups G1 and G2, designated 'cancer groups', to study the effects of crocin on the progression of colon cancer. Groups G3 and G4, designated 'toxicity groups', to study the cytotoxic effects of crocin *in vitro*. Groups G2 and G4 received a weekly injection of crocin (400 mg/kg). Animals in Groups G1 and G3 received no treatment.	Life span was extended and colon cancer growth was slower in crocin-treated female rats, but not in male rats. *In vitro*, crocin had a potent cytotoxic effect on human and animal adenocarcinoma cells.
	Experimental animal model: Wistar albino rats.[63]	After gastric cancer induction in rats, different concentrations of saffron aqueous extract (SAE) were administered. The stomach tissue was then investigated.	Inhibition of gastric cancer induction in a dose-dependent manner; 20% of cancerous rats treated with higher doses of SAE was completely normal at the end of experiment.
Other actions	Experimental animal model: Wistar rats.[64]	Saffron extract as topical cream was applied 24 h after thermal induced burn wounds, in comparison with sil¬ver sulfadiazine (SSD). Animals were divided into four groups: control, base, saffron (20%) or SSD (1%).	The wound size of saffron group was signifi¬cantly smaller than other groups. Saffron increased re-epithelialization due to the anti-inflammatory and antioxi¬dant effects.
	Experimental animal model: virgin Wistar rats.[65]	Evaluation of the aphrodisiac activities of *Crocus sativus* stigma aqueous extract. The aqueous extract of crocus (80, 160 and 320 mg/kg body wt.), pure crocin (100, 200 and 400 mg/kg body wt.), pure safranal (0.1, 0.2 and 0.4 ml/kg), sildenafil (60 mg/kg – as a positive control) and normal saline were administered intraperitoneal to male rats.	Crocin, at all doses, and the aqueous extract (doses 160 mg/kg, 320 mg/kg) showed ameliorated sexual behaviour. Safranal did not show any aphrodisiac effects.

Table 2 (*Continued*)

Diseases	Type of study	Intervention	Results
	Experimental animal model: Wistar albino rats.[66]	Randomly divided in five groups to receive intraperitoneally: (1) single dose of corn oil 1 ml/kg, (2) hexachlorobutadiene (HCBD) 50 mg/kg), (3–5) safranal at doses of 0.5, 0.25 and 0.1 ml/kg 1 h before HCBD (50 mg/kg) injection. Nephrotoxicity was assessed by urine samples and kidneys analysis.	Safranal at doses of 0.25 and 0.5 ml/kg had a protective effect against HCBD-induced nephrotoxicity in rats.
	Experimental animal model: male albino Swiss–Webster mice.[67]	Ethanolic (14.12% crocin, 15.3% safranal) and aqueous extract (22.5% crocin, 18.4% safranal) of saffron *Crocus sativus* at different doses and its constituents safranal (1, 5 and 10 mg/kg; i.p.) and crocin (1, 5 and 10 mg/kg; i.p.) were administered. Mice were exposed to a trial of electroshock stress for 7 days.	Intraperitoneal administration of crocin and the aqueous extract but not the ethanolic extract significantly reduced the side effects of electroshock stress (anorexic time, weight gain).
	Experimental animal model: 3-month-old Wistar rats.[19]	Rats randomly divided into six experimental groups of 8 as follows: vehicle + vehicle; vehicle + crocins 30 mg/kg; vehicle + crocins 50 mg/kg; [1-(3-chlorophenyl)piperazine hydrochloride] mCPP 0.6 mg/kg + vehicle; mCPP 0.6 mg/kg + crocins 30 mg/kg; and mCPP 0.6 mg/kg + crocins 50 mg/kg	Crocins might alleviate the mCPP-induced excessive self-grooming by an antagonistic action at the 5-HT_{2C} receptor site.

5-HT2C, 5-hydroxytryptamine (serotonin) receptor 2C; AMPK/ACC, AMP-activated protein kinase/acetyl-CoA carboxylase; BD-IX, Berlin Duckrey IX rat strain; CK-MB, Creatine kinase-MB; CPP, Conditioned Place Reference; DA, dopamine; DLA, Dalton's lymphoma ascites; EAC, Ehrlich ascites carcinoma; GSH, antioxidant enzymes; HCBD, hexachlorobutadiene; HDL, high-density lipoprotein; ISO, isoproterenol; LDH, lactase dehydrogenase; LDL, low-density lipoprotein; MAPKs, mitogen-activated protein kinases; mCPP, 1-(3-chlorophenyl) piperazine hydrochloride or meta-chlorophenylpiperazine; NMRI, Naval Medical Research Institute mice strain; OHDA, 6-hydroxydopamine; SAE, saffron aqueous extract; SH, thiol group; SSD, silver sulfadiazine; STZ, streptozotocin; TBARS, thiobarbituric acid reactive substance.

disorders and related memory impairments. The induction of long-term potentiation (LTP) in CA1 and dentate gyrus regions of the hippocampus essentially requires the activation of the N-methyl-d-aspartate (NMDA) type of glutamate receptor. Crocin is likely to prevent ethanol-induced inhibition of hippocampal LTP as an antagonist of the inhibitory effect of ethanol on NMDA receptor.[72,73,75] However, it remains unclear whether crocin acts directly on the NMDA receptor channel complex or indirectly modulates NMDA receptor function.[76] Similarly, saffron extract may also prevent the LTP induced by acetaldehyde (ethanol metabolite).[73]

Furthermore, saffron extract and its components have been tested in Alzheimer's and Parkinson's disease.[33,54,55] Alzheimer's disease is characterized pathologically by deposition of amyloid β-peptide (Aβ) fibrils. Oxidation is thought to promote Aβ fibril formation and deposition. The antioxidant properties of extract of *C. sativus* stigmas and its effect on Aβ1–40 fibrillogenesis were evaluated in double-blind, placebo-controlled study in 46 patients with Alzheimer's disease.[54]Patients group that received 15 mg of saffron extract twice daily for 16 weeks had better outcome on cognitive functions than the placebo group. In a second study of the same research group,[33] the same saffron extract dose was administered to 54 adults with mild to moderate Alzheimer's disease, age older than 55 years, in comparison with standard administration of 10 mg/day of donepezil, for 22 weeks. Treatments were found similar in efficacy both decreasing the score in Alzheimer's Disease Assessment Scale and the score of Clinical Dementia Rating Scale by 3.7 and 0.7–0.8, respectively. Furthermore, adverse effects exerted by saffron were similar to placebo,[54] while some adverse effects, such as vomiting, were observed in donepezil and not in saffron-treated patients[33] It should be mentioned that both studies were well designed as double-blind multicentre clinical studies. However, the use of saffron for the treatment of Alzheimer's disease needs more and larger studies with well-defined patient inclusion criteria.

Concerning the underlying mechanisms of saffron effect on Alzheimer's disease, pathological analyses of human brains in another study revealed the crocetin-induced inhibition of aggregation and deposition of Aβ.[77] In particular, the main carotenoid constituent, *trans*-crocin-4, the digentiobiosyl ester of crocetin, inhibited Aβ fibrillogenesis at lower concentrations than dimethylcrocetin, revealing that the action of the carotenoid is enhanced by the presence of the sugars.

As far as Parkinson's disease is concerned, the neuromodulatory effect of crocetin in the rat model of Parkinson's disease, induced by 6-hydroxydopamine, was investigated by Ahmad *et al.*[55] The histopathological analyses demonstrated the significant amelioration of neurons degeneration in the treated groups rather than untreated rats. Besides this, the preventing potential of crocetin in Parkinson's disease was also demonstrated. However, further animal as well as human studies are needed to elucidate safety and efficacy of saffron extract use as alternative in preventing and treating Parkinsonism.

Effect of saffron on glucose levels and diabetes

Diabetes is a complex metabolic disorder characterized by hyperglycaemia that leads to an increased production of ROS (reactive oxygen species). The resulting oxidative stress (the imbalance between ROS production and the antioxidant defences) can play a key role in diabetes pathogenesis.[78,79] There are indications that crocin is effective in blood glucose lowering. The involvement of several mechanisms such as the stimulation of Langerhans islets or the insulin-sensitizing effects on peripheral muscles has been proposed. Most importantly, it was suggested that the strong antioxidant properties of crocin by scavenging the ROS[56] may help β-pancreatic cells to increase insulin secretion and reduce elevated blood glucose levels.[57,80] Recently, Kang *et al.*[58] investigated the hypoglycaemic actions of saffron in C_2C_{12} skeletal muscle cells. Saffron strongly enhanced glucose uptake and the phosphorylation of AMPK (AMP-activated protein kinase)/ACC (acetyl-CoA carboxylase) and MAPKs (mitogen-activated protein kinases), but not PI 3-kinase (phosphatidylinositol 3-kinase)/Akt. Interestingly, the cotreatment of the C_2C_{12} skeletal muscle cells line with saffron and insulin further improved their insulin sensitivity via both insulin-independent (AMPK/ACC and MAPKs) and insulin-dependent (PI 3-kinase/Akt and mTOR) pathways. The authors concluded the mediating role of AMPK in saffron-induced glucose uptaking and insulin sensitization of skeletal muscle cells. Nevertheless, a pure, in-vivo, antidiabetic impact of saffron and crocin is still elusive. Accordingly, clinical studies are required to investigate the potential glucose lowering of saffron and to quantify the contribution of each of the active components.

The antioxidant activity and properties of saffron

The majority of studies related to the therapeutic properties of saffron constituents indicate that saffron has a potent antioxidant activity, which is mostly due to the presence of unique carotenoids.[81] The antioxidant activity of saffron carotenoids is more effective than safranal, whereas crocin is regarded as the most potent component against oxidative stress. However, the synergistic effect of all the bioactive constituents gives to saffron spice a significant antioxidant activity. These compounds can protect DNA and tRNA from harmful chemical reaction through the formation of ligand–polynucleotide complexes.[10,81] Generally, saffron components can bind to proteins, nucleic acids (DNA, tRNA)[82,83] and lipids (linoleic acid)[84] and protect these molecules from free radicals.[85] Assimopoulou *et al.*[80] found methanolic crocin solution from *C. sativus* L. to have high radical scavenging activity, whereas Soeda *et al.*,[9] suggested a Glutamate Dehydrogenase, GDH-dependent mechanism for crocin protective effect against stress-induced cell death. Mousavi *et al.*[86] reported reduced ROS production in glucose-induced neurotoxicity in PC12 cells pretreated with 10–50 μμ of crocin. The latter yield confirms the previous results of Ordoudi *et al.*[87] who reported that saffron extract is equally efficient to phenolic antioxidants in reduction of intracellular ROS production in human monocyte system. Concerning the multifactorial regulatory role of oxidative stress in clinical pathological conditions, this may raise the potential therapeutic applications of saffron. Up to now, numerous antioxidant molecules have been tried as therapeutic modalities in a wide spectrum of diseases. Unfortunately, they have failed to show clinical efficacy despite their promising antioxidant yields in initial experimental studies. In addition, the multidisciplinary regulation of oxidative stress raises many obstacles in oxidative stress monitoring. Therefore, the emerging antioxidant activity of saffron is likely to require further investigation in the context of a complex regulation of oxidative stress across nosology.

Effect of saffron on atherosclerosis, plasma lipids and cardiac ischaemia

Adhesion and migration of the leucocyte to endothelial cells is one of the early key steps in the pathogenesis of atherosclerosis. Advance glycation end-products (AGEs) may promote this migration possibly by the expression of the intercellular adhesion molecule-1 (ICAM-1) protein. Crocetin (the major metabolite of crocin) was found to inhibit the AGE-induced growth suppression of bovine endothelial cells (BEC) and significantly reduce the adhesion rate of leucocyte to BEC, in parallel to the down-regulation ICAM-1 expression.[88] In addition, crocin has shown protective effects against endothelial cells apoptosis by increasing Bcl2/Bax ratio expression.[89,90] Crocin also decreases cholesteryl ester deposition in macrophages and the uptake of oxidized low-density lipoprotein, LDL (Ox-LDL) and thereby may slow down the formation of foam cell, which constitutes the primary element of

atherosclerosis progression,[59,91] while another proposed atheroprotective mechanism of crocin and crocetin may be illustrated by their ability to increase the plasma oxygen diffusivity.[92] That could attenuate the artery damage and cholesterol insertion, which is of clinical relevance.[6] Furthermore, in a recent in-vivo study,[61] the administration of aqueous saffron extract in atherosclerosis prone Apo E transgenic mice significantly reduced the mean area of the aortic atheromatic plaques and beneficially changed their composition. By measuring collagen and elastin concentrations within atheromatic plaques, saffron (and especially crocin) treatment seemed to enhance plaque stability and attenuate the risk for plaque rupture.

Safranal has also shown interesting pharmacological properties concerning cardiac ischaemia in animal models. According to several in-vivo studies, safranal can reduce infarct size and enhance left ventricular functions and myocardium haemodynamic status generally. It was shown that following myocardial ischaemia–reperfusion, safranal increases phosphorylation of Akt/GSK-3b/eNOS and decreases IKK-b/NFκB protein expressions.[60,93–95] However, clinical data are still missing. Therefore, well-controlled clinical trials with sufficient number of patients are required.

Chemopreventive and anticancer effects

Cancer continues to represent the second major cause of mortality worldwide by claiming over 6 million deaths per year.[96] An extremely promising strategy for cancer prevention today is chemoprevention, which is defined as the use of synthetic or natural agents (alone or in combination) to block the development of cancer in humans. The chemical composition of saffron has attracted the interest of several research groups during the last decades due to its three main active constituents (crocins, picrocrocin and safranal). According to a growing number of studies, strong evidence indicates that saffron and its components possess anticarcinogenic and antitumour effects *in vitro* and *in vivo*.[15,62,97,98] Saffron extract, either applied topically or administered *per os* or intravenously, has given excellent results against skin neoplasia (retardation or even inhibition) The anticarcinogenic properties of saffron are attributed to the presence of crocin, crocetin, picrocrocin and safranal at concentrations ranging from 2 μM to 3 mM.[99] Most carotenoids from other sources used in chemoprevention and chemotherapy are insoluble in water. In contrast, saffron contains water-soluble carotenoids that are convenient for oral administration. Most importantly, all saffron-treated mice demonstrated marked improvement of the skin texture. Skin histology of the saffron-treated mice showed a near normal appearance in comparison with normal control mice.[100] Moreover, the use of crocetin in lung cancer was found to decrease the lipid peroxidation, glutathione-metabolizing enzymes and reverse the histopathological changes relevant to tumour development, implicating its antitumour properties.[101–103] Saffron extract has been proved capable of inhibiting and/or retarding a variety of neoplasias using in-vivo experimental models. For example, saffron aqueous extract and crocetin itself inhibit the progression of gastric cancer in rats, in a dose-dependent manner.[63] *Crocus sativus* seems to induce the mechanism of cell apoptosis in many cancer types such as colorectal, pancreatic and bladder cancer.[21,63,104] The antitumour or anticarcinogenic effect of saffron may be due to different mechanisms. It has been proposed that saffron and its components have an inhibitory effect on cellular DNA and RNA synthesis, but not on protein synthesis.[62,105] Another possible antitumour mechanism is the inhibitory effect on free-radical chain reaction, as most carotenoids are lipid-soluble and act as membrane-associated high-efficiency free radical scavengers (antioxidant properties).[106,107] A third proposed anticarcinogenic mechanism is the metabolic conversion of naturally occurring carotenoids to retinoids. However recently, it was reported that conversion of carotenoids to vitamin A is not a prerequisite for anticancer activity.[108] Finally, it has been also suggested that the cytotoxic effect of saffron is associated with the interaction of carotenoids with topoisomerase II, an enzyme-regulating cellular DNA and proteins synthesis.[107]

Although *C. sativus* stigma and petal extracts have been widely studied for their antitumour properties, all studies and experiments concern only in-vitro cell lines and in-vivo animal models, as mentioned above. There is a lot of clinical investigation to be done regarding the impact of saffron extracts and their components on human carcinomas in conjunction with conventional therapy used nowadays.

Other possible applications

Saffron and its active constituents have been also ascribed some different from the above-mentioned applications. First, studies have shown the effect of *C. sativus* L. extract on second-degree burn wounds in an experimental animal model.[64] Histological comparison revealed that saffron significantly increased re-epithelialization in burn wounds. Although the exact mechanism of saffron is unclear, anti-inflammatory and antioxidant effects may have contributed to the greater wound healing.

A study on the immunomodulatory effect of the alcoholic extract of saffron and its action on the selective Th2 upregulation was also conducted.[109] It has been proven that *C. sativus* significantly modulates immune reactivity in all animal models applied. The potential therapeutic mechanism of saffron involves its relaxant, inhibitory effect on histamine (H1) and muscarinic receptors. Saffron was also found to have relaxant effect on tracheal smooth muscle

leading to bronchodilatory effect and may be used, therefore, for asthma symptoms relief.[110] All the above-mentioned data were derived from in-vitro and animal studies, so their clinical importance has to be established by far.

Furthermore, saffron has been proposed for sexual disorders therapy (especially due to major depression problems) in both males and females and erectile dysfunction in males. It has been reported to possibly have beneficial aphrodisiac effects in both animal and human studies.[65] Men and women treated with Selective Serotonin Reuptake Inhibitors (SSRIs) experienced beneficial effects on their sexual function by simultaneous administration of saffron extract.[111] Moreover, saffron seemed to ameliorate the sexual arousal, lubrication and pain domains of fluoxetine-related sexual dysfunction in women.[112] However, there is no clear clinical evidence regarding neither the aphrodisiac effect of saffron nor its effectiveness on erectile dysfunction in males. In fact, inconsistent results were found in two clinical trials evaluating the efficacy of saffron on erectile dysfunction.[113,114] In the trial of Shamsa *et al*.,[113] saffron extract was administered orally as a 200-mg tablet daily to 20 male patients for 10 days. It was observed an overall beneficial effect of saffron with significant increase in erectile and orgasmic function, as well as sexual desire and overall satisfaction. In contrast, Safarinejad *et al*.[114] reported no improvement of erectile dysfunction in a crossover study comparing efficacy of sildenafil citrate and saffron, among 346 men who received either sildenafil for 12 weeks followed by 30-mg saffron twice daily for another 12 weeks or vice versa, separated by a 2-week washout period.

Safranal has been tested on its action to hexachlorobutadiene-induced (HCBD) nephrotoxicity in rats proving its protection against it in certain doses per rat weight. The evaluated indicators were serum concentrations of urea, creatinine, glucose, protein and malondialdehyde along with the histological analysis of kidneys.[66] Finally, stress-induced anorexia in mice has been treated with both crocin and safranal, showing that the aqueous rich in crocin extract reduces anorexia as a side effect of electroshock stress in mice.[67]

Safety and toxicity

The investigation of saffron's toxicity is of main importance. According to studies reported in the literature, rats administered daily with intraperitoneal doses of aqueous extract of crocus stigma and petal for 2 weeks showed that stigma extract did not affect any organ deleteriously. However, petal extract induced necrosis in liver and lung cells. The results indicated that petal and stigma extracts caused normochromic normocytic anaemia, whereas petal extract induced toxic effects on liver and lung.[115,116] Also, Hosseinzadeh *et al*.[117] studied in 2010 both acute and sub-acute toxicity of crocin in mice and rats. Acute toxicity was evaluated by single oral and intraperitoneal crocin administration (doses: 0.5, 1, 1.5, 2, 2.5 and 3 g/kg) after a 24 and 48-h observation. Chronic toxicity was established by administering daily 15, 45, 90 and 180 mg/kg intraperitoneal for 21 days. All results, including gaining body weight, haematological, biochemical and pathological parameters evaluation and mortality, confirmed that crocin is practically a low-toxic agent.[117] Concerning safranal, Hosseinzadeh *et al*.[118] presented a study in 2013 discussing its acute and subacute toxicity. The acute toxicity was estimated by the final evaluation of LD50 after oral and intraperitoneal administration of safranal to male Wistar rats and to both male and female mice. Animals were observed for signs of toxicity and mortality for a 48-h period after treatment. Intraperitoneal LD50 value was calculated at 1.48 ml/kg and at 1.88 ml/kg for male and female mice respectively. The maximum non-fatal dose of safranal was 0.75 ml/kg. Oral LD50 values were 21.42 and 11.42 ml/kg for male and female mice respectively. In male Wistar rats ,intraperitoneal and oral LD50 were 1.5 ml/kg and 5.53 ml/kg respectively, whereas the maximum non-fatal doses were 0.75 and 3.5 ml/kg respectively. All results, according to the toxicity classification, showed that safranal is practically non-toxic in acute oral administration and low toxic when administered intraperitoneally. In subacute toxicity studies, after oral and intraperitoneal administration of safranal (doses 0.1, 0.25 and 0.5 ml/kg), all parameters evaluated concluded that safranal shows mortality in mice and rats as well as abnormalities in kidneys and lungs followed by induced decrease in haematological parameters such as haemoglobin.[118]

Generally, doses of up to 1.5 g of saffron daily are considered to be safe.[3] In clinical studies conducted in healthy volunteers, administration of 200 and 400 mg saffron tablets for 1 week revealed no changes in the general health status of volunteers,[119] while after 1-month treatment with 20-mg crocin tablets, only minor changes of haematological parameters and no major adverse effects were reported.[120] As the dose of 30 mg/day seems to be efficacious in a number of studies (e.g. depression-related studies) and toxic effects are reported with 5 g and above, with a lethal dose of approximately 20 g,[68,105,121] saffron could be considered as safe non-toxic extract to use in therapeutics. However, long-term and large-scale investigations are required to elucidate the effect of saffron and its constituents on human health.

Conclusion

In summary, saffron and especially crocin and safranal components possess significant pharmacological properties, including hypolypidaemic, anticancer and antioxidant, that

may be applied to a wide spectrum of diseases, such as atheromatosis, diabetes, neurodegenerative diseases and various types of cancer. Despite the worldwide reports of the quite promising therapeutic properties of saffron, yet, there is a lot of research to be done because the vast majority of scientific findings derive from in-vitro and animal in-vivo studies. In the limited number of clinical trials carried out up to now, the administration of saffron extract, at doses ranging from 20 to 200 mg/day for 10 days to several weeks, was found effective and safe with no major adverse effect, mainly in patients with CNS diseases and pshychological disorders such as Alzheimer's and depression, respectively. However, further studies are required to elucidate the possible use of saffron extract and its main constituents crocin and safranal, as natural alternatives to conventional treatment of the above-mentioned as well as of many other diseases (such as, cardiovascular related disease etc). Up to now, the limited clinical evidence restrains saffron usage as herbal therapy and food supplement. Because the precise mechanisms of its action remain obscure, there is a great need of new, pharmacokinetic and bioavailability studies in conjunction with clinical trials that will establish the role of saffron as a novel therapeutic approach.

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