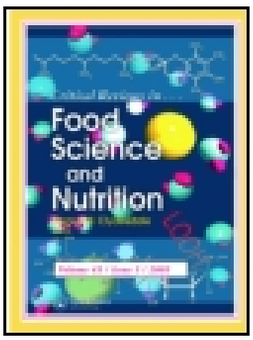

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REVIEW

Saffron bioactives crocin, crocetin and safranal: effect on oxidative stress and mechanisms of action

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ABSTRACT

Saffron (*Crocus sativus* L.) is used as a spice for its organoleptic characteristics related to its coloring and flavoring properties, and it has been also used in traditional medicine to treat various diseases. The main chemical components responsible for these properties are crocin, crocetin and safranal. These compounds have been shown to have a wide spectrum of biological activities, including several properties as antigenotoxic, antioxidant, anticancer, anti-inflammatory, antiatherosclerotic, antidiabetic, hypotensive, hypoglycemic, antihyperlipidemic, antidegenerative and antidepressant, among others. This review article highlights the antioxidant effects of these bioactive compounds to reduce reactive oxygen species (ROS) and the mechanisms of action involved, since there are a multitude of diseases related to oxidative stress and the generation of free radicals (FRs). Recent studies have shown that the effects of crocin, crocetin and safranal against oxidative stress include the reduction in lipid peroxidation (malondialdehyde [MDA] levels) and nitric oxide (NO) levels, and the increase in the levels of glutathione, antioxidant enzymes (superoxide dismutase [SOD], catalase (CAT) and glutathione peroxidase [GPx]) and thiol content. Therefore, due to the great antioxidant effects of these saffron compounds, it makes saffron a potential source of bioactive extracts for the development of bioactive ingredients, which can be used to produce functional foods.

KEYWORDS

Saffron apocarotenoids; functional ingredients; saffron supplementation; antioxidant activity; nutrients; health

Introduction

Crocus sativus L., commonly known as saffron, is a perennial herb which belongs to the *Iridaceae* family with more than 85 species. It is a traditional Mediterranean plant, and it is widely cultivated in different areas, such as Iran, India, Morocco, Azerbaijan, Spain, Greece, Italy, Turkey and China, among others (Karabagias et al. 2017). *C. sativus* L. is a sterile low-rise plant developed and propagated by corms, a bulbous tuberous structure. The flower is composed of six purple tepals, three golden yellow stamens and one red pistil, which culminates with three red branched stigmas (filaments) that when dried up give the spice saffron (Lotfi et al. 2015; Mathew 1977) (Figure 1). Therefore, only that part of the saffron flower is used to obtain the spice. It is the most expensive spice in the world because of its production costs, since to produce 1 kg of saffron are necessary around 230.000 flowers (350 kg of tepals) (Kafi et al. 2006).

Saffron is mainly used in agro-food and cosmetic industries due to its organoleptic characteristics related to its coloring and flavoring properties, and it has been used for centuries as part of a healthy Mediterranean diet. The chemical composition of saffron consists mostly on carbohydrates (starch, gums, pentosans, reducing sugars, pectin and dextrins) (63%), amino acids and proteins (12%), moisture (10%), fat (5%), minerals (5%), crude fiber (5%), and vitamins specially vitamin B1 (thiamine) and vitamin B2 (riboflavin). Other important constituents of saffron are carotenoids, monoterpenes, anthocyanins and flavonoids (Rios et al. 1996).

Traditionally, it has been also used in medicine to treat various diseases, since it is considered as a medicinal plant in many cultures. The main chemical components responsible for these properties are apocarotenoids such as crocin, crocetin and safranal considered as bioactive compounds (Melnyk, Wang, and Marcone 2010). These compounds have been shown to have a wide spectrum of biological activities, including several properties as antigenotoxic, antitumor (Festuccia et al. 2014), anticancer (Abdullaev and Espinosa-Aguirre 2004), antioxidant (Ghadrdoost et al. 2011; Karimi et al. 2010; Verma and Bordia 1998), anti-inflammatory (Amin and Hosseinzadeh 2012), antidiabetic (Sheng et al. 2008), antiatherosclerotic (He et al. 2005), hypotensive, hypoglycemic, antihyperlipidemic (Lee et al. 2005), antidepressant (Schmidt, Betti, and Hensel 2007) and antidegenerative (Soeda et al. 2016), among others.

One of the greatest interests of saffron bioactive compounds is their impact on the human health due to their high antioxidant capacity and free radical (FR) scavenging activity (Hosseinzadeh, Shamsaie, and Mehri 2009). The

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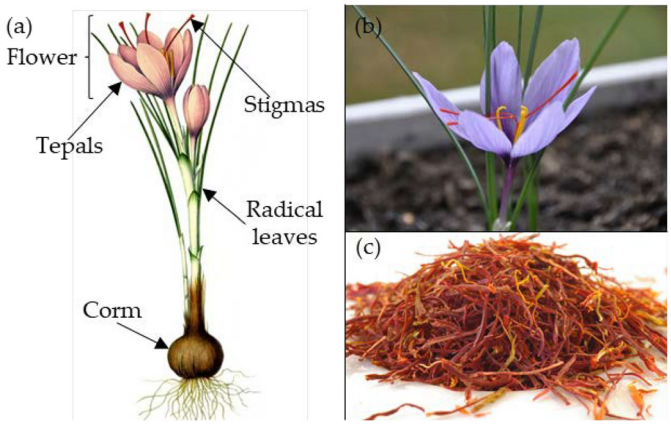


Figure 1. (a) Diagram of the different parts of saffron plant (Brandt et al. 1898); (b) The saffron flower; (c) Dry stigmas used as saffron spice.

generation process of reactive oxygen species (ROS) takes place in normal cells, but oxidative stress originates as a consequence of the uncontrolled generation and increase of ROS levels (Valko et al. 2007). Oxidative stress is a harmful process because ROS and FRs can attack and lead to extensive damage to biological molecules, such as DNA, lipids and proteins, causing a variety of diseases: cancer, metabolic syndrome, neurological disorders, inflammation and cardiovascular diseases (CVDs), affecting almost all organs in the body (Fang, Yang, and Wu 2002; Seifried 2007). The oxidative systems may be disturbed by various physical, chemical and biological agents. Therefore, a wide range of factors, such as psychological stress, diet (high in fat, sugar and processed foods), lifestyle, drugs, alcohol, smoking, obesity, infections and environmental factors, such as the exposure to corrosive chemicals and toxins, excessive exposure to x-rays, sunlight and radioactive materials, or extreme temperatures can contribute to the excess FR production, causing the generation of abnormally high levels of oxidative stress (Gholamnezhad, Keyhanmanesh, and Boskabady 2015; Makhlouf et al. 2011).

In order to combat the oxidative damage, cells have evolved a complex antioxidant system including exogenous and endogenous antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). SOD is an endogenous antioxidant enzyme that constitutes an important defense system against ROS. It catalyzes the dismutation of two molecules of superoxide anion to hydrogen peroxide and oxygen, rendering the superoxide anion less hazardous (Fridovich 1995). CAT is an antioxidant enzyme which is present almost in all living tissues that use oxygen and completes the detoxification process started by SOD, catalyzing the degradation or reduction of hydrogen peroxide to water and oxygen (Chelikani, Fita, and Loewen 2004). GPx is an important intracellular enzyme which also plays an important role against oxidative stress. It breaks down hydrogen peroxides to water and decomposes lipid peroxides to their corresponding alcohols mainly in the mitochondria, acting against a wide range of peroxides which react with glutathione (Goth, Rass, and Pay 2004). Glutathione, a non-protein intracellular thiol, is generated by cells as an antioxidant against oxidative stress. In normal cells, more than 90% of glutathione is in the reduced form (GSH), which is maintained by glutathione reductase (GR). Glutathione S-transferase (GST) conjugates GSH to a variety of substrates for detoxification (Hayes, Flanagan, and Jowsey 2005). Other important scavengers of FRs and ROS are sulfhydryl (thiol, -SH) groups, highly-reactive constituents of protein molecules which play important functions in several biochemical and metabolic processes, such as the activation of antioxidant enzymes. Other interesting markers of oxidative stress are malondialdehyde (MDA) and nitric oxide (NO). MDA is one of the end products in the lipid peroxidation process, one of the reactions induced by oxidative stress that leads to an increase in phospholipids rigidity (Gawel et al. 2004). NO is an unstable FR which can be generated endogenously in several cells by nitric oxide synthases (NOS) and has different physiological and biochemical functions. In oxidative stress conditions, it is produced in large excess or produced with ROS concurrently, causing tissue damage and inducing apoptotic cell death (Mayer and Hemmens 1997).

Thus, the antioxidant properties of saffron bioactive compounds could be an effective way to fight against several disorders and to prevent diseases *via* modulation of oxidative stress markers, depressing ROS-derived oxidative stress. The effects of saffron extracts and its bioactive compounds have been reviewed previously (Bathaie and Mousavi 2010; Broadhead et al. 2016; Giaccio 2004). However, this article is focused on reviewing the studies conducted to date about the antioxidant effects of crocin, crocetin and safranal to inhibit the oxidative stress, featuring the most recent studies with new findings, and trying to explain their possible mechanisms of action. Therefore, this review article highlights the antioxidant effects of saffron bioactive compounds crocin, crocetin and safranal against oxidative stress to reduce ROS and promote the reduction of lipid peroxidation (MDA levels) and the increase of GSH level and antioxidant enzymes (SOD, CAT and GPx), among other possible mechanisms of action involved. Besides, this review will examine the beneficial effects of these saffron bioactive compounds on several diseases, and the future perspectives to use saffron as a potential source of bioactive extracts for the development of bioactive ingredients to produce functional foods.

Saffron bioactive compounds: crocin, crocetin and safranal and their physicochemical properties, bioavailability and toxicity

Crocin

Crocins are glycosyl esters of crocetin, formed by esterification of crocetin with different glycosides, being the geometric isomers *trans* the majority and *cis* isomers the minority (Carmona et al. 2006). Crocetin molecule is modified by the activity of glucosyltransferases, adding different numbers of glycosidic molecules to produce crocins, the major components of the stigmas of saffron and which confer solubility in water (Ahrazem et al. 2015). The content of crocetin esters in saffron represents 16–28% (Moratalla-López et al. 2019).

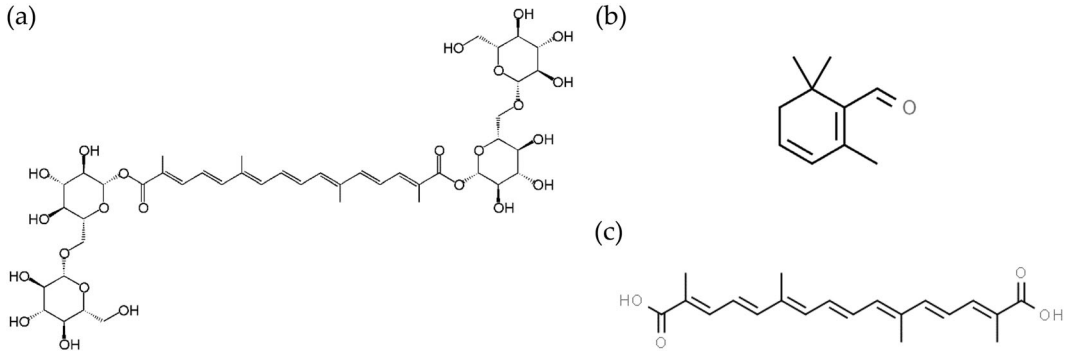


Figure 2. Structural formula of the saffron bioactive compounds: (a) crocin; (b) safranal; (c) crocetin.

The most abundant carotenoid glycoside in saffron is α-crocin or crocin-1 ($C_{44}H_{64}O_{24}$, *trans*-crocetin di-(β-d-gentiobiosyl) ester), a diester formed from the disaccharide gentiobiose and the dicarboxylic acid crocetin, with a molecular weight of 976.96 g/mol and a melting point of 186 °C (Figure 2). It is also the most important and studied due to its antioxidant activity, protecting against oxidative stress (Rahaiee et al. 2015). It easily dissolves in water, unlike most carotenoids, because of the saccharide link with sugars, and is barely soluble in alcohol, ether and other organic solvents (Carmona et al. 2006). Crocin has a golden-yellow color and it is responsible for the intense characteristic red color of saffron, used as a natural food colorant (Lage and Cantrell 2009; Pfander and Schurtenberger 1982). Besides, the maximum absorbance showed is at 440 nm (Shahi, Assadpour, and Jafari 2016).

Crocins lose most of their functionality after exposure to oxygen, light, heat and acidic environment due to their low stability. They also have low bioavailability, and poor absorption, being not absorbed after oral administration, until they are hydrolyzed due to enzymatic processes in the intestinal epithelial cells and by the fecal microbiome to deglycosylated *trans*-crocetin in the intestinal tract. *Trans*-crocetin is absorbed by passive diffusion through the intestinal barrier (Lautenschläger et al. 2015).

The safety of crocins has been evaluated in some studies in mice. The acute and sub-acute toxicity of α-crocin were evaluated in mice and rats. The acute toxicity during 2 d with doses administration orally and intraperitoneally up to 3 g/kg b.w. did not produce death. The sub-acute toxicity during 21 d administered intraperitoneally at doses of 154,590 and 180 mg/kg b.w. did not show toxic effects at pharmacological doses on any pathological, biochemical and hematological parameters (Hosseinzadeh, Shamsaie, and Mehri 2010). Regarding genotoxicity and mutagenicity, crocin did not exhibit mutagenic and toxic effects in the Ames test (Abdullaev et al. 2003; Alavizadeh and Hosseinzadeh 2014). A summary of the toxicological effects of crocin is presented in Table 1.

Crocetin

Crocetin ($C_{20}H_{24}O_4$, 8,8′-Diapocarotenedioic acid) is a natural carotenoid dicarboxylic acid with a molecular weight of 328.40 g/mol and a melting point of 285 °C (Figure 2) (Samarghandian and Borji 2014). It is a hydrophobic compound, insoluble in water and most organic solvents, except for pyridine and dimethylsulfoxide. In its anionic form is water soluble, easily dissolves in dilute aqueous sodium hydroxide or other aqueous alkali solutions (Escribano et al. 1996). Crocetin is the precursor of crocin, and it is obtained as the result of crocin glycosides hydrolysis (Moraga et al. 2004).

Crocetin just like crocins have low stability, but a higher bioavailability and rapid absorption. After the oral administration of crocins which are hydrolyzed to *trans*- and *cis*-crocetin, they are incorporated into the bloodstream. Several studies have reported that levels of crocetin in blood were very high by the oral administration of crocin that could be due to the crocins hydrolysis to *trans*-crocetin in the gastrointestinal lumen and the intestinal mucosa, which is rapidly absorbed by passing to the blood stream through the portal vein (Christodoulou et al. 2019; Moratalla-López et al. 2019).

Respect to crocetin toxicity, some studies have revealed that crocetin is not teratogenic and genotoxic, but there is no data about its LD_{50} value (Martin, Goh, and Neff 2002; Ozaki et al. 2002). Several studies showed that LC_{50} values for saffron and its constituents against normal cells could be higher than pharmacological doses (Hashemi and Hosseinzadeh 2019). Besides, the safety of crocetin was evaluated in healthy adult volunteers (37.5 mg per day) for four weeks and the results did not show significant changes in blood biochemistry and hematology (Yamashita et al. 2018). A summary of the toxicological effects of crocetin is presented in Table 1.

Safranal

Safranal ($C_{10}H_{14}O$, 2,6,6-trimethyl-1,3-cyclohexadiene-1-carboxaldehyde) is a monoterpene aldehyde with a molecular weight of 150.21 g/mol and a melting point of below 25 °C (Figure 2). Safranal represents about 60–70% of volatile fraction of saffron and it is responsible for its characteristic aroma. Safranal has a low aqueous solubility and its maximum absorbance is at 330 nm (Carmona et al. 2007; Tarantilis and Polissiou 1997).

Safranal is a cyclic terpenic aldehyde which is produced *via* enzymatic and thermal degradation during saffron storage and drying process (Rezaee and Hosseinzadeh 2013). Greater amounts of safranal are obtained at higher temperatures (80 °C) and less processing time (Himeno and Sano 1987).

Table 1. Summary of toxicological effects of crocin, crocetin and safranal

Bioactive compound	Toxicity	Administration	Dose	Effects	References
Crocin	Acute	Orally and intraperitoneally in mice and rats, 2 d	1–3 g/kg b.w.	No toxic effects	Hosseinzadeh et al. (2010)
	Sub-acute	Intraperitoneally in mice and rats, 21 d	15–180 mg/kg b.w.	No toxic effects	Hosseinzadeh et al. (2010)
Crocetin	Sub-acute	Orally in healthy adult volunteers, 4 weeks	37.5 mg/d	No changes in blood biochemistry and hematology	Yamashita et al. (2018)
Safranal	Acute	Orally and intraperitoneally in mice and rats, 2 d	0.1 – 0.5 ml/kg b.w.	Low toxicity in intraperitoneal administration	Hosseinzadeh et al. (2013)
	Sub-acute	Orally in mice and rats, 21 d	0.1 – 0.5 ml/kg b.w.	Changes in biochemical and hematological parameters	Hosseinzadeh et al. (2013)

However, the biomedical and pharmacological properties of saffron are attributed mostly to the carotenoids, crocetin and crocins, and not to the aldehyde safranal, the major compound of the volatile fraction of saffron, because its bioactivity is more variable and less known than that of carotenoids (Bolhassani, Khavari, and Bathaie 2014; Kyriakoudi et al. 2015; Moratalla-López et al. 2019). The safety of safranal has been evaluated in some studies. The acute and sub-acute toxicity of safranal was evaluated in mice and rats. The acute toxicity during 2 d with different doses of safranal administered orally and intraperitoneally and the sub-acute toxicity during 21 d, administrating safranal orally at doses of 0.1, 0.25 or 0.5 ml/kg b.w. per day. The LD_{50} values indicated that safranal was nontoxic in acute oral administration and of low toxicity in acute intraperitoneal administration, however in sub-acute toxicity, safranal induced some changes in biochemical and hematological parameters (Hosseinzadeh et al. 2013). A summary of the toxicological effects of safranal is presented in Table 1.

Bioactive compounds, antioxidant effects and the mechanism of action of crocin, crocetin and safranal

Crocin

Crocin is a high potency antioxidant with enormous pharmacological properties. Its beneficial physiological effects against certain diseases have been tested. Bandegi et al. (2014) investigated the protective effects of saffron and crocin extract against chronic stress-induced oxidative stress in adult male rats. To develop the study, the authors investigated whether saffron and crocin extract can protect the kidney, liver and brain against chronic stress. The levels of the lipid peroxidation (MDA content), and the activity of the antioxidant enzymes (SOD, GPx and GR) were measured after the end of the induced chronic stress. Stress was induced by restriction of the animals for 6 h per day for 21 d. After 6 h of restriction, the rats were injected intraperitoneally with saffron and crocin extract at doses of 30 mg/kg b.w. both the control and the stressed groups. The results showed that the administration of crocin and saffron could reverse the changes in stressed rats and had a positive effect to prevent the induced oxidative stress. Xiao et al. (2019) evaluated the antidepressant activity of crocin in male mice C57BL/6L. The depression was induced into three groups with corticosterone (20 mg/kg b.w.) by subcutaneous injection during 28 d. The control group was treated with saline solution. Depression was detected through the attenuation of depression-like behaviors, such as the forced swim test, tail suspension test, and open field test. After detecting depression, two experimental groups were treated with 20 and 40 mg/kg b.w. of crocin administered orally during 2 weeks. Liver function and histomorphology, body weight, neuroinflammation and oxidative stress were investigated. The results showed that the oral administration with 40 mg/kg b.w. of crocin acted as a good antidepressant through the reduction of oxidative stress and inflammation. However, none of the administered doses had an effect in restoring the histomorphological findings compared to those of the control group. Only when 40 mg/kg b.w. of crocin was administered, a reduction in ballooning degeneration (a histomorphologic change seen in liver pathology) was observed in the liver of the mice treated with corticosterone.

The therapeutic effect of crocin after intracerebral hemorrhage that may be related to oxidative stress was tested in vivo with male mice. Intracerebral hemorrhage was induced with collagenase injection. Mice were randomly divided in two groups; one group was treated with crocin (40 mg/kg b.w.) and the other with saline solution through oral gavage 6 h after intracerebral hemorrhage and then every 12 h for up to 7 d. The results showed that crocin could reduce the neurological deficit, myelin loss, neuronal degeneration and iron deposition (as the principal factor for ROS generation) after intracerebral hemorrhage. A reduction was also observed in hemo-oxigenase expression and in ROS production during the early stage of intracerebral hemorrhage (Duan et al. 2019).

The effect of crocin has also been investigated as a potential anti-inflammatory in acute pancreatitis induced by cerulein in rats. Animals were randomized into five groups: normal control, cerulein control, crocin control (100 mg/kg b.w.), low dose of crocin with acute pancreatitis (30 mg/kg b.w.) and high dose of crocin with acute pancreatitis (100 mg/kg b.w.). The results showed that crocin could protect against acute pancreatitis, being able to observe the enormous reduction in pancreatic edema and the levels of inflammatory pancreatic cytokines (Godugu et al. 2020). Qiu et al. (2020) reported protection of renal and hypoglycemic

properties with the administration of crocin in obese and type 2 diabetic (db/db) and nondiabetic (db/m) mice. The db/db and db/m were fed with a high-sucrose/hig-fat diet and with a normal diet, respectively. Mice were treated with normal saline (8 ml/kg b.w.), metformin (positive control group) (100 mg/kg b.w.) and crocin (50 mg/kg b.w.) by gavage dissolved in the physiological saline with a daily dose for 8 weeks. The administration of crocin showed that the regulation of the reduced nuclear factor-kappa B (NF-κB) signal suppressed its activation through the nuclear factor erythroid 2-related factor 2 (Nrf2) activation signal, and this effect was in relation to the modulation of oxidative stress. At the same time, the anti-inflammatory properties of crocin showed hypolipidemic, hypoglycemic and renal protective activities. In another study, the daily oral administration of crocin by gavage (25 mg/kg b.w.) for 28 d showed a potential effect as an effective therapy against bleomycin-induced idiopathic pulmonary fibrosis in rats. The idiopathic pulmonary fibrosis was induced by a single dose of bleomycin dissolved in physiological saline on day 7. Bleomycin administration produced pathological changes in the lung, which may be related to the suppression of antioxidant enzymes and the induction of inflammatory and oxidative markers. However, the crocin-treated rats showed a decrease in the damage caused by the administration of bleomycin (tumor necrosis factor-α [TNF-α], MDA levels, CAT and GPx activities), except in SOD activity and lung index, that may be related to the antioxidant capacity of crocin (Mehrabani et al. 2020). Therefore, it is necessary to continue conducting studies to know the protection mechanisms because they are still unclear.

The cardioprotective effect of crocin has been evaluated through the study of different CVDs. Myocardial fibrosis was induced by isoproterenol in mice. The animals were randomly divided into 5 groups, the crocin groups were injected intraperitoneally with 100 mg/kg b.w. and 200 mg/kg b.w., respectively, for 14 d. Crocin administration decreased oxidative stress, apoptosis and inflammation associated with myocardial fibrosis, which may be related to reduced NF-κB, p65 and toll-like receptor (TLR-4) expression (Jin et al. 2020). The possible cardioprotective effect of crocin against doxorubicin (a common drug to treat a wide range of tumors) that induces cardiotoxicity as the main adverse reaction. Therefore, the cardioprotective effect of crocin against doxorubicin was tested in 4 groups of male rats. Each group was given an intraperitoneal injection: control group with a daily dose of normal saline solution and low, medium and high dose groups with crocin with 30 mg/kg b.w., 60 mg/kg b.w. and 120 mg/kg b.w., respectively, for 6 d. Finally, the results showed that the administration of crocin protected against cardiotoxicity induced by doxorubicin by inhibiting inflammation, correcting cardiomyocyte calcium dysomeostasis, oxidative stress and mitochondria damage (Chu et al. 2020). Another study investigated the cardioprotective effect on isoprenol-induced cardiotoxicity in rats in the form of myocardial infarction. The groups of animals were treated daily with crocin at 5, 10 and 15 mg/kg b.w. using a gastric tube for 21 d. This study also demonstrated the cardioprotective potential of crocin administration through modulation of oxidative stress (Goyal et al. 2010). Wang et al. (2018) also reported the potential antioxidant effect of crocin to improve myocardial infarction in rats treated with crocin 20, 40 and 60 mg/kg b.w., intraperitoneally, for 7 d. However, further studies are necessary to know other pathways and possible mechanisms involved with cardioprotective effects.

In addition, in the animal model and in vitro culture of human cancer cells, administration of crocin also had promising results through suppression of tumor cell proliferation, apoptosis and inhibition of telomerase activity in different types of cancer, such as colorectal (Aung et al. 2007), skin (Sun et al. 2011), prostate (Festuccia et al. 2014), lung, liver and breast (Ashrafi et al. 2015; Khosrojerdi et al. 2012; Nasimian et al. 2020).

Although it is well established that crocin has a potential antioxidant effect to prevent oxidative stress and related diseases, the underlying mechanisms are still unclear. Therefore, more studies are needed to delve into other pathways and possible mechanisms involved in the effects of crocin in different diseases. Furthermore, most of the studies are carried out in vitro or with animal models. Thus, it is necessary to further investigate the effects of crocin in oxidative stress-related pathologies in intervention studies. A summary of the studies conducted to date of the antioxidant effects of crocin is presented in Table 2 and the possible mechanisms of action involved are shown in Figure 3.

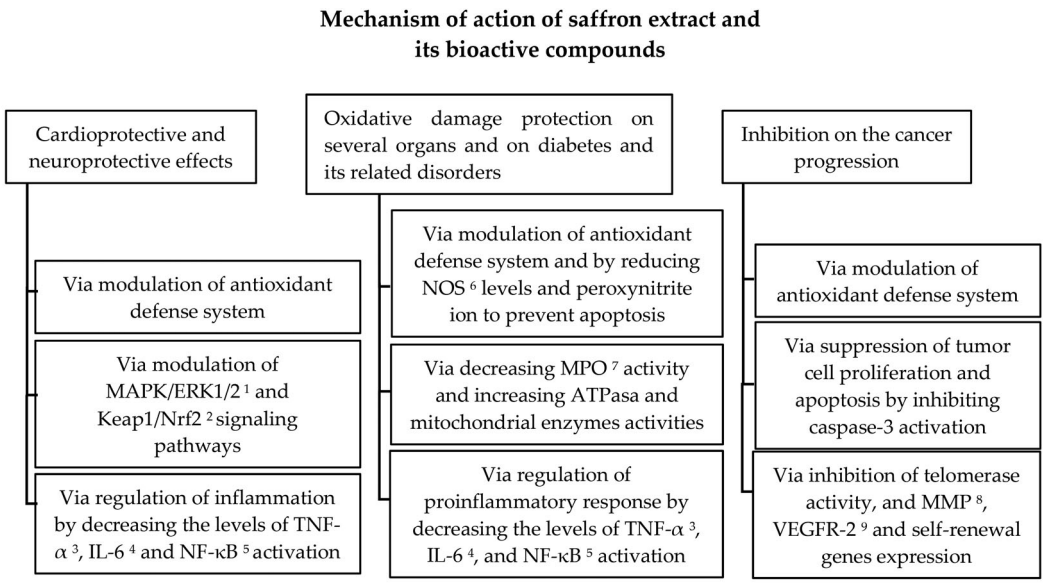
Crocetin

Several researchers have investigated the antioxidant activity of crocetin against oxidative stress. Regarding in vitro studies, Karimi et al. (2020) investigated the effects of crocetin, on an in vitro retinal pigment epithelium model (aged ARPE19 cells) of oxidative stress induced by tert-butyl hydroperoxide (TBHP). ARPE19 cells were incubated for 24 h with crocetin (0, 100 and 200 μM), and it was able to pass through the blood–retinal-barrier. The mechanism of action of crocetin to protect stressed cells was through the activation, in the first minutes of TBHP exposure, of the extracellular signal-regulated kinase 1/2 (ERK1/2), a member of mitogen-activated protein kinases (MAPK) signaling cascade involved in regulation of many cellular processes, and in the preservation of energy production pathways. Thus, crocetin may be a potential agent to prevent neurodegenerative diseases as age-related macular degeneration. Furthermore, Sapanidou et al. (2016) studied the antioxidant effects of crocetin on bovine spermatozoa during in vitro fertilization against oxidative stress. After the incubation of bovine sperm samples with crocetin (1, 2.5 and 5 µM) for 120 or 240 min, the results showed an improvement in bovine sperm quality and its fertilization ability with 2.5 µM of crocetin by modulating ROS production and lipid peroxidation, decreasing superoxide anion and hydrogen peroxide generation.

Other studies have also evaluated the antioxidant effects of crocetin against oxidative stress in cells. Zheng et al. (2015) indicated that crocetin had positive effects on

Table 2. Summary of studies conducted to date of the antioxidant effects of crocin

Experimental model	Crocin administration	Effects	References
Adult male rats with chronic stress	Intraperitoneally, with saffron and crocin extract at 30 mg/kg b.w., for 21 d	Reverse the changes in stressed rats and had a positive effect to prevent the induced oxidative stress	Bandegi et al. (2014)
Male mice C57BL/6L with depression induced by corticosterone	Orally, 20 and 40 mg/kg b.w., for 2 weeks	40 mg/kg b.w. of crocin acted as a good antidepressant through the reduction of oxidative stress and inflammation	Xiao et al. (2019)
Male mice with ntracerebral hemorrhage induced by collagenase	Oral gavage, 40 mg/kg b.w., 6 h after intracerebral hemorrhage and then every 12 h for up to 7 d	Reduction in the neurological deficit, myelin loss, neuronal degeneration and iron deposition after intracerebral hemorrhage	Duan et al. (2019)
Rats with acute pancreatitis induced by cerulein	30 and 100 mg/kg b.w.	Protection against acute pancreatitis. Enormous reduction in pancreatic edema and the levels of inflammatory pancreatic cytokines	Godugu et al. (2020)
Obese and type 2 diabetic and nondiabetic mice	By gavage, 50 mg/kg b.w., for 8 weeks	The anti-inflammatory properties of crocin showed hypolipidemic, hypoglycemic and renal protective activities	Qiu et al. (2020)
Rats with idiopathic pulmonary fibrosis induced by bleomycin	By gavage, 25 mg/kg b.w, for 28 d	Effective therapy against bleomycin-induced idiopathic pulmonary fibrosis	Mehrabani et al. (2020)
Mice with myocardial fibrosis induced by isoproterenol	Intraperitoneally, 100 and 200 mg/kg b.w., for 14 d	Decrease in oxidative stress, apoptosis and inflammation associated with myocardial fibrosis	Jin et al. (2020)
Male rats with cardiotoxicity induced by doxorubicin	Intraperitoneally, 30, 60, and 120 mg/kg b.w, for 6 d	Protection against cardiotoxicity, correcting cardiomyocyte calcium dysomeostasis, oxidative stress and mitochondria damage	Chu et al. (2020)
Rats with cardiotoxicity induced by isoprenol	Gastric tube, 5, 10, and 15 mg/kg b.w., for 21 d	Cardioprotective potential through modulation of oxidative stress	Goyal et al. (2010)
Rats myocardial infarction-induced	Intraperitoneally, 20, 40, and 60 mg/kg b.w., for 7 d	Improve myocardial infarction in rats treated with crocin	Wang et al. (2018)



[1]MAPK/ERK1/2: mitogen-activated protein kinases/extracellular signal-regulated kinase 1/2; [2]Keap1/Nrf2: kelch-like ECH-associated protein 1/ nuclear factor erythroid 2-related factor 2; [3]TNF-α: tumor necrosis factor-α;[4] IL-6: interleukin-6; [5]NF-κB: nuclear factor-kappa B; [6]NOS: nitric oxide synthases; [7]MPO: myeloperoxidase; [8]MMP: matrix metalloproteinases; [9]VEGFR-2: vascular endothelial growth factor receptor 2.

Figure 3. Possible mechanisms of action involved in the effects of saffron and its bioactive compounds in different diseases.

angiotensin II-induced vascular cell adhesion molecule-1 (VCAM-1) and monocyte-endothelial cell adhesion in human umbilical vein endothelial cells. Crocetin (0.1, 1 and 10 μM) was able to inhibit angiotensin II-induced VCAM-1 expression and monocyte-endothelial cell adhesion by blocking of NF-κB activation that could be due to its antioxidant

properties by decreasing ROS generation and increasing antioxidant content. Papandreou et al. (2011) demonstrated that crocetin protected human neuroblastoma cells viability against H_2O_2-induced toxicity. Cells were incubated for 18 h with crocetin (1–125 μM) and this carotenoid decreased ROS production and repressed caspase-3 activation involved in apoptosis, showing its protective effects. Cai et al. (2009) showed that crocetin protected primary rat cultured cardiac myocytes against cardiac hypertrophy *via* modulating MAPK/ERK1/2 signaling pathway. Crocetin (1–10 μM, for 61,224 and 48 h) dose-dependently inhibited cardiac hypertrophy through blocking ROS-dependent MAPK/ERK1/2 pathway and GATA binding protein 4 activation and had anti-inflammatory effects by blocking NF-κB signaling. Tseng et al. (1995) studied the protective effects of crocetin against genotoxicity induced by paraquat in rat primary hepatocytes. Crocetin (5, 10 and 20 μM, for 1 h) prevented hepatotoxicity through decreasing lipid peroxidation (MDA levels) and superoxide anion formation, by its FR-quenching capacity.

According to in vivo investigation with animal models, some studies have reported cardiovascular protective effects of crocetin. Higashino et al. (2014) studied the cardioprotective effects of the oral administration of crocetin (25 or 50 mg/kg b.w. per day) for 3 weeks in stroke-prone spontaneously hypertensive male rats. After the treatment, the levels of 8-hydroxy-2-deoxyguanosine (8-OHdG), a marker of oxidative stress (DNA damage) and NO metabolites nitrite/nitrate in urine were significantly decreased. Shen and Qian (2006) evaluated the antioxidant properties of crocetin against cardiac hypertrophy induced by norepinephrine in rats. The treated group with crocetin, administered intraperitoneally at 25 and 50 mg/kg b.w. for 15 d, showed a decrease in the lipid peroxidation and an increase in endogenous antioxidant enzymatic activities (SOD and GPx) in cardiac tissue. Therefore, these studies demonstrated the cardioprotective effect of crocetin, that might be related to the modulation of antioxidant enzymatic activities and it could restrict the effects of ROS-related mechanisms, ameliorating cardiovascular damages and being useful to treat CVDs.

Other in vivo studies have focused on the neuroprotective effects of crocetin. Khan et al. (2012) studied the effects of crocetin in neurodegenerative disorders, combined with certain natural antioxidants (extracts of roots of *Nardosatchys jatamansi* and sodium selenite) against oxidative stress in male albino rats with cognitive impairment induced by streptozotocin. The treatment (crocetin 25 μg/kg b.w., orally) for 15 d showed a synergic potential effect that significantly reduced the lipid peroxidation (MDA levels), and increased GSH levels and the activity of the antioxidant enzymes, GPx, CAT and GST, in rat hippocampus and frontal cortex, compared to the control group (normal saline). Thus, this combination that includes crocetin could provide a protective effect to neurodegeneration. Ishizuka et al. (2013) researched the effect of crocetin to inhibit retinal ischemic damage which leads to neuronal cell death in male mice. The oral administration with crocetin (20 mg/kg b.w. twice a day) for 4 d showed a protective effect against retinal ischemic damage partly due to its antioxidant properties. In the crocetin treated group, oxidative stress was reduced, since the expression of 8-OHdG in the retina was significantly lower than in the control group (oral administration of 20 mg/kg b.w. sodium carboxymethyl cellulose). Furthermore, crocetin prevented the activation of MAPK and redox-sensitive transcriptional factors, which play important roles in the retinal cell death induced by oxidative stress and they also showed anti-apoptotic properties, inhibiting retinal cell death. Crocetin reduced the phosphorylations of MAPK, c-Jun N-terminal kinases (JNK) and p38, and those of the redox-sensitive transcription factors c-Jun and NF-κB in the retina. These results indicated that this bioactive compound may be a potential preventive treatment against retinal ischemic injury. Yoshino et al. (2011) showed that crocetin reduced oxidative stress induced by ROS in the brain of stroke-prone spontaneously hypertensive male rats, a high-oxidative stress model. After the oral administration of crocetin (100 mg/kg b.w.), there was a reduction of oxidative damage in the brain of treated rats, acting crocetin as a scavenger of ROS, such as the hydroxyl radical, exhibiting its antioxidant properties, compared to the control group (administration of sodium carboxymethyl cellulose). Therefore, these findings demonstrated that crocetin could provide protective effects to neuronal disorders due to its antioxidant capacity.

Crocetin also showed protective effects on skin oxidative damage. Ohba et al. (2016) investigated its effects on skin-induced oxidative damage by ultraviolet-A in vivo and in vitro. After oral crocetin administration (100 mg/kg b.w. at 2, 4 and 6 h after the initiation of UV-A irradiation) in month old male kelch-like ECH-associated protein 1 (Keap1)-dependent oxidative stress detector transgenic mice, the oxidative stress was ameliorated, since lipid peroxidation in the skin was decreased. Regarding in vitro effects, in human skin-derived fibroblasts cells (NB1-RGB), after the incubation with crocetin (1 μM, for 1 h), ROS production was significantly reduced, and the expression levels of cleaved caspase-3 were decreased. Therefore, this carotenoid may be useful to protect skin against oxidative damage, decreasing ROS generation and subsequent cell apoptosis.

Besides, crocetin had antioxidant properties against oxidative damage in lungs. Several studies demonstrated the antioxidant capacity of this carotenoid against oxidative stress in lungs. Venkatraman et al. (2008) investigated its effects against mitochondrial damage induced by benzo(a)-pyrene. Crocetin was administered intraperitoneally (20 mg/kg b.w.) in male albino mice for 18 weeks and the levels of ROS, lipid peroxides, GSH and the activities of ATPase and mitochondrial enzymes were assessed in the lung. After the treatment, the results revealed that crocetin modulated lung mitochondria oxidative damage, increasing ATPase and mitochondrial enzymes activities and GSH levels, decreasing ROS generation and normalizing lipid peroxides, compared to the control group (corn oil intraperitoneal injection). Magesh et al. (2006) evaluated the intraperitoneal administration of crocetin (20 mg/kg b.w. dissolved in dimethyl sulfoxide) for 4 weeks in lung cancer-bearing male albino mice.

The results of the treatment showed a decrease in lipid peroxidation levels, and an increase in the activities of antioxidant enzymes (SOD, CAT and GPx,) and GSH metabolizing enzymes (GR and GST) in lung and liver tissues from the treated mice, compared to the control group (orally corn oil). Crocetin, due to its antioxidant capacity that influences in the detoxification processes, may be a potential chemotherapeutic agent and be useful to treat lung disorders. Yang et al. (2012) examined the effect of crocetin on acute lung injury induced by lipopolysaccharide in male and female mice. Crocetin was administered intragastrically (50 and 100 mg/kg b.w. for 1,122,436 and 48 h) and after the treatment, the results showed that this bioactive compound was able to protect against acute lung injury by reducing oxidative stress indices, compared to the control group (normal saline solution). Lung myeloperoxidase (MPO) activity was significantly reduced, and the SOD activity was significantly increased in mice lung.

Other studies examined crocetin effects to inhibit oxidative stress in intestine and colon. Zhou et al. (2015) researched its protective effects on intestinal injury burn-induced in male rats. Crocetin was administered intraperitoneally immediately after burn injury (100 and 200 mg/kg b.w.), and animals in the control group received a subcutaneous injection of normal saline without crocetin treatment. The results showed that this bioactive compound was able to inhibit oxidative stress, increasing levels of the antioxidant enzymes (SOD, CAT and GPx) and decreasing the lipid peroxidation (MDA content) in intestinal tissue. In addition to its antioxidant activities, crocetin also reduced the levels of proinflammatory response, TNF-α, interleukin-6 (IL-6) and NF-κB activation in intestinal tissue. Kazi and Qian (2009) studied the protective mechanism of crocetin on ulcerative colitis induced by 2,4,6-trinitrobenzene sulfonic acid in female BALB/c mice. The treatment with crocetin (50 mg/kg, intragastrically) for 8 d significantly reduced the lipid peroxidation (MDA levels), the MPO activity and NO levels in the inflamed colon. Therefore, crocetin might be useful as a supplement to ameliorate diarrhea and the disruption of colon, and also to treat intestinal disorders, inhibiting oxidative tissue damage and improving the antioxidant defense system.

Therefore, these in vivo and in vitro studies conducted to date demonstrated the antioxidant properties of crocetin to inhibit oxidative stress, preventing and protecting against oxidative damage, but further research is needed for a deeper understanding of its mechanisms of action and more in vivo intervention studies with patients, including human clinical trials. A summary of the studies conducted to date of the antioxidant effects of crocetin is presented in Table 3 and the possible mechanisms of action involved are shown in Figure 3.

Safranal

Recently, a large number of studies have been focused on the antioxidant activity of safranal to inhibit the oxidative stress which is related with a multitude of diseases. Regarding several in vitro studies, Rahiman et al. (2018) investigated the protective effect of safranal against oxidative damage and apoptosis in endothelial cells. Bovine aortic endothelium cell line was incubated with safranal (21,020 and 40 μM) for 24 h. After the treatment, safranal reduced intracellular ROS levels and the rate of cell apoptosis, which was mediated *via* MAPK signaling pathways, activating stress-activated protein kinases (SAPK)/JNK and ERK1/2. Safranal may be a potential drug for CVDs therapies due to its antioxidant and anti-apoptotic activities. Pan, Qiao, and Wen (2016) studied the potential effect of safranal on Parkinson's disease using an in vitro model induced by rotenone which increased ROS generation and cell apoptosis. Primary dopaminergic cells isolated from rat embryos were incubated with safranal (1,01,520 and 50 μg/ml) for 4 h. The results revealed that safranal decreased intracellular ROS level and inhibited apoptosis. Furthermore, safranal inhibited the expression of Keap1 and upregulated the nuclear translocation of Nrf2. Nrf2 regulates antioxidant genes to protect against oxidative damage, and its transcription activity is controlled by Keap1. Safranal also promoted the antioxidant capacity, since the downstream antioxidant enzyme genes of Nrf2, such as glutamate-cysteine ligase catalytic subunit, GST, heme oxygenase 1, and NADPH-quinone oxidoreductase 1 were induced by this bioactive compound in the dopaminergic neurons. Therefore, safranal could be a potential and therapeutic agent to treat neurodegenerative diseases, protecting neurotoxicity associated with the Keap1/Nrf2 signaling pathway. Bukhari et al. (2015) showed that safranal reduced oxidative damage and prevented apoptosis in normal human bronchial epithelial cells asthma-induced. Cells were incubated with different concentrations of safranal (10 and 100 ng/ml, 1 μg/ml) and after the treatment, NOS levels were significantly reduced, that led to a significant suppression of NO production and a reduction of peroxynitrite ion production, which was related to epithelial cell apoptosis. Therefore, this agent decreased oxidative stress in bronchial epithelial cells *via* reducing NOS and preventing apoptosis in these cells. Hosseinzadeh, Shamsaie and Mehri (2010) evaluated the antioxidant capacity of safranal under in vitro methods, such as deoxyribose assay, erythrocyte membrane lipid peroxidation, and liver microsomal non-enzymatic lipid peroxidation. The results indicated that safranal (0.5, 1, and 2 mM) decreased MDA levels in red blood cells and liver microsomal non-enzymatic lipid peroxidation. In addition, safranal, in deoxyribose assay, showed hydroxyl radical-scavenging effect, increasing antioxidant activity in a dose-dependent manner.

According to in vivo studies with animal models, safranal presented neuroprotective effects due to its antioxidant properties. Samarghandian et al. (2017) investigated about the antioxidant effects of safranal against chronic stress in rat brain. Chronic stress leads to several disorders that induce oxidative damage, producing a higher amount of FRs, generating an imbalance between ROS production and the antioxidant system. Albino rats were treated with safranal (0.25, 0.50, and 0.75 mg/kg b.w. per day, intraperitoneally) for 21 d. In the stressed animals, the levels of GSH and SOD and CAT antioxidant enzymes in the brain tissues

Table 3. Summary of studies conducted to date of the antioxidant effects of crocetin

Experimental model	Crocetin administration	Effects	References
In vitro retinal pigment epithelium model (aged ARPE19 cells)	0, 100 and 200 μM, for 24 h	Activation of the extracellular signal-regulated kinase 1/2	Karimi et al. (2020)
Bovine spermatozoa	1, 2.5 and 5 μM, for 2 or 4 h	Improvement in bovine sperm quality and its fertilization ability by modulating ROS [a] production and lipid peroxidation	Sapanidou et al. (2016)
Human umbilical vein endothelial cells	0.1, 1 and 10 μmol/l	Inhibition of nuclear factor-kappa B activation by decreasing ROS[a] generation and increasing antioxidant content	Zheng et al. (2015)
Human neuroblastoma cells	1–125 μM, for 18 h	Decrease in ROS[a] production and repression of caspase-3 activation	Papandreou et al. (2011)
Rat primary cardiac myocytes	1–10 μM, for 48 h	Inhibition of ROS[a]-dependent MAPK/ERK1/2[b] pathway, GATA binding protein 4 activation and nuclear factor-kappa B signaling	Cai et al. (2009)
Rat primary hepatocytes	5, 10 and 20 μM, for 1 h	Decrease in the lipid peroxidation and superoxide anion formation	Tseng et al. (1995)
Stroke-prone spontaneously hypertensive male rats	Orally, 25 or 50 mg/kg b.w. per day, for 3 weeks	Decrease in 8-hydroxy-2-deoxyguanosine and nitric oxide metabolites nitrite/nitrate levels in urine	Higashino et al. (2014)
Rat with cardiac hypertrophy induced by norepinephrine	Intraperitoneally, 25 and 50 mg/kg b.w., for 15 d	Decrease in the lipid peroxidation and an increase in endogenous antioxidant enzymatic activities in cardiac tissue	Shen and Qian (2006)
Male albino rats with cognitive impairment induced by streptozotocin	Orally, 25 μg/kg b.w., combined with roots of *Nardosatchys jatamansi* and sodium selenite, for 15 d	Decrease in the lipid peroxidation and increase in glutahtione levels and the activity of antioxidant enzymes in rat hippocampus and frontal cortex	Khan et al. (2012)
Male mice with retinal ischemic damage	Orally, 20 mg/kg b.w. twice a day, for 4 days	Reduction in 8-hydroxy-2-deoxyguanosine expression and inhibition of the activation of mitogen-activated protein kinases and redox-sensitive transcriptional factors in the retina	Ishizuka et al. (2013)
Stroke-prone spontaneously hypertensive male rats	Orally, 100 mg/kg b.w.	Decrease in oxidative damage, reducing hydroxyl radicals in the brain	Yoshino et al. (2011)
Month old male Keap1 [3]- dependent oxidative stress detector transgenic mice; human skin-derived fibroblasts cells	Orally, 100 mg/kg b.w. at 2, 4 and 6 h after the initiation of ultraviolet-A irradiation; in vitro: 1 μM, for 1 h	Decrease in the lipid peroxidation in the skin; in vitro effects, reduction in ROS[a] generation and the expression levels of cleaved caspase-3	Ohba et al. (2016)
Male albino mice with mitochondrial damage induced by benzo(a)pyrene	Intraperitoneally, 20 mg/kg b.w., for 18 weeks	Increase in ATPase and mitochondrial enzymes activities and glutathione levels; decrease in ROS[a] generation and lipid peroxides in lungs	Venkatraman et al. (2008)
Lung cancer-bearing male albino mice	Intraperitoneally, 20 mg/kg b.w. dissolved in dimethyl sulphoxide, for 4 weeks	Decrease in the lipid peroxidation, and increase in the activities of antioxidant enzymes and glutathione metabolizing enzymes in lung and liver tissues	Magesh et al. (2006)
Male and female mice with acute lung injury induced by lipopolysaccharide	Intragastrically, 50 and 100 mg/kg b.w., for 1,122,436 and 48 h	Reduction in lung myeloperoxidase activity and increase in superoxide dismutase activity in lungs	Yang et al. (2012)
Male rats with intestinal injury burn-induced	Intraperitoneally, 100 and 200 mg/kg b.w., immediately after burn injury	Increase in antioxidant enzymes levels, decrease in the lipid peroxidation and the levels of proinflammatory response in intestinal tissue	Zhou et al. (2015)
Female BALB/c mice with ulcerative colitis induced by 2,4,6-trinitrobenzene sulfonic acid	Intragastrically, 50 mg/kg, for 8 d	Reduction in the lipid peroxidation, the lung myeloperoxidase activity and nitric oxide levels in the colon	Kazi and Qian (2009)

[a]ROS: reactive oxygen species.
[b]MAPK/ERK1/2: mitogen-activated protein kinases/extracellular signal-regulated kinase 1/2; [3]Keap1: kelch-like ECH-associated protein 1.

were significantly lower and MDA levels were significantly higher than non-stressed rats. The results demonstrated that safranal had a neuroprotective effect because the serum levels of MDA in the brain were significantly lower and levels of GSH and antioxidant enzymes were significantly higher in the treated group compared to the control. These findings indicated that the antioxidant effect of safranal could be mediated *via* increasing antioxidant enzymatic levels and decreasing the lipid peroxidation, being a potential compound to enhance the brain oxidative response in rats

subjected to chronic stress. Baluchnejadmojarad, Mohamadi-Zarch, and Roghani (2019) evaluated the antioxidant effects of safranal on neurodegenerative disorders using male rats with Alzheimer's disease (AD) induced by intrahippocampal amyloid beta. During AD progression, an increment of oxidative damage occurred. Safranal was administered orally (0.025, 0.1, and 0.2 ml/kg b.w./d) for 1 week. The results showed that safranal was able to ameliorate oxidative stress, since it reduced the hippocampal level of MDA decreasing the lipid peroxidation, ROS levels and protein carbonyl content (an index of protein oxidation), and improved SOD activity with no significant and beneficial effect regarding nitrite, CAT, and GSH in hippocampal tissue. Therefore, safranal may prevent learning and memory impairment at a molecular level by reducing oxidative stress. Sadeghnia et al. (2017) studied the neuroprotective effect of safranal on brain injuries in adult male rats of transient focal cerebral ischemia induced by middle cerebral artery occlusion (MCAO). In ischemia, the oxygen restoration could lead to the generation of toxic levels of FRs, which end in lipid peroxidation, inhibition of protein synthesis, and cell death (Ginsberg 2009). Safranal was administered intraperitoneally (72.5 and 145 mg/kg b.w.) at 0, 3, and 6 h after MCAO induction. Markers of oxidative stress were evaluated using left cerebral portions, and the results demonstrated that safranal inhibited the oxidative stress caused by ischemia in the brain tissues of rats treated, since the lipid peroxidation was decreased, reducing MDA levels at both doses of safranal and there was an increment in the antioxidant capacity, and a significant increase in the total-SH content, compared to the control group that received saline solution. Thus, safranal administration had protective effects on ischemic reperfusion by the modulation of oxidative stress markers and increasing the antioxidant activity in brain tissues. Sadeghnia et al. (2013) also demonstrated the protective effect of safranal to the oxidative damage induced by quinolic acid in the hippocampus of adult male rats. The intraperitoneal administration of high doses of safranal (145.5 and 291 mg/kg b.w., after the induction) decreased quinolinic acid-induced lipid peroxidation and the oxidative DNA damage (% tail DNA, comet assay), and restored thiol redox status and the antioxidant power in the hippocampus the treated group, compared to the control group (normal saline solution), showing that safranal could be a useful therapy to prevent and treat neurodegenerative disorders. Another study also indicated the potential effect of this bioactive compound to protect the oxidative damage induced by ischemia-reperfusion injury which leads to overproduction of ROS in the hippocampus of adult male rats. After intraperitoneal safranal administration (727.5, 363.75, 145.5, and 72.75 mg/kg b.w. for 72 h after the induction), the treated group showed a significant increase in the antioxidant capacity and total-SH content and a significant decrease in MDA levels at higher doses in hippocampal tissue compared to the control group, in which saline solution was given intraperitoneally (Hosseinzadeh and Sadeghnia 2005).

In addition to the neuroprotective effects of safranal in the brain, several studies have demonstrated that the antioxidant properties of safranal had a positive effect regarding oxidative stress on other disorders, such as asthma, diabetes, and other pathologies that affects several organs like liver, heart, or skeletal muscle. The study of Hazman and Bozkurt (2015) indicated the potential antioxidant activity of safranal and free radical scavenging activity on the oxidative stress in male albino rats with type 2 diabetes (diabetic nephropathy) induced by high-fat diet and streptozotocin. Safranal, administered intraperitoneally (30 mg/kg b.w.) for 4 weeks, decreased the levels of oxidative stress in kidney by increasing total antioxidant capacity levels and decreasing total oxidant capacity and NO levels and oxidative stress index values (calculated by using total antioxidant capacity and total oxidant capacity levels) in the diabetic group compared to the control group (composed of healthy rats), but did not have a positive effect on GSH. Therefore, safranal due to its antioxidant activity may improve kidney damage in renal tissue and could be effective to treat the diabetic nephropathy. Another study also showed that safranal might be effective in the treatment of type 2 diabetes and its related gastric disorders, since the treatment with safranal (0.25, 2, 5 ml/kg b.w., orally) inhibited gastric lesions, induced by indomethacin in male adult nondiabetic and diabetic rats. Safranal decreased the gastric ulcer index (by dividing total number of ulcer spots by the number of animals), and the lipid peroxidation in the gastric mucosa, and increased GSH levels of gastric tissue in the treated group, compared to the control group (physiological saline). Safranal, like omeprazole, may be a potential antiulcer agent to prevent the gastric mucosa damage in rats (Kianbakht and Mozaffari 2009). Regarding antioxidant effects of safranal against oxidative damage in other pathologies, Bukhari et al. (2015) presented that safranal ameliorated asthma in BALB/c mice. During asthma, NOS levels were induced, and NO production was increased, causing bronchorelaxation and it reacted with superoxide ions, producing peroxynitrite ion that leads to epithelial cell damage. Safranal, administered orally (1 and 10 mg/kg b.w.) for 7 d, reduced the oxidative damage in inflamed lungs due to allergic reaction in mice lungs, decreasing significantly NOS levels and preventing the epithelial cell injury which was associated to peroxynitrite ion production. Farahmand et al. (2013) were focused on the safranal effects during aging in male aged rats, in which the oxidative damage was increased. Safranal was administered intraperitoneally (0.5 mg/kg b.w. per day) for 1 month, and after the treatment in aged rats, the hepatic antioxidant enzymes SOD, CAT, and GST levels increased significantly, and MDA level in liver homogenate and NO serum content decreased significantly. This study elucidated the potential treatment of safranal to prevent oxidative damage in liver and as antiaging compound in old rats.

In addition to these protective effects, other researchers investigated the cardioprotective effect of safranal and its anti-ischemia activity. Mehdizadeh et al. (2013) evaluated the potential effect of safranal against the oxidative damage in heart tissue induced by isoproterenol which leads to myocardial infarction followed by several biochemical alterations

such as lipid peroxidation in male albino rats. Safranal was administered intraperitoneally (0.025, 0.050, and 0.075 ml/kg b.w.) for 8 d, and the results revealed that MDA level in heart tissue was significantly decreased in the treated group, compared to the control group (normal saline solution for 9 d). Safranal protected myocardium from myocardial functional and structural damage through the modulation of antioxidant defense system, reducing the lipid peroxidation. Therefore, it could be effective against heart tissue oxidative damages. Hosseinzadeh, Modaghegh, and Saffari (2009) studied the protective effect of safranal against oxidative damage induced by ischemia-reperfusion injury in skeletal muscles of male rats. Intraperitoneal safranal administration (0.1, 0.25, and 0.5 ml/kg b.w.) showed anti-ischemia activity due to its antioxidant properties by decreasing MDA levels and increasing antioxidant capacity and total thiol content in rat skeletal muscle tissue.

Besides, Tamaddonfard et al. (2014) compared the antioxidant activity of safranal with that of the vitamin E (α-tocopherol), which is a potent antioxidant compound. The effects of safranal on sciatic nerve function in adult male rats were studied after induction of crush injury, being the oxidative stress one of the main causes of nerve damage. Rats were treated with safranal (0.05, 0.2, and 0.8 mg/kg b.w./d, intraperitoneally) during 10 consecutive days or with subcutaneous injection of vitamin E (100 mg/kg b.w./d). Safranal showed a positive effect decreasing blood levels of MDA at doses of 0.2 and 0.8 mg/kg and the same effect was observed in vitamin E in treated groups. The effects of safranal were comparable with those of vitamin E, and both ameliorated sciatic nerve function induced by the modulation of the oxidative stress pathway.

In addition of the rats and mice models, Boskabady, Byrami, and Feizpour (2014) evaluated the effect of safranal on ovalbumin sensitized guinea pigs, who drank water containing three concentrations of the bioactive compound (4, 8, and 16 μg/ml). The results showed that total NO and nitrite serum levels were significantly reduced in treated groups, reporting that safranal might prevent tracheal responses due to its antioxidant effects.

Therefore, these studies conducted to date showed the antioxidant capacity of safranal against oxidative stress, but further in vivo research including human clinical studies is needed to deepen and have a broader knowledge of its mechanisms of action. A summary of the studies conducted to date of the antioxidant effects of safranal is presented in Table 4 and the possible mechanisms of action involved are shown in Figure 3.

The effect of saffron supplementation to improve the health

This point explores the potential mechanisms of action of saffron supplementation to prevent several diseases by in vitro and in vivo studies.

Effects of saffron on cancer

Cancer is the result of uncontrolled cell division due to the effects of genetic and environmental factors. Many strategies have been developed against cancer development, by saffron administration to prevent the growth of cancer cells.

Recently, Akbarpoor et al. (2020) have carried out in vitro investigations to evaluate the effects of different saffron distillate concentrations (20, 40 and 100 μg/ml) on the expression of some self-renewal genes in gastric adenocarcinoma tumor cell line. The results showed that saffron administration reduced the expression of self-renewal genes, but this reduction was depended of the concentration of saffron extracts and on the duration of the treatment. The best concentrations were 40 and 100 μg/ml and the incubation time of 48 h. Bathaie et al. (2013) explored the therapeutic potential of saffron aqueous extract on gastric cancer. Three groups of albino rats were administered with 100,150 and 175 mg/kg b.w./d, respectively, of the extract by intraperitoneal injection for 50 d. The results indicated that the higher doses of saffron aqueous extracts inhibited the cancer progression.

Positive results have also been reported in breast cancer using MCF7 cell line human. Saffron aqueous extract in different concentrations (100, 200, 400 and 800 μg/ml) was tested on matrix metalloproteinases (MMP) gene expression. The MMP play functions by degrading and modifying the cell-extracellular matrix and cell–cell contacts. In this in vitro test, all concentrations showed an inhibitory effect on *MMP* gene expression respect to the control sample. However, the inhibitory effect was not dose-dependent. The results showed that the MCF-7 cells treated with saffron at 200 μg/ml showed the best reduction (18%) in gene expression (Mousavi, Baharara, and Asadi-Samani 2014). The same author investigated the synergic effect of saffron and electromagnetic field (EMF) on the vascular endothelial growth factor receptor 2 (*VEGFR-2*) gene expression on the same cell line culture and with the same saffron concentrations. The VEGFR-2 activation is considered critical in the proliferation and survival of the endothelial cells and is the primary receptor involved in angiogenesis. The results showed that both treatments induced a considerable reduction on VEGFR-2 gene expression respect to the control group when administered separately. However, the synergic administration showed higher reduction on the VEGFR-2 gene expression with 29%, 35%, and 36% reduction using 200,400 and 800 μg/ml of saffron, respectively. A higher reduction using the synergy method was observed from the lowest concentration of saffron (100 μg/ml) with a 38% of reduction (Mousavi, Baharara, and Shahrokhabadi 2014). Therefore, these preliminary investigations proved that saffron could be a good chemotherapeutic agent in breast cancer treatment. A study conducted by Makhlouf et al. (2016) showed that the saffron extract administration on Jurkat cells was better to exert antiproliferative activities against human acute lymphoblastic cells than the crocin and safranal mixture administration. However, these in vitro studies must be corroborated by further in vivo research.

Table 4. Summary of studies conducted to date of the antioxidant effects of safranal

Experimental model	Safranal administration	Effects	References
Bovine aortic endothelium cells	21,020 and 40 μM, for 24 h	Reduction of intracellular ROS[a] levels and the rate of cell apoptosis	Rahiman et al. (2018)
Primary dopaminergic cells from rat embryos	1,01,520 and 50 μg/ml, for 4 h	Decrease of intracellular ROS[a] level and inhibition of apoptosis	Pan, Qiao, and Wen (2016)
Human bronchial epithelial cells asthma-induced; BALB/c mice asthma-induced	10 ng/ml, 100 ng/ml, and 1 μg/ml; orally, 1 and 10 mg/kg b.w. for 7 d	In vivo and in vitro effects: suppression of nitric oxide and peroxynitrite ion production and nitric oxide synthase levels	Bukhari et al. (2015)
Rat liver microsomes and red blood cells	0.5, 1, and 2 mM	Decrease of the lipid peroxidation in red blood cells and liver microsomal non-enzymatic lipid peroxidation	Hosseinzadeh, Shamsaie, Mehri (2010)
Albino rats with chronic stress	Intraperitoneally, 0.25, 0.50, and 0.75 mg/kg b.w. per day, for 21 d	Reduction of MDA[b] and glutathione levels, and increase of the antioxidant enzymes content in the brain	Samarghandian et al. (2017)
Male rats with Alzheimer's disease induced by intrahippocampal amyloid beta	Orally, 0.025, 0.1, and 0.2 ml/kg b.w. per day, for 1 week	Decrease of MDA[b] and ROS[a] hippocampal levels, the protein carbonyl content, the index of protein oxidation, and improvement of superoxide dismutase activity	Baluchnejadmojarad, Mohamadi-Zarch, and Roghani (2019)
Male rats with transient focal cerebral ischemia	Intraperitoneally, 72.5 and 145 mg/kg b.w. at 0, 3 and 6 h after the induction	Reduction of the lipid peroxidation, an increment in the antioxidant capacity and in the total sulfhydryl content	Sadeghnia et al. (2017)
Male rats with oxidative damage induced by quinolic acid in the hippocampus	Intraperitoneally, 145.5 and 291 mg/kg b.w. after the induction	Decrease of the lipid peroxidation and the oxidative DNA damage, and restoration of thiol redox status and the antioxidant power in the hippocampus	Sadeghnia et al. (2013)
Male rats with oxidative damage induced by ischemia-reperfusion injury in the hippocampus	Intraperitoneally, 727.5, 363.75, 145.5 and 72.75 mg/kg, for 72 h after the induction	Increase of the antioxidant capacity and total sulfhydryl content and a decrease of MDA[b] levels in hippocampal tissue	Hosseinzadeh and Sadeghnia (2005)
Male albino rats with diabetic nephropathy induced by high-fat diet and streptozotocin	Intraperitoneally, 30 mg/kg b.w., for 4 weeks	Decrease of the total oxidant capacity, nitric oxide levels and the oxidative stress index values; increase of total antioxidant capacity levels in kidney	Hazman and Bozkurt (2015)
Diabetic and nondiabetic male rats with gastric ulcers induced by indomethacin	Orally, 0.25, 2 and 5 ml/kg b.w.	Decrease of the gastric ulcer index and the lipid peroxidation, and increase of glutathione levels in the gastric mucosa	Kianbakht and Mozaffari (2009)
Aged male rats	Intraperitoneally, 0.5 mg/kg b.w. per day, for one month	Increase of the hepatic antioxidant enzymes, and decrease of MDA[b] level in liver homogenate and nitric oxide serum content	Farahmand et al. (2013)
Male albino rats with oxidative damage in heart tissue induced by isoproterenol	Intraperitoneally, 0.025, 0.050 and 0.075 ml/kg b.w., for 8 d	Reduction of MDA[b] level in heart tissue	Mehdizadeh et al. (2013)
Male rats with oxidative damage induced by ischemia-reperfusion injury in skeletal muscles	Intraperitoneally, 0.1, 0.25 and 0.5 ml/kg b.w.	Decrease of MDA[b] levels and increase of antioxidant capacity and total thiol content in skeletal muscle tissue	Hosseinzadeh, Modaghegh, Saffari (2009)
Male rats crush injury induction of sciatic nerve	Intraperitoneally, 0.05, 0.2 and 0.8 mg/kg b.w. per day, for 10 d	Decrease of MDA[b] blood levels	Tamaddonfard et al. (2014)
Ovalbumin sensitized guinea pigs	Orally, 4, 8 and 16 μg/ml	Reduction of total nitric oxide and nitrite serum levels	Boskabady, Byrami, and Feizpour (2014)

[a]ROS: reactive oxygen species.
[b]MDA: malondialdehyde.

Effects of saffron on the neurological disorders

Neurological disorders exert a great influence on our society worldwide. Although the symptoms derived from these diseases are well known, the causes and mechanisms are complex and depend on multiple factors (Fernández, Valero-Cases, and Rincón-Frutos 2019).

Currently, there is a controversy in the results regarding the saffron administration and its beneficial effects on depressive disorder. In one double-blind, controlled clinical trial with 30 mg/d of saffron capsule (containing saffron extract) or 40 mg/d of citalopram during 6 weeks as a treatment of major depressive disorder with anxious distress, 66 patients were recruited during visits to the psychiatric clinic to participate in the study. The results did not show any difference between the two experimental groups. Therefore, the saffron administration could be a good alternative to treat major depressive disorder with an anxious distress (Ghajar et al. 2017). In another double-blind, controlled clinical trial,

Table 5. Summary of studies conducted to date of the effects of saffron supplementation to improve health

Experimental model	Saffron administration	Effects	References
Gastric adenocarcinoma tumor cell line	Saffron distillates concentrations, 20, 40 and 100 μg/ml	Reduction in the expression of self-renewal. The best concentrations were 40 and 100 μg/ml and the incubation time of 48 h	Akbarpoor et al. (2020)
Albino rats with gastric cancer	Saffron aqueous extract, intraperitoneally, 1,00,150 and 175 mg/kg b.w. per day, for 50 d	The higher doses of saffron aqueous extracts inhibited the cancer progression	Bathaie et al. (2013)
Breast cancer using MCF7 cell line human	Saffron aqueous extract, 100,200,400 and 800 μg/ml	MCF-7 cells treated with saffron at 200 μg/ml showed the best reduction (18%) in gene expression	Mousavi, Baharara, and Asadi-Samani (2014)
MCF7 cell line human	Synergic effect of saffron aqueous extract at 100,200,400 and 800 μg/ml and electromagnetic field	A higher reduction (38%) on the VEGFR-2 gene expression using the synergy method with the lowest concentration of saffron	Mousavi, Baharara, and Shahrokhabadi (2014)
Jurkat cells	Crocin and safranal mixture administration	Antiproliferative activities against human acute lymphoblastic cells	Makhlouf et al. (2016)
Double-blind, controlled clinical trial	Saffron capsules, 30 mg/d, for 6 weeks	The same efficacy to treat major depressive disorder with an anxious distress compared to reference treatment	Ghajar et al. (2017)
Double-blind, controlled clinical trial	Saffron capsules, 30 mg/d, or fluoexetin, 40 mg per day, for 6 weeks	The same efficacy in improving depression compared to reference treatment	Shahmansouri et al. (2014)
Double-blind, controlled clinical trial	Saffron capsules, 15 mg twice a day, for 12 weeks	The results did not show significant differences between placebo or saffron groups	Moazen-Zadeh et al. (2018)
Meta-analysis	Saffron extract administration	Significant reduction in beck anxiety inventory and Pittsburgh sleep quality index and beck anxiety inventory respect to antidepressant common drugs	Ghaderi et al. (2020)
Systematic review	Saffron extract supplementation	Similar effect in improving cognitive impairment to the reference Alzheimer's drug	Avgerinos et al. (2020)
Systematic review	Saffron extract administration	Positive impact on fasting blood glucose	Giannoulaki et al. (2020)
Meta-analysis	Saffron extract supplementation	Beneficial effect on fasting plasma glucose and WC after 12 weeks with saffron supplementation	Rahmani, Saberzadeh, and Takhshid (2020)
Randomized double-blind	Powered saffron, 100 mg/d, for 8 weeks	Improve the inflammation status and significant effect on the reduction of glucose levels in patients with type 2 diabetes mellitus	Mobasseri et al. (2020)

40 patients with a mild to moderate depression in post percutaneous coronary intervention were randomized to receive 30 mg/d of saffron in capsules or 40 mg/d of fluoexetine during 6 weeks. The results showed no significant differences between the two experimental groups. Therefore, saffron administration showed the same efficacy in improving depression compared to treatment with fluoexetine (Shahmansouri et al. 2014).

On the other hand, in a double-blind, controlled clinical trial, patients undergoing coronary artery bypass grafting (CABG) with depression and anxiety were randomized to receive saffron capsules (15 mg twice a day) or placebo during 12 weeks. However, the results did not show significative differences between placebo or saffron groups. Therefore, these results did not support the saffron efficacy in improving anxiety and depression related to CABG (Moazen-Zadeh et al. 2018).

These studies showed controversial results, although they administered the same dose of saffron, and showed the same limitations: short observation periods and reduced sample size. Moreover, the studies reported by Ghajar et al. (2017) and Shahmansouri et al. (2014) did not consider a placebo group in experimentation.

Therefore, to summarize all research carried out to evaluate the saffron effect on mental health parameters and C-reactive protein a recent meta-analysis with 21 randomized clinical trials was conducted by Ghaderi et al. (2020). This meta-analysis concluded that the saffron administration did not affect C-reactive protein levels, Hamilton depression rating scale, and Hamilton anxiety rating scale levels. However, saffron administration showed a significant reduction in beck anxiety inventory and Pittsburgh sleep quality index and beck anxiety inventory respect to antidepressant common drugs such as: imipramine, fluoxetine, and citalopram. However, the results of this meta-analysis should be interpreted with caution due to heterogeneity of factors such as the period of administration, the concentration of saffron used, the differences between the samples of saffron (crocin, saffron liquid extract, or powder) that were used. All these variabilities led to different effects on the parameters of mental health investigated.

In a recent systematic review, the effect of saffron supplementation in relation to cognitive impairment such as mild cognitive impairment or AD was also investigated. A total of five randomized controlled trials were included: one study

with cognitively normal individuals and four studies with subjects with mild cognitive impairment or AD. Saffron supplementation was compared with Alzheimer's medication or placebo. Results showed that saffron supplementation had a similar effect in improving cognitive impairment to the reference Alzheimer's drug. Those results could be in relation with the potential effect of saffron against oxidative stress that takes place in the Alzheimer's brain. However, the results should be interpreted carefully because the study presents a potential high risk of bias due to known and unknown factors. Therefore, studies with more individuals to reduce the risk of bias are required to confirm the supplementation of saffron as an alternative treatment for these diseases (Avgerinos et al. 2020).

Effects of saffron on the cardiovascular diseases

CVDs are the major cause of mortality and morbidity worldwide. Among the risk factor of CVDs, diabetes, metabolic syndrome, genetics, high blood pressure, obesity, and hyperlipidemia are the most common (Armenian et al. 2017; Chen et al. 2020).

A recent systematic review evaluated the effect of saffron supplementation on the metabolic profile in patients with metabolic syndrome or diabetes mellitus. This systematic review included 14 randomized control trials: one studied the impact of saffron in patients with prediabetes, one with coronary artery disease, four with metabolic syndrome and eight patients with diabetes mellitus. The results showed that the saffron supplementation had an impact on fasting blood glucose. However, authors commented some limitations such as the high heterogeneity in the studies, no report of titration in the administered supplement and few different protocols. Nevertheless, being a systematic review neither analysis nor meta-analysis was carried, only the research trails were evaluated qualitatively (Giannoulaki et al. 2020).

However, a recent meta-analysis conducted by Rahmani, Saberzadeh, and Takhshid (2020) reviewed the saffron effect on fasting plasma glucose, waist circumference (WC), and hemoglobin A1c (HbA1c). These parameters are used to detected and monitor the metabolic syndrome and diabetes mellitus. After removing the studies that were not in compliance with the inclusion criteria, 9 randomized clinical trial studies were selected. The results showed that saffron supplementation has not a significant effect on HbA1c. However, the meta-analysis found beneficial effect on fasting plasma glucose and WC after 12 weeks with saffron supplementation.

At the same time, the effect of saffron supplementation on inflammation and glycaemia in patients with type 2 diabetes mellitus (DM2) has been investigated in a randomized double-blind, placebo-controlled clinical trial study. Two groups with 30 patients with DM2 were randomized in each one. For 8 weeks, one group was treated with 100 mg/d of powered saffron and another group, was treated with a placebo. The results showed that saffron supplementation reduced the TNF-α and IL-6, which play an important role as inflammatory markers and are present in a DM2 before detection of the disease. This reduction improved the inflammation status. At the same time, the saffron administration also had a significant effect on the reduction of glucose levels respect to the control placebo group (Mobasseri et al. 2020).

A summary of the studies conducted to date of the effects of saffron extracts supplementation to improve the health is presented in Table 5 and the possible mechanisms of action involved are shown in Figure 3.

Conclusions and future perspectives

The results of the in vivo and in vitro studies conducted to date about the antioxidant effects of the supplementation with saffron or its bioactive compounds crocin, crocetin, and safranal show encouraging effects in the prevention of certain diseases related to oxidative stress, such as cardiovascular and respiratory diseases, cancer and neurological disorders, among others. The acute and sub-acute toxicity of crocin, crocetin, and safranal have been studied in different in vivo research carried out in mice and rats, after oral or intraperitoneal administration, demonstrating that they did not show any toxic effects. Some studies also revealed that they are not genotoxic or teratogenic.

However, although there are many studies carried out with crocin, crocetin, and safranal showing the effects of these compounds as oxidative stress inhibitors, their mechanisms of action are not still completely elucidated. Therefore, further in vivo research on human clinical studies with a larger number of individuals is needed, expanding the administration time, and with the inclusion of control groups for a deeper understanding of the pathways and possible mechanisms of action involved in these antioxidant effects. Moreover, the great antioxidant effect against oxidative stress of crocin, crocetin, and safranal, makes saffron a promising source of bioactive extracts. Therefore, future research in the field of the potential of saffron derived bioactive ingredients for the development of functional foods is needed. This point explores the potential mechanisms of action of saffron supplementation to prevent several diseases by in vitro and in vivo studies.

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