

Therapeutic effects of saffron (*Crocus sativus* L.) in digestive disorders: a review

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ABSTRACT

Saffron, the dried red-orange stigmas of *Crocus sativus* L, has been known as a flavoring agent, food coloring and traditional herbal medicine. Pharmacological effects of saffron are mainly attributed to crocin, crocetin, picrocrocin and safranal. These components especially crocin, have significant effects including antidepressant and anticonvulsant, analgesic, anti-cancer and other therapeutic effects on different parts of our body namely cardiovascular, immune, respiratory, genital-urinary and central nervous system. According to the reports and findings, saffron plays a key role to cure different digestive system disorders via chemopreventive, inhibition of cell proliferation, induction of apoptosis, antioxidant effects and radical scavenging, genoprotective property, prevention of lipid peroxidation and anti-inflammatory processes. The outcome of the above mentioned mechanisms shows potential therapeutic properties of saffron against liver cancer, hepatotoxicity, fatty liver, hyperlipidemia, stomach cancer, peptic ulcer, colon cancer, ulcerative colitis, diabetes and pancreas cancer and ileum contractions. According to global statistics, the susceptibility to intestinal diseases is considered as a significant matter and can be important in health planning in any community. Several strategies for treatment and prevention of the digestive system diseases have provided that the use of herbal remedies seems effective and useful. Considering the available findings, the present study aims to introduce saffron as a prophylactic and therapeutic agent against gastrointestinal tract disorders. However, further clinical studies seem necessary in various aspects of saffron effects in different parts of body to verify these findings.

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Introduction

Saffron is a dietary spice derived from the flowers of *Crocus sativus* - Iridaceae. There are more than 150 different compounds in saffron comprising carbohydrates, polypeptides, lipids, H₂O, minerals and vitamins. Beside these constituents, saffron has four main bioactive components including crocin (C₄₄H₆₄O₂₄), crocetin (C₂₀H₂₄O₄), picrocrocin (C₁₆H₂₆O₇) and safranal (C₁₀H₁₄O) (Figure 1). The chemical structure of crocin consists of crocetin as a central core and two sugars that are responsible for the color of the compound. It is hydrolyzed and absorbed as the active metabolite crocetin in the intestines. Picrocrocin is another constituent of saffron and has a bitter taste. The aroma and odor of saffron is due to the presence of safranal which is the metabolite of picrocrocin (1-3).

From a long time ago, saffron has been used as an herbal medicine. The ancient Persians and Egyptians and Medieval Europeans used it as an aphrodisiac (4), lumbar pain remedy (5), a general-use antidote against poisoning (6), a treatment for dysentery and

measles (7). Also, it has been used to cure pre-eclampsia (8), inflammation, wounds and abscesses (9). At present, modern pharmacological investigations have indicated that saffron and its constituents has a lot of therapeutic roles including anti-cancer and anti-tumor activity, anti-neuropathic pain effects (10-12), anti-genotoxic and chemoprotective properties (13), prevention of renal ischemia-reperfusion (14) and human rhabdomyosarcoma (RD) cells (15), anti-diabetic (16), antitussive (17), prevention of liver and spleen enlargement (18), reducing withdrawal syndrome (19), a potent relaxant effect on smooth muscles (20). Additionally, improving disease condition in adjuvant-induced arthritis (21), reduction of oxidative damages in kidney, skeletal muscle and hippocampus are among other saffron properties that can be mentioned (22, 23). Moreover, saffron shows various medicinal effects on different parts of body that have been summarized in Table 1.

The above mentioned findings reveal that much research has been conducted on saffron and

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Table 1. Various effects of saffron in different parts of body

Site of Action	Effects	Effective material	Dosage	Reference	
Central nervous system	Anticonvulsant	Safranal	(0.15, 0.35 ml/kg)	24	
		Safranal	(72.75,145.5,291 mg/kg)	25	
		Aqueous/ ethanolic extracts of saffron	(0.08-0.80 g/kg)/(0.2-2.0 g/kg)	26	
	Reducing neurotransmitters release	Safranal	(291 mg/kg)	27	
		Antidepressant	Kaempferol	(100, 200 mg/kg in mice) (50 mg/kg in rats)	28
	Neuroprotective Anti-Parkinson, anti-Alzheimer, improves memory and learning skills	Aqueous extract of saffron	Aqueous extract of saffron	(40, 80 and 160 mg/kg/day)	29
			Crocic	(12.5,25,50 mg/kg)	30
		Safranal Crocin	Aqueous and ethanolic extracts of stigma	(0.5 ml/kg) (50-600 mg/kg) (0.2-0.8 g/kg)	31
			Crocic	(10-50 μM)	32
		Crocic	-----	-----	10
			Crocetin	(8 mg/kg)	33
		Anxiolytic and hypnotic	Safranal	(0.15,0.35 ml/kg)	34
	Against cerebral ischemia, brain damage and memory deficits	Saffron aqueous extract	Saffron aqueous extract	(0.56 g/kg)	34
			Crocic	(25 mg/kg)	35
		Saffron extract	(250 mg/kg)	35	
Crocic-Safranal		(different dosage)	36		
Saffron extract		(20, 40, 80, 160 mg/kg/day)	37		
Safranal		(0.025, 0.050, 0.075 ml/kg)	37		
Crocic		(25,50 mg/kg/day)	38		
Effect on atherosclerosis	Crocic	(25,50,100 mg/kg/day)	39		
Effect on hypertension	Crocic	(12.5, 25,50 mg/kg)	40		
Hyperlipidemia (reduction of cholesterol, triglyceride and low-density lipoprotein cholesterol)	Crocic	(25 to 100 mg/kg/day)	41		
Reduction of hematological toxicity	Safranal	(0.025, 0.05, 0.1 ml/kg)	42		
		Crocic	(50, 100, 200 mg/kg)	42	

	No effect on coagulant and anticoagulant system	Saffron	(200, 400 mg/week)	43
Immunity system	Decreasing of red blood cells, hemoglobin, hematocrit, platelets and increasing of sodium, blood urea nitrogen and creatinine	Saffron	(400 mg/week)	44
	Against of infectious disease (measles, smallpox and scarlet fever) and antibacterial, antiseptic, antifungal effects	Saffron components	-----	10
	Anti-nociceptive effect	Saffron	-----	45
	Analgesic and anti-inflammatory (earache, tooth-ache, swelling, otitis, anal pain, gout, cancer pain, gingivitis). Reduce the discomfort of teething infants	Safranal Saffron components	(0.1, 0.3, 0.5 ml/kg) -----	46 10, 47
Genital and urinary system	Ejaculation latency and erection disorders	Crocin Aqueous extract of saffron Saffron	(100, 200, 400mg/kg) (160 and 320mg/kg)	4
	Effects on male Sexual behavior and female Sexual behavior such as premenstrual Syndrome (PMS)	Saffron	(200mg) -----	48 16
	Improving renal function	Saffron	-----	49
Eye	Lacrimation, poor eyesight, day blindness, retina and corneal disease and cataract. Increases blood flow in choroid	Saffron components	-----	10
Skin	Sunscreen and moisturizing properties	Liposomes containing safranal	(8%)	50
	Used as emollient and anti-pruritic agent, add the shine, lighten the skin, reduce dark pigments, dark circles under the eyes, acne, pimple and treatment of other skin problems extensively	Lotions containing saffron	(4%)	51

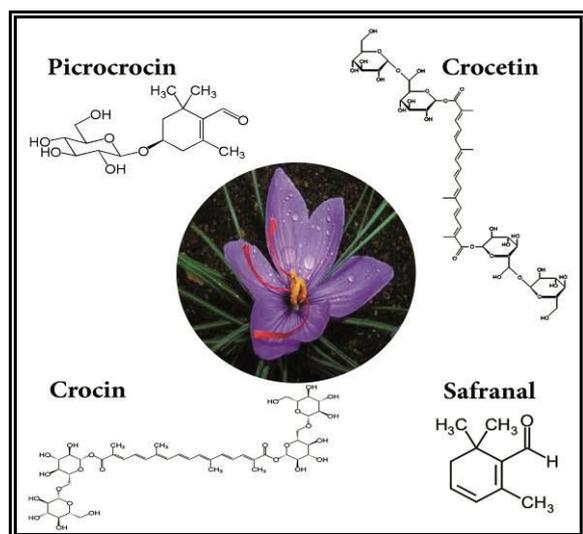


Figure 1. Major constituents of saffron

beneficial properties of this amazing spice has been confirmed. The studies show that the prophylactic and therapeutic role of saffron is done through its antioxidant effects, inhibition of cell proliferation, induction of apoptosis and genoprotective property. Regarding to potential therapeutic properties of saffron, we try to review various aspects of beneficial medicinal effects of saffron in varying parts of gastrointestinal system such as stomach, liver, pancreas, colon and ileum and provide comprehensive documentations to introduce traditional herbal medicines namely saffron.

Methods

To meet our objectives, we searched SciVerse (Science Direct and Scopus), PubMed, SpringerLink, Wiley Online Library and Google Scholar databases. We made use of a list of keywords such as saffron, *Crocus sativus*, crocin, crocetin, picrocrocin, safranal, stomach, liver, intestine, gastrointestinal and digestive system. We collated all relevant data published from 1991 up to the present.

Effects of saffron on gastrointestinal system

To date, a lot of researches have been done to determine the therapeutic applications and side effects of chemical drugs. Although there is substantial progress in this connection, recently a new requirement has been felt in medical science and novel replacement sources instead of chemical drugs seems necessary. This source is plants that is effective, affordable and harmless and also has therapeutic effects. Accordingly, the scientists are keen to evaluate various effects of medicinal plants. From the distant past to the present time, saffron is one of medicinal plants that its miracle effect in the treatment of many kinds of disorders in different parts of body is notable. But the effects of saffron and

its main constituents in gastrointestinal system have not been taken into consideration comprehensively and completely. Because of the above mentioned reasons, in this review, we evaluate the properties of saffron in almost all parts of gastrointestinal system.

Absorption of saffron and its ingredients

Studies showed that crocin is not absorbed throughout the gastrointestinal tract. Following oral administration of crocin, it is hydrolyzed to crocetin. After intestinal absorption, crocetin is partially metabolized to mono- and di-glucuronide conjugates. It is worth mentioning that crocetin concentrations of plasma do not tend to rise with frequent oral doses of crocin (52). Liu in 2002, Chryssanthi in 2011 and Mohammadpour in 2013 introduced an HPLC method for the quantification of crocetin in rat and human plasma. The results of their findings suggested that crocetin is absorbed into the blood through the gastrointestinal tract quickly and the peak concentration of crocetin appearance after little time in plasma and can remain for long time (53-55). In 2004, Jin stated that crocin cannot be absorbed in the whole intestinal tract. This research indicated that reduction of crocin-1 in various intestine segments such as duodenum, jejunum, ileum and colon were 13.81%, 9.89%, 10.07%, 10.04% respectively. On the other hand, degradation of crocin-1 during incubation in refluxing solution of various intestinal segments were 13.01%, 10.11%, 9.95%, 10.45% for duodenum, jejunum, ileum and colon respectively and crocin-1 cannot be traced in blood. Based on the above-mentioned data, crocin cannot be absorbed equally throughout all parts of the intestinal tract (56). In connection with absorption of saffron, in another study, it has been found that starch could be able to enhance bioavailability of crocin in gastric lumen. Crocin in foods is mixed with salivary nitrite in the oral cavity and it is mixed into gastric juice in the stomach, then reactions between crocin and nitrite in the gastric lumen occurs. Crocin reduces nitrite to nitric oxide (NO) under the gastric conditions of (pH= 2.0). The formation of starch/crocetin complexes can repress the redox reaction. Therefore, transportation of crocin to the intestine can be increased (57). In 2007 Xi and colleagues evaluated the pharmacokinetic properties of crocin following oral administration in rats. After investigation, it was found that orally administered crocin is not absorbed either after a single dose or repeated doses. Also crocin is excreted largely through the intestinal tract following oral administration. In addition, crocetin levels in plasma do not tend to accumulate with repeated oral doses of crocin and the intestinal tract serves as an important site for crocin hydrolysis (58). Zhang *et al* investigated the pharmacokinetic characteristics and intestinal absorption of crocin-1. They indicated that

crocin-1 is absorbed as crocetin. It was proposed that the *in vivo* pharmacological activities of crocin-1 are mostly due to crocetin (59). Also, Li *et al* conducted a study about the association between Tibetan medicine Zuotai and pharmacokinetics of crocin-1. Plasma concentration of crocin-1 was determined by reversed-phase high-performance liquid chromatography (RP-HPLC). Analysis the data demonstrated that the absorption degree of crocin-1 after administration of Zuotai in rats is significantly augmented and the clearance rate is significantly diminished (60). In addition, Asai and colleagues compared the absorption of crocetin and crocin. Their findings showed that orally administered crocetin is rapidly absorbed into the blood circulation and can be traced in plasma as an intact free form and as glucuronide conjugates (crocetin-monoglucuronide and crocetin -diglucuronide). Crocetin and its glucuronide conjugates were also found in crocins-administered mouse plasma, whereas intact crocins (glycoside forms) were not detected. Based on this research, orally administered crocins are hydrolyzed to crocetin before or during intestinal absorption and absorbed crocetin are partly metabolized to mono- and di-glucuronide conjugates (61).

Effects on abnormalities of stomach

Saffron and its pharmacological activities have been described by Avicenna long time ago. He mentioned various biological effects of saffron in Canon of Medicine including strengthens the stomach, reduction of appetite and gastric acidity and improving digestion (62).

Peptic ulcer

Gastric ulcer is peptic ulcer and the result of caustic effects of acid and pepsin. Kianbakht and colleagues compared the effects of the ethanol extract of saffron (its active constituents, crocin and safranal) and omeprazole against gastric ulcer induced by indomethacin in non-diabetic and streptozocin diabetic rats. This study provided evidence that the effect of saffron in treatment of gastric ulcer as compared with omeprazole was satisfactory. Having antioxidant properties, saffron can be regarded as an agent to prevent the gastric mucosa damage through enriching the glutathione levels as well as reducing the lipid peroxidation (8). In a similar study, Inoue *et al* demonstrated anti-ulcer properties of N-095, a nutrient drug containing 90 mg of saffron per daily dose. The results proved that saffron can inhibit stress ulcers and histamine-induced ulcers (63). Also, Nabavizadeh *et al* investigated the effects of saffron extract on gastric acid and pepsin secretion. The results of the research revealed that aqueous extract of saffron increased basal, pepsin and stimulated acid secretion through

an increase in nitric oxide (NO) (64). Al-Mofleh expressed that saffron is an agent against gastric ulcer. To prove this hypothesis, he and his colleagues induced gastric ulcer by pylorus ligation, indomethacin and various necrotizing agents including (80% ethanol, 0.2 M NaOH and 25% NaCl). Also they evaluated gastric wall mucus, non-protein sulfhydryl contents, histopathological assessment of rat stomach, basal gastric secretion and ulcer index. According to the results, they demonstrated that saffron has significant antisecretory and anti-ulcer activities (65). In recent research, saffron as a whole has been examined but the efficacy of each saffron ingredients and statistical comparison are still unclear. Although the above mentioned studies revealed positive results, the findings shows that peptic ulcer can be originated from different factors, on the other hand a great many drugs may influence peptic ulcer and their interaction with saffron is not still known. Therefore, further studies and clinical trials seems necessary to compare saffron to other medical therapies in the treatment of peptic ulcer resulting from various causes.

Stomach cancers

Stomach cancers are classified according to the type of tissue where they originate. The most common type of stomach cancer is adenocarcinoma, which starts in the glandular tissue of the stomach. There are various chemotherapeutic drugs for treatment of this kind of cancer but their side effects are unavoidable. Considering safety, cost-effectiveness and efficiency, plants can be regarded as a promising source in new drug enhancement. He *et al* illustrated that crocetin is able to treat gastric cancer without any specific and serious side effects. To detect the efficiency of crocetin, the apoptosis of BGC-823 human GC cells and mitochondrial performance was considered. The results revealed that BGC-823 human GC cells were treated with crocetin (66). Also in another study, Bathaie *et al* introduced crocetin as an anti-cancer agent in both human and rat adenocarcinoma gastric cancer cells. In this research, 1-methyl-3-nitro-1-nitrosoguanidine (MNNG) was used to induce gastric cancer in rats. For evaluation of crocetin effects, some parameters such as caspases activity, Bcl-2 and Bax expression, antioxidant activity and lactate dehydrogenase in serum were considered. Based on these parameters, antioxidant, antiproliferative and apoptotic activities are the main effects of crocetin against gastric cancer (67). In the same study, Hoshyar *et al* displayed that crocin, another saffron active ingredients, has anti-cancer property like crocetin. They selected alteration of the Bax/Bcl-2 ratio as a marker of apoptosis. In conclusion, they found that crocin is a potential anti-cancer agent and it is able to treat gastric adenocarcinoma cells by

induction of apoptosis as a biological phenomenon can lead to death of the cancer cells (68). From the above studies, it is apparent that in coping with cancer, saffron ingredients act in different ways such as antioxidant activity, antiproliferative activity and induction of apoptosis. Considering interaction between all above mentioned different ways, various components of saffron, various anti-stomach cancer drugs and different causes of stomach cancer, more studies are needed for definitive conclusions on cancer prevention, especially with saffron supplements.

Effects on abnormalities related to liver function

Liver is one of the most important and vital organ of the digestive system that has various essential functions including metabolism, detoxification, storage, production and immunity (69). Considering the influential functions of liver, it can play a key role in measuring the impact varying drugs have on body as well as the therapeutic effects of saffron. According to Avicenna's book of the Canon of Medicine, there are various hepatoprotective herbs such as *C. sativus*, *Pistacia lentiscus*, and *Cinnamomum spp* for treatment of liver injury from many years ago. Among these ancient medicinal plants, it seems that *C. sativus* has the high therapeutic potential on liver disorders (70). Modaresi *et al* stated that the consumption of saffron has an impact on the synthesis of serum proteins like albumin via changing the status of liver cell function (71).

Antioxidant effect on liver

Oxidative stress can lead to a range of diseases such as subclinical hepatitis without jaundice, inflammatory necrotic hepatitis, liver cirrhosis, cancer and other liver disorders. Antioxidant agents can reduce destructive effects of oxidative stress. In a study, the hepatoprotective properties of ethanolic extract of saffron were evaluated in streptozotocin-induced diabetic rats. Several parameters were considered such as aminotransferases, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) to determine the concentration of intracellular hepatic enzymes, alkaline phosphatase (ALP) and bilirubin as a marker of hepatocyte injury, albumin as a liver synthetic function, the lipid peroxidation product, malondialdehyde (MDA) and reduced glutathione (GSH) content to distinguish free radical activity in the liver tissues, the enzymatic activities of glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and catalase (CAT) as an indicators of anti-oxidation in liver tissue. Based on the assessing of these parameters, the result indicated the hepatoprotective effect of ethanolic extract of saffron (*C. sativus* L. stigma) in liver injury and

streptozotocin-induced diabetic rats because of antioxidant effect of saffron (72). Farahmand *et al* showed that aging can cause increasing of free radical production and oxidative stress in liver and how safranal can modify it. In different ages, the alteration of antioxidant enzymes activities (superoxide dismutase, glutathione-S-transferase and catalase), lipid peroxidation levels and serum nitric oxide occurring in liver were observed. Considering alteration of these materials proved that safranal is a candidate to stop the development of age-induced damage by protecting against oxidative stress and increment of antioxidant defense. It was proposed that safranal properties led to suppress lesions from liver and increasing the activation of antioxidative enzymes (73). Based on the study of Ramadan, the application of the ethanolic extracts of *C. sativus* and propolis does not have any toxicological effects, mortalities, meaningful changes in liver and kidney functions of rats because of the antioxidant activities of *C. sativus* (74). The antioxidant capacity of crocetin and crocin was evaluated *in vivo* by Chen *et al*. Also, superoxide dismutase (SOD) in liver and kidney, glutathione peroxidase (GSH-Px) in liver, total antioxidant capacity (TAOC) in heart and kidney and malondialdehyde (MDA) were measured. The fluctuation of these indicators during the examination demonstrated that liver and kidney are the main target organs for crocetin and crocin due to their antioxidant activity (75). Also, in the same study, Bandegia indicated that chronic stress-induced oxidative stress damage of brain, liver and kidneys can be inhibited by saffron (crocin) due to the antioxidant effects (76). In 2014, Lari *et al* evaluated how diazinon can influence the regulation of lipid metabolism (hyperlipidemia), extracellular signal-regulated kinase (ERK) and LDLr (low-density lipoprotein receptor) expression in the liver of rats. Also, they tried to find the interaction between crocin and diazinon. In conclusion, the findings suggested that diazinon causes increasing of concentrations of cholesterol, triglyceride, LDL and reduction of ERK1/2 protein phosphorylation and LDLr transcript. But crocin declined inhibition of ERK performance and hyperlipidemia. According to all collected data, crocin inhibit hepatotoxicity due to its antioxidant activity. Also, in 2015 Lari and colleagues, in addition to consider the improving effect of crocin on lipid peroxidation and pathological changes in rat liver, they evaluated the protein levels of malondialdehyde (MDA), total caspases-3 and -9 and Bax/Bcl-2 ratio. Regarding the obtained results, they stated that crocin is able to prevent and remove negative effect of diazinon (77, 78). Pan *et al* described the ability of saffron ethanolic extract on remedy of hepatic ischemia-reperfusion (IR) injury. IR is known as a common

clinical disorder that intracellular ROS concentration, inflammation, oxidative damage and cell apoptosis are considered as some of its symptoms. As a result, saffron ethanol extract was introduced as a protective agent against IR via prevention of the intracellular ROS concentration, restoring the content of antioxidant enzymes, modulation and regulation of protein synthesis. (79). Negative effects on lipids, proteins, and DNA, destructive changes of enzyme levels, abnormalities in gene expression and protein synthesis, damage to different organs and tissues are among the results of imbalance between oxidative stress and various antioxidant agents. In view of the existing deficiencies in the conducted research projects, pharmacokinetic and toxicological studies concerning antioxidant activity of saffron and harmful consequences of oxidative stress in different parts of body, interaction of saffron with other substances that have antioxidant effects and saffron replacement therapy with chemical drugs are recommended.

Effect in liver cancer

A number of hypotheses have been proposed for the anti-tumor actions of the major therapeutic effects of saffron and its components such as a) the inhibitory effect on cellular DNA and RNA synthesis, but not on protein synthesis; b) the inhibitory effect on free radical chain reactions; c) the metabolic conversion of naturally occurring carotenoids to retinoids; d) the interaction of carotenoids with topoisomerase II, an enzyme involved in cellular DNA-protein interaction (22). According to these characteristics, Noureini *et al* demonstrated that down regulation of hTERT expression and reduction of telomerase activity of HepG2 cells are some of the ways that crocin can treat hepatic cancer cells significantly (80). In similar research, Yang *et al* proved that crocetin can protect liver from activation of apoptotic cell death and it is an acceptable treatment option for hemorrhagic shock (81). Amin *et al* demonstrated that two anti-cancer functions for saffron can be referred: 1) inhibition of cell proliferation at early time by inducing cell cycle arrest and it may directly target DNA sequences and modulate gene expression; 2) killing of cancer cells via apoptosis. The purpose was to investigate the chemopreventive action and the mechanisms of saffron against diethylnitrosamine (DEN)-induced liver cancer in rats. DEN increases the number and the incidence of hepatic dyschromatic nodules while saffron significantly reduced them. Based on the results, saffron has a significant chemopreventive effect against liver cancer through inhibition of cell proliferation, induction of apoptosis, modulating oxidative damage and suppressing inflammatory response (82). Besides the remedial advantages of anti-tumor drugs, this kind of cancer therapy faces a

number of disadvantages including their toxicity effects that spread to the healthy cells. Cisplatin (CDDP) is one of the common anti-tumor drugs that has destructive side effects. The hepatotoxicity is the result of cisplatin damage in two ways: a) induction of oxidative stress; b) apoptosis in the liver. The function of crocin against oxidative stress includes reduction of malondialdehyde level, improving the levels of glutathione and antioxidant enzymes such as superoxide dismutase, catalase and glutathione peroxidase. Also, crocin prevent apoptosis by decreasing the levels of phospho-p38 mitogen-activated protein kinase (MAPK), tumor protein 53 (p53) and reducing of caspase-3 activation (83). Also, Cyclophosphamide (CP) is used to treat cancers and autoimmune disorders. As a prodrug, it is converted by liver cytochrome P450 (CYP) enzymes to form the metabolite 4-hydroxy cyclophosphamide that has chemotherapeutic activity. On the other hand, CP has severe and life-threatening adverse effects such as acute myeloid leukemia, bladder cancer, hemorrhagic cystitis, permanent infertility. In this connection, Jnaneshwari *et al* revealed the relevance between positive effects of crocin against negative effects of CP. Hence, they measured the rising and falling levels of different biochemical agents including endogenous reactive oxygen species, oxidation of lipids and proteins (the hallmarks of oxidative damage in liver and serum), glutathione, total thiol and antioxidant enzymes such as superoxide dismutase, catalase, glutathione-S-transferase, glutathione peroxidase, aspartate aminotransferase, alanine aminotransferase along with acid and alkaline phosphatase. Based on these alterations, it was suggested that crocin, as a natural substance, can prevent adverse chemotherapeutic activity of CP such as oxidative damage, inflammation and organ toxicity like hepatotoxicity (84).

Tavakkol-Afshari *et al* proposed that ethanol saffron extract has the ability to induce apoptosis in tumor cells. They proved specific role of saffron in the death of human hepatocellular carcinoma cells (HepG2) and human cervical carcinoma cells (HeLa) since it plays an effective role in killing tumor cells without inducing toxicity in normal cells (85). In another study, Parizadeh found an interaction between aqueous saffron extract and nitric oxide (NO) production as an intercellular messenger molecule in many pathological and physiological processes. Two lines cell were considered including the hepatocellular carcinoma cell line (HepG-2) and laryngeal carcinoma cell line (Hep-2). This study revealed that the saffron extract had a cytotoxic effect on HepG-2 and Hep-2 cell lines by limitation of NO concentration (86). In 2011, Rongjie and colleagues investigated that how crocetin can influence the hepatic apoptotic pathways and

hemorrhagic shock. In this research hepatic apoptosis parameters including hepatic cytosolic cytochrome c, caspase-3 and bcl-2 were gauged. The findings suggested that crocetin can protect the liver from activation of apoptotic cell death (87). According to above information, saffron has an anti-cancer activity through two mechanisms including inhibition of cell proliferation and killing of cancer cells via apoptosis. But many questions still remain unanswered and these investigators did not attempt to elucidate that which constituent of saffron, which approach and dose has the best results, what interaction is between saffron and other anti-cancer drugs in liver, can saffron prevent chemotherapy and radiation therapy side effects, which stage of cancer saffron has the highest efficiency on?

Hepatoprotective effects of saffron

Carbon tetrachloride (CCl₄) induced hepatotoxicity, fatty degeneration and vacuole formation in mice. Also, it increased the levels of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) in plasma. On the other hand, the aqueous and ethanolic extracts of saffron stigmas and petals significantly decreased the levels of AST and ALT in plasma and reduced the incidence of liver lesions induced by CCl₄. It was proposed that the hepatoprotective effects of saffron against liver damages induced by CCl₄ may have three reasons: a) fixation of hepatic cell membrane; b) antioxidant effects and radical scavenging; c) reduction of CCl₄ metabolic activation by inhibition of cytochrome P450 (88). Wang *et al* illustrated the effects of crocetin on the hepatotoxicity and hepatic DNA binding of aflatoxin B1 in rats. To achieve accurate results, the levels of aspartate aminotransferase, alanine aminotransferase, lactate dehydrogenase, gamma-glutamyl transpeptidase as serum marker enzymes, hepatic glutathione (GSH), glutathione S-transferase (GST) and glutathione peroxidase (GSH-Px) were measured. Following the data analysis, it was suggested that crocetin reduced AFB1-DNA adduct formation. Also, the positive performance of crocetin on AFB1 hepatotoxicity might be due to the hepatic tissues defense mechanisms that elevated the cytosol GSH and the activities of GST and GSH-Px (89). In this connection, it was demonstrated that the aqueous extract of saffron and honey syrup declined aluminum chloride-induced hepatotoxicity via improvement of liver biochemical markers and diminishing of lipid peroxidation (90). In another research, Hariri *et al* studied about protective effects of crocin and safranal on sub-acute toxicity of diazinon (DZN). For detection of the fact, biomarkers and biochemical indices, enzymes levels, serum urea, creatinine, cholesterol, triglyceride, total and direct bilirubin levels, serum TNF- α , direct 8-iso-prostaglandin F2 α ,

soluble protein-100 β (S100 β), total protein and albumin concentrations were evaluated. The results obtained in this work revealed that safranal and crocin in combination with vitamin E could prevent the abnormality effects of diazinon (6). Hepatotoxicity is considered as a common liver injury caused by some natural products, industrial and pharmaceutical chemicals. Saffron, as a herbal remedy, is an agent that can prevent hepatotoxicity via its properties namely antioxidant effects, maintaining of hepatic cell membrane and favorable effect on liver enzymes. So, it seems that saffron can effectively remove adverse factors that cause toxicity in the liver.

Genoprotective property of saffron in liver

In another study, Hosseinzadeh *et al* distinguished that *C. sativus* stigma extract and crocin have protective effects against DNA damage. Methyl methanesulfonate is a chemical substance that can induce DNA damage in various organs in mice. These findings suggest that DNA damage was reduced in liver, kidney, lung and spleen in result of the genoprotective property of saffron (91). In a similar study, Hosseinzadeh and colleagues introduced safranal that has a protective effect against DNA damage like crocin (92). Regarding the research findings, saffron is believed to be able to prevent DNA break and defects. Following further clinical trial studies, saffron may be introduced as a beneficial agent for genomic abnormalities originated from varying reasons.

Saffron against fatty liver and hyperlipidemia

The accumulation of triglyceride fat in liver cells is responsible for fatty liver. Shi *et al* studied about the effect of crocetin in alcoholic fatty liver rats. Alcoholic fatty liver was induced by feeding high lipid diet plus alcohol. Different factors were measured including liver index, FFA, rate of VLDL-secretion, activity of aniline hydroxylase (ANH), alcohol dehydrogenase (ADH), aldehyde dehydrogenase (ALDH), alanine aminotransferase (ALT) activity and TG concentration in serum. The results highlighted the protective role of crocetin against alcoholic fatty liver since it causes the enhancement of mitochondrial- β -oxidation, decline of fatty sediment, prevention of lipid peroxidation and acceleration of the removing of alcohol and aldehyde (93). Hyperlipidemia includes several conditions, but it usually means that blood has a high cholesterol and triglyceride levels. Hyperlipidemia can influence various organs especially liver, kidney and heart. There are several classes of hypolipidemic drugs but Asdaq *et al* tried to prove the hypolipidemic and antioxidant potential of saffron (crocin). In order to reach the aim, different biochemical factors such as triglyceride (TG), total

cholesterol (TC), high-density lipoprotein (HDL), low-density lipoprotein (LDL), alkaline phosphatase (ALP), aspartate transaminase (AST), alanine aminotransferase (ALT), malondialdehyde (MDA), glutathione peroxidase enzyme activity (GSHPx), total glutathione (GSH), oxidized glutathione (GSSG) in serum and superoxide dismutase (SOD), catalase (CAT), thiobarbituric acid reactive species (TBARS), ferric reducing/antioxidant power (FRAP) and total sulfhydryl (SH) groups in liver tissue homogenate were considered. Based on the variation of aforementioned biochemical factors, it was proposed that saffron, as a medicinal plant, enjoy the anti-hyperlipidemic and antioxidant properties (94). Dietary and lifestyle change, increased desire to consume fast foods and junk foods, alcohol intake and insulin resistance are regarded as some of fatty liver and hyperlipidemia reasons that can lead to different diseases. Therefore, consumption of some herbal remedies like saffron beside the main meal may reduce the risk of fatty liver.

Therapeutic effects on colon

Colorectal cancer is a malignant tumor arising from the inner wall of the large intestine. It is regarded as the third leading cause of cancer in males and fourth in females. Risk factors for colorectal cancer include family history of colorectal cancer, colon polyps, and long-standing ulcerative colitis. Although surgery, radiation therapy and chemotherapy are employed to destroy the cancer cells, recently, medicinal plants are viewed as beneficial as well as efficient options in treating various types of cancer. Saffron and its ingredients are known as cancer therapy agent.

Anti-cancer effect

Bajbouj *et al* proved that saffron has an anti-cancer activity against colorectal cancer in two ways: a) antiproliferative; b) pro-apoptotic effects. Many genes and their proteins products play a leading role in the cell division and apoptosis including p53, pRb and Bcl-2 family. p53 has a starring role in the prevention of tumor development since it can regulate a cell to live or die. Following the evaluation of the relevance between saffron, p53, cell cycle and induced apoptosis, it seems that saffron can turn into a beneficial natural drug for treatment of colorectal cancer (95). In a similar study, Li considered p53 to evaluate the efficiency of crocetin to improve colon cancer cells. The findings introduced crocetin as a chemotherapeutic drug that could induce cytotoxicity in colon carcinoma cells via p53-independent mechanisms (96). Also, García-Olmo investigated the anti-cancer effect of crocin on colon adenocarcinoma. Animal and human colon adenocarcinoma cells (DHD/K12-PROb and HT-29) were used to examine the cytotoxicity of crocin. This

natural carotenoid can be considered as a promising treatment in colorectal cancer because of decline of tumor growth, destruction of cancer cell and powerful cytotoxic effect against cancer cell (97). Rastgoo *et al* studied on the anti-cancer role of crocin. To enhance the performance and efficiency of crocin, they developed a PEGylated nanoliposomes containing crocin for *in vitro* cytotoxicity against colon carcinoma (C-26) cells. The results illustrated that liposomal encapsulation of crocin could promote significantly its anti-tumorigenic effect (98). Crocin was utilized as an anti-proliferation on three colorectal cancer cell lines (HCT-116, SW-480, and HT-29). The antiproliferative effect of crocin was evaluated by MTS assay. Based on the results, it was concluded that antiproliferative effect of crocin on tumoral cells was meaningful while it does not have any effect on non-cancer cells. It seems that crocin is a safe and natural substance that can treat human colorectal cancer via prevention of cancer cell growth (99). All things considered, the results confirmed that saffron is effective on colon cancer via different ways such as regulation of proteins involved in gene mutation, cytotoxicity against carcinoma cells and prevention of cell proliferation. But it should be noted that the correlation between different animal and human colon carcinoma cells and various components of saffron, possible side effects, different causes of colon cancer, severity of cancer and more statistical information are unclear and it seems that more investigations should be done regarding this issue.

Ulcerative colitis

Ulcerative colitis (UC) describes an inflammation of the large intestine (colon, caecum and rectum) that includes characteristic ulcers or open sores. Oxidative and nitrosative stress, leukocyte infiltration and up regulation of pro-inflammatory cytokines can cause UC. Kazi and colleagues induced colitis by 2, 4, 6- trinitrobenzene sulfonic acid (TNBS) and studied anti-inflammatory effect of crocetin. Undesirable alteration in intestinal lesions including neutrophil infiltration and level of malondialdehyde (MDA), the expression of TH1 and TH2 cytokines, inducible NO synthase due to the down regulation of nuclear factor-kB (NFkB) were observed. But after treatment the colitis by crocetin, the obtained information demonstrated that crocetin can shift undesirable alteration to the safe and healthy situation by regulation of protein that has been linked to cancer (100). In the same research, however, the prevention effects of crocin (another active substance of saffron) were studied against inflammation that leads to mouse colon carcinogenesis. Azoxymethane (AOM) and dextran sodium sulfate (DSS) were used to induce colitis. The final conclusion demonstrated that crocin and

crocetin have anti-inflammatory effects-associated colon carcinogenesis. In addition, crocin can regulate mRNA expression of pro-inflammatory cytokines and inducible inflammatory enzymes. All of the mentioned properties of crocin and crocetin lead to prevent or treat colitis (101).

Saffron against pancreatic disorders

High-fat diet is considered as one of the most important reasons that can cause insulin resistance. Crocetin can influence insulin resistance due to its hypolipidemic effect and regulation of lipid metabolism. Also, crocetin raised hepatic non-esterified fatty acid uptake and oxidation, accelerated triglyceride clearance in plasma, enhanced lipoprotein lipase activity in liver, reduced the accumulation of detrimental lipids (DAG and long-chain acyl CoA) in liver and muscle. Genes involved in hepatic lipid metabolism which are regulated by peroxisome proliferator-activated receptor- α , were modulated to accelerate lipid uptake and oxidation. Based on all current evidence, crocetin can treat different defects related to reduction of insulin efficiency (102). In 2005, Xi prepared the examination to find the therapeutic effects of crocetin on insulin resistance and abnormalities related to pancreatic gland. So insulin resistance was induced by dexamethasone in rat model. Serum insulin, free fatty acids (FFA), triglyceride (TG) and tumor necrosis factor (TNF- α) were meaningfully increased in these animals. Also, the diminution of hepatic glycogen content and enhancement of pancreatic islet beta cells function were seen. After usage of crocetin, the results confirm that crocetin could remove or decline all of mentioned abnormal items (103). In another study, Xi again evaluated crocetin activity on insulin resistance and its associated abnormalities induced by high-fructose diet. Insulin resistance, hyperinsulinemia, dyslipidemia, hypertension, increasing tumor necrosis factor (TNF- α), decreasing in the expression of both protein and mRNA of adiponectin are some signs of high-fructose diet. Analysis the variables after treatment proved that crocetin has the ability to eliminate or modulate destructive effects of high-fructose diet (104). In 2009, Mohajeri *et al* compared anti-hyperglycemic effect of ethanolic saffron extract and tolbutamide as a standard hypoglycemic drug. In order to reach the aim, they induced diabetes in rats with alloxan. In addition to fasting blood glucose (FBG) levels, histopathological and immunohistochemical studies were performed on pancreatic islet cells of control and diabetic rats. The obtained result from the research showed that saffron extract has an anti-hyperglycemic effect that can help damaged pancreas (105).

Antioxidant effect

Rajaei *et al* revealed that streptozotocin-induced diabetes in rat causes hyperglycemic and oxidative situation and also treatment role of crocin in the pathogenesis of different organs was investigated. The reduction of the blood glucose level, increase in thiobarbituric acid reactive substance (TBARS) levels and decrease in total thiol (SH) groups in the liver and kidney of diabetic animals were observed. Crocin showed an antioxidative activity and decrement of lipid peroxidation levels in these organs. The findings of this study proved that crocin, the most important component of saffron, has anti-hyperglycemic and antioxidant properties in diabetic patients (106). Kianbakht studied the performance of saffron in diabetic rats induced by alloxan. In these animals, the alterations of some biochemical substances were observed. Saffron reduced the fasting blood glucose and HbA1c levels and increased the blood insulin levels. Also, there were no meaningful effects on the blood SGOT, SGPT and creatinine levels as liver and kidney function tests (8). Bajerska studied about combination of rye bread (RB) and saffron (S) powder and their anti-diabetic influence in the high-fat (HF) diet in streptozotocin (STZ)-induced wistar rats. Some parameters were evaluated such as fasting blood glucose (FBG), glycemia, thiobarbituric acid-reactive substances (TBARS), the ferric-reducing ability of plasma (FRAP) and triglyceride (TG). According to the data, both S and RB can be used in diabetic therapy due to antioxidant property, but there is no synergic metabolic effect was achieved after usage of RB+S (107).

Anti-cancer effect

One of the practical methods for cancer therapy is the induction of apoptosis. In connection with this subject, Bakshi *et al* recognized that there is relevance between saffron and the induction of apoptosis in different cancer types. In order to reach the aim, human pancreatic cancer cell lines (BxPC-3) were studied. Although the molecular mechanism of crocin is not clear, the development of apoptosis and G1-phase cell cycle arrest of BxPC-3 cells and cell growth reduction were seen (108). Dhar and colleagues reported that crocetin could reduce the destructive effects of cancer via inhibition of tumoral cells proliferation. To prove this hypothesis, they evaluated some parameters including MIA-PaCa-2 cells, Capan-1 and ASPC-1 pancreatic cancer cells, Cdc-2, Cdc-25C, Cyclin-B1 and epidermal growth factor receptor. Crocetin induced the apoptosis through increasing the Bax/Bcl-2 ratio. Based on evaluation these parameters, it was concluded that pancreatic cancer could cure by crocetin due to its anti-tumorigenic and anti-proliferation activities (109). Pancreas is regarded as one the most

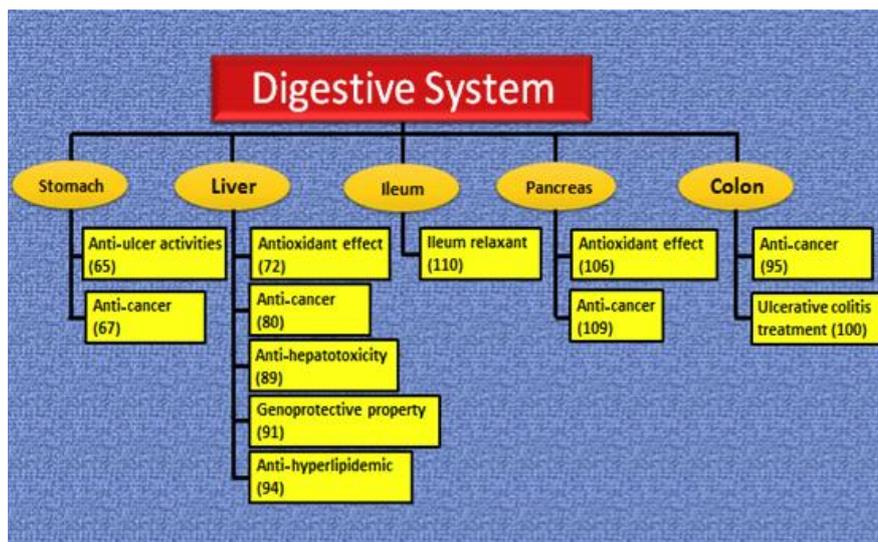


Figure 2. Saffron effects on the gastrointestinal tract

significant glands due to insulin production that is a peptide hormone for regulation the metabolism of carbohydrates and fats. Any defects in pancreas can lead to varying abnormalities in different parts of body. On the other hand, due to the beneficial effects of saffron, more clinical trial studies can prevent problems and diseases related to the pancreas and insulin performance.

Effects on ileum

In 2003, Fatehi investigated the effects of petal extracts of *C. sativus* on blood pressure in anaesthetized rats and on responses of the isolated rat vas deferens and guinea-pig ileum induced by electrical field stimulation (EFS). The aqueous and ethanol extracts of *C. sativus* petals diminished the blood pressure and reduced the evoked contractions in isolated rat vas deferens and guinea-pig ileum. Decrement of contractile responses is related to relaxant activity of this plant (110). All mentioned properties of saffron on the gastrointestinal aspects were summarized in Figure 2.

Conclusion

Research projects demonstrated that saffron has the great potential, various applications and therapeutic effects nearly on various parts of the body. In this review, we tried to consider some pharmacological actions of saffron in relation with different parts of gastrointestinal system such as stomach, liver, pancreas, ileum and colon. Antioxidant, anti-proliferation, anti-cancer, anti-genotoxic, anti-inflammatory, apoptogenic, chemoprotective, cytotoxic, anti-diabetic, anti-hyperlipidemic effects and relaxatory action are some of the mechanisms action of saffron in treating gastrointestinal disorders. However, most of the investigations have been done in animal models and

isolated tissues. It seems that clinical trials studies, medicinal products of saffron, study about interaction between saffron and cells, tissue, organs and various systems of body, combination of saffron and other herbal medicine, effective dose and optimal duration of use this plant seem necessary. From the therapeutic point of view, there are no comprehensive clinical trial research projects about the various effects of saffron such as antiemetic, anti-diarrheal, the reduction of gastric acid secretion and other therapeutic roles of saffron in different parts of body. It is clear that more clinical trial studies can open a new window of knowledge about unknown aspects of saffron effects and its biological constituents to treat or prevent different diseases and disorders in the future.

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