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Crocus Sativus (Saffron): An Immunoregulatory Factor in the Autoimmune and Non-autoimmune Diseases

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ABSTRACT

It has been reported that patients with arthritis, osteoarthritis, atherosclerosis, coronary artery disease, brain ischemia, diabetes, and inflammatory bowel disease (IBD) suffer from pro-inflammatory and oxidant related responses. Therefore, anti-inflammatory and anti-oxidant therapies are used to improve the quality of life of the patients. Saffron is a herbal drug that has immunomodulatory and antioxidant properties. Hence, Saffron and its components have been proposed as therapeutic agents for the treatment of the diseases. Therefore, this review article was designed to collect recent information regarding the effects of saffron and its components on the amelioration of the inflammatory symptoms in the autoimmune and non-autoimmune diseases and anti-cancerous effects from 1999 up to now via searching the Pubmed, Google Scholar, and Scopus databases. Due to fact that several investigations have reviewed the roles played by Saffron on autoimmune and non-autoimmune diseases such as multiple sclerosis, mood disorders, and Alzheimer's disease, this review article focuses on other diseases to keep the novelty of the present review for readers.

Keywords: Arthritis; Atherosclerosis; Brain ischemia; Cancer; Coronary artery disease; Diabetes; Inflammatory bowel disease; Leukemia; Osteoarthritis; Saffron

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progressively more in recent years.[1] Accordingly, the herbal drugs, which have anti-inflammatory effects, can be considered as new targets to improve the quality of life the patients who are suffering from the diseases

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that are associated with inflammation, such as arthritis, osteoarthritis, atherosclerosis, coronary artery disease, brain ischemia, type 1 and 2 diabetes, inflammatory bowel disease (IBD), cancer and leukemia.

Crocus sativus L., with its dried red-orange stigmas, is commonly known as saffron, a well-known herb of the Iridaceae family. Crocus sativus L. stigma is the most important part of the plant and its pharmaceutical applications have been evaluated during the last decades.[2] Moreover, due to the results obtained from the clinical investigations, it appears that Saffron and its active constituents may have immunomodulatory properties[2]in various diseases.

Because the roles played by the Saffron and its components have been reviewed in the autoimmunity diseases previously, this review article was designed to evaluate recent information regarding the roles played by Saffron and its active constituents, as a herbal drug, in the pathobiology of the disorders.

Crocus Sativus L

As mentioned in the previous section, Crocus sativus L is categorized as a species of the Crocus genus in the iris family Iridaceae. The saffron spice is produced from the filaments inside the Crocus sativus L flower.[3] Saffron is used for nutrition and fragrance for more than 3,500 years in various cultures.[3] The important constituents of Saffron are related to five families, including Monoterpene aldehydes, Carotenoids, Monoterpenoids, Isophorones and flavonoidsColor, odor, and flavor of Saffron are due to crocins, safranal, and picrocrocin, respectively.[3]

Saffron consists of some known molecules, such as water (14–16%), volatile oil (0.6–0.9%), nitrogenous matters (11–13%), fibers (4–5%), sugars (12–15%) and total ashes (4–6%).[4] Saffron also contains two important vitamins, riboflavin 56 to 138 μg/g and thiamine 0.7 to 4 μg/g, sterols (campesterol, stigmasterol, and β-sitosteral), palmitoleic, ursolic, palmitic, oleanolic and oleic acids.[4,5] It has been reported that 70% of the volatile fraction is 'Safranal', the main fragment responsible for the saffron aroma.[3]

As mentioned, crocin is the saffron fragment and is the molecule responsible for color. Additionally, the fragment has a metabolite entitled "crocetin", which mainly refers to its antioxidant properties. Due to the various fragments, recent studies demonstrated the significant Saffron medicinal properties such as antioxidants,[6-9] anti-depressants and anxiolytics,[10,11] antitumor,[12] aphrodisiac,[13] antitussive[14] and neuroprotective.[15]

Due to the medicinal properties of Saffron, investigations have been focused on the application of this herbal drug for the improvement of the diseases that are associated with inflammation.

Design of the Study

This article was designated to review the role of Saffron and its active components in nine various diseases including arthritis; osteoarthritis; atherosclerosis; coronary artery disease; brain ischemia; diabetes; IBD; cancer and leukemia via searching in the three main databases as Medline (Pubmed), Google Scholar and Scopus. To write the present review, more than 600 articles were collected at the first step and then the combination of other therapies with Saffron was excluded and resulted in 111 relevant articles either *in vitro* or *in vivo* studies which finally employed to write the present review.

Saffron and Its Effects on the Arthritis

Arthritis and osteoarthritis are associated with the inflammation and high rates of free radicals, which damage the tissues.[16-18] An investigation which has explored the anti-inflammatory effects of Saffron in both *in vitro* and *in vivo* conditions employingcomplete Freund's adjuvant-induced arthritis mouse model reported that the administration of Saffron was associated with decrease in interleukin (IL)-6, IL-1β, Vascular endothelial growth factor (VEGF), and tumor necrosis factor (TNF)-R1 expression independent of HO-1/nuclear factor erythroid 2 (Nrf2) and also down-regulation of nuclear factor-kappa B (NF-κB).[19] The study also demonstrated that Saffron administration led to down-regulation of cyclooxygenase-2 (COX-2), which catalyzes prostaglandin E2 (PGE2), and inducible nitric oxide synthase (iNOs) that catalyzes nitric oxide (NO) production in a dose-dependent manner.[19] The results were proved by Li et al who reported that Saffron is a key immunoregulatory factor to decrease expression of TNF-α, IL-1β, and IL-6 in a mouse model of collagen-induced arthritis (CIA), via up-regulation of inhibitory NF-κB factor (Iκκ) and consequently decreased function of NF-κB signaling.[20] The anti-osteoarthritis and anti-inflammatory effects of Saffron through alleviating oxidative stress and decreased expression of pro-inflammatory cytokines in c-Jun N-terminal kinases (JNK), but not extracellular

signal-regulated protein kinases (ERK), to repress NF-κB activation-dependent pathways have been documented by Chinese investigators.[21] Investigation of the Indian population had the same results, so Saffron is a key therapeutic factor to ameliorate arthritis symptoms via alleviating oxidative stress, inflammation and cartilage deteriorating enzymes, including matrix metalloproteinase 13 (MMP-13), MMP-3, MMP-9 and hyaluronidases (HAases).[22] Down-regulation of MMP-1, MMP-3 and MMP-13 and suppressing degradation of inhibitory-kappa-B-α, which led to decreased expression of pro-inflammatory cytokines, have been reported in a rabbit osteoarthritismodel.[23] Our previous investigation on the patients, who suffered from osteoarthritis also confirmed the immunoregulatory effects of Saffron.[24]

Due to the roles played by Saffron in arthritis, it seems that this herbal drug can suppress production of pro-inflammatory cytokines and their receptors and the inflammatory molecules, such as PGE2, NO, and cartilage deteriorating enzymes via inhibition of NF-κB, COX-2, and, iNOs via activation of HO-1/Nrf2 and JNK pathways and also Iκκ molecule, which bind to NF-κB and inactivate this transcription factor (Figure 1).

Saffron and Its Effects on the Cardiovascular Diseases

Saffron modulates immune responses and oxidant pathways in patients suffering from cardiovascular diseases. Saffron can ameliorate inflammatory responses in the rat coronary atherosclerosis via either down-regulation of NF-κB or inhibition of itstranslocation into the nucleus.[25] NF-κB plays key roles in the pathogenesis of atherosclerosis.[26] The dose-dependent anti-atherosclerotic and plaque-stabilizing effects of the Saffron components, by down-regulation of pro-inflammatory cytokines and tissue lysis enzymes, have also been demonstrated by Christodoulou and colleagues.[27]Mahdavifard et al., also reported that oxidant production, athermanous plaque formation, and inflammation were significantly decreased in the diabetic-atherosclerotic rats treated

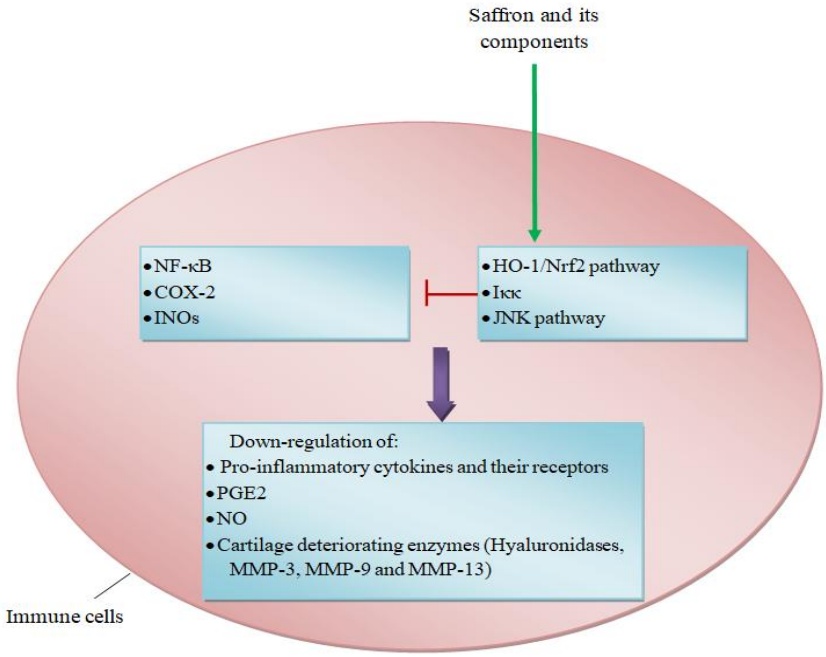

Figure 1. Saffron and arthritis. Saffron and its components suppress the production of pro-inflammatory cytokines, their receptors and molecules, such as PGE2, NO, and cartilage deteriorating enzymes via inhibition of NF-κB, COX-2, and iNOs indirectly. Accordingly, Saffron inhibits the enzymes via activation of HO-1/Nrf2 and JNK pathways and also Iκκ molecule, which binds to NF-κB and in activate this transcription factor.

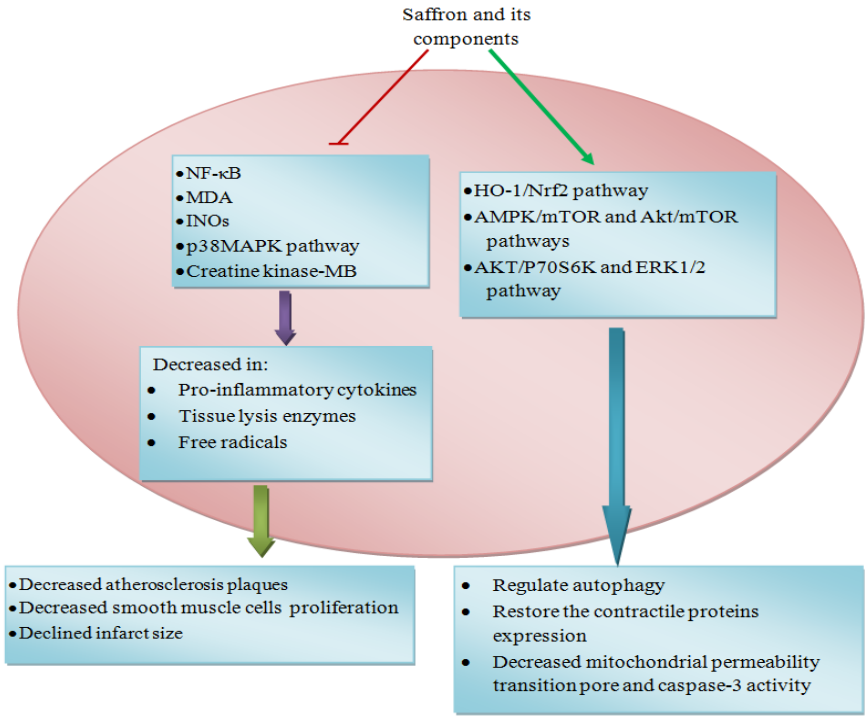

Figure 2. Saffron and cardiovascular diseases. Saffron and its components up-regulate HO-1/Nrf2, AMPK/mTOR and Protein kinase B(Akt)/mTOR and AKT/P70S6K and ERK1/2 pathways, which lead to autophagy regulation, restore the expression of the contractile protein and decreased mitochondrial permeability transition pore and caspase-3 activity. Additionally, Saffron directly inhibits NF-κB, MDA, INOs, p38 pathway and creatine kinase-MB that lead to decreased production of pro-inflammatory cytokines, tissue lysis enzymes and free radicals and consequently decreased in atherosclerosis plaque, smooth muscle cells proliferation and infarct size.

with Saffron.[28] Hydroalcoholic and aqueous extract of saffron increase the concentration and activities ofthe anti-oxidant agents and consequently decreaseanti-atherosclerotic conditions in the diabetic animal models.[29] Saffron can prevent atherosclerosis through suppression of p38Mitogen-activated protein kinase (MAPK) pathway and inhibition of smooth muscle cell proliferation.[30] Appetite, dietary intakes, and body composition were improved in the patients with coronary artery disease by using 8 weeks randomized, double-blind, and placebo-controlled trial treatment with Saffron.[31] One study on an ischemia/reperfusion rat model revealed that Saffron reduces infarct size and activity of malondialdehyde (MDA), creatine kinase-MB (CK-MB) and TNF-α.[32] Saffron also increases the levels of total superoxide dismutase (T-SOD) and IL-10 in the animal model.[32] Other investigations proved a potential anti-arrhythmic, anti-myocardial infarction and a prorelaxing properties for Saffron.[33-39] Moreover, Saffron provides nutritional preconditioning against myocardial ischemia-reperfusion by increasing the production of Nrf2,[37]AMP-activated protein kinase (AMPK)/mammalian target-of-rapamycin (mTOR) and protein kinase Band Akt/mTOR pathways to regulate autophagy,[40] AKT/P70S6K, and ERK1/2 pathways to restore the contractile protein expression, and decreased mitochondrial permeability transition pore and caspase-3 activity.[41,42]Additionally, the guanine nucleotide exchange factor (Rho)/Rho kinase (ROCK)/NF-κB[43] and p38 MAPK[42] pathways are inhibited by Saffron.

Figure 2 shows the effects of Saffron on patients suffering from cardiovascular diseases. As it is illustrated in the figure, Saffron and its components can up-regulate HO-1/Nrf2, AMPK/mTOR and Akt/mTOR and AKT/P70S6K and ERK1/2 pathways, which leads to autophagy regulation, restores the contractile protein expression and decreases mitochondrial permeability transition pore and caspase-3activity.Saffron directly suppresses NF-κB, MDA, iNOs, p38 pathway and CK-MB, which is associated with decreased production of pro-inflammatory cytokines, tissue lysis enzymes and

free radicals and the consequent decrease in atherosclerosis plaque, smooth cell proliferation, and infarct size.

Saffron and Its Effects on the Brain Ischemia

All of the investigations on the brain ischemia demonstrated that Saffron has neuroprotective roles. Saffron significantly reduces infarcted areas, which are induced by occlusion of the middle cerebral artery (MCA) in mice, by increased expression of gamma-glutamylcysteinyl synthase (gamma-GCS), the main enzyme for glutathione synthesis.[44] Saffron therapy led to decreased inflammation and increased effects of hypothermia therapy in mice hypoxic ischemia-induced brain injury.[45] Several animal model investigations showed that Saffron and its components have neuroprotective roles via inhibition of the production of free radicals and enhanced antioxidant activities in the ERK1/2 pathway-dependent manner. [15,46-50] The protective roles played by Saffron against neuropathological alterations in the hippocampus and consequent improvement of spatial learning memory via down-regulation of oxidant factors have also been documented by previous investigations.[51,52]

The same results have been reported regarding the neuroprotective roles played by Saffron in humans. Accordingly, a clinical trial study revealed that Saffron therapy is associated with short and long-term neuroprotective effects on ischemic stroke in humans.[53]

As it is illustrated in Figure 3, Saffron and its components play neuroprotective roles through the down-regulation of pro-inflammatory cytokines and free radicals and activation of gamma-glutamylcysteinyl synthase and ERK1/2 pathway.

Saffron and Its Effects on the Diabetes

It has been reported that type 1 diabetes is an autoimmune disease with the participation of Th1 immune responses in delayed-type hypersensitivity (DTH) manner.[54] The pro-inflammatory mechanisms in the induction of type 2 diabetes also have been proved.[55,56] Thus, therapeutic agents that target pro-inflammatory pathways and cytokines, as well as oxidant factors, can be considered as suitable candidates to use against diabetes. Several studies have been performed on both animal and human models. For example, Samaha et al reported that crocin, as the main active ingredient of Saffron, administered to the mouse model of type-1 diabetes, STZ-induced diabetes mellitus, significantly increased expression of pancreatic insulin and anti-oxidant defenses and reduced blood glucose (BG) levels and oxidative burden as well as inflammatory cytokines.[57] Another study on the STZ-induced diabetes mouse model showed that Saffron extract administration in high doses is associated with reduced BG, via increased expression of glucokinase and increased insulin production.[58] The investigation also reported that oxidative stress markers were down-regulated in the animal model by treatment with the Saffron extract.[58]

Another study on the STZ-induced diabetes mouse model had similar results and revealed that the hydroalcoholic extract of Saffron can reduce BG, pro-inflammatory cytokines, such as IL-17A, nitric oxide, and reactive oxygen substances, while increasing insulin secretion, anti-inflammatory IL-10 and transforming growth factor-β (TGF-β) in the pancreatic cell population.[59] Jiang et al also confirmed the positive roles played by Saffron in the STZ-induced diabetes mouse model, which was associated with reduced BG and LDL as well as repaired pancreas.[60] Saffron also improved diameter of seminiferous tubules, spermatogenesis index, sperm morphology, count, motility, and viability in the rat diabetic model.[61] Saffron administration in the rat diabetic model can improve learning and memory impairments and cardiac dysfunction via the up-regulation of anti-inflammatory, antioxidant and antiapoptotic molecules as well as normalizing autophagy.[62] The positive roles played by Saffron to improve electrocardiography (ECG), biochemical and histopathological changes, regulate oxidative burden, inflammatory cascade and improve the diabetes complications, such as atherogenesis, encephalopathy, cataract, retinal ischemic damage, liver steatosis and nephropathy progression in the rat diabetic animal models have been demonstrated previously.[28, 63-67] Interestingly, Saffron plays the rolesvia down-regulation of inflammation and decreased oxidants.[68] For example, studies on a rat model of nephropathy and retinal ischemic damage revealed that Saffron improves the tissue functions by down-regulation of inflammation, via decreased expression of IL-6, toll-like receptor-4 (TLR4) and adhesion molecules[37,69] and elevation in PI3K/AKT pathway.[70] Moreover, an in vitro study on the microglial cells derived from mouse, N9, and rats, BV-2, revealed that Saffron suppresses oxidative stress and pro-inflammatory responses in PI3K/Akt signaling

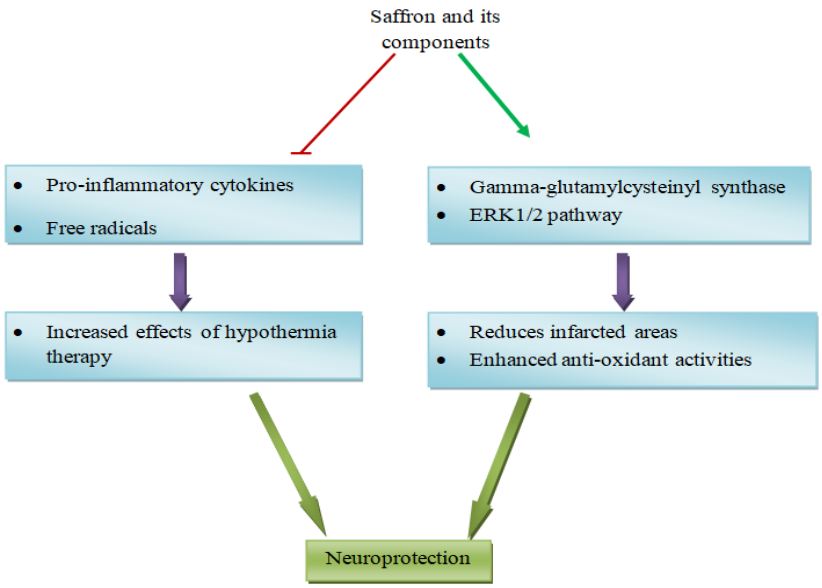

Figure 3. Saffron and Brain ischemia. Saffron and its components have neuroprotective roles via inhibition of production of pro-inflammatory cytokines and free radicals and increased gamma-glutamylcysteinyl synthase and ERK1/2 pathway. The effects lead to increase the routine therapies, such as hypothermia and reduce infarcted areas. The effects result in neuroprotection in treated patients.

dependent manner.[71] Another study showed that Saffron in either *in vivo* or *in vitro* conditions improves glucose uptake in glucose transporter type 4 (GLUT4)/AMPK pathway.[72] Saffron, as a protein tyrosine phosphatase 1B inhibitor, can also activate insulin signaling and consequently increase glucose uptake in a diabetic mouse model.[73] An in vitro study demonstrated that Saffron improved the insulin sensitivity via AMPK/acetyl-CoA carboxylase (ACC) and MAPKs, as both insulin-independent, and PI 3-kinase/Akt and mTOR, as insulin-dependent, pathways.[74] Interestingly, human treatment with high doses of Saffron also had similar results. Moravej Aleali and colleagues reported that treatment of the patients with 15 mg of Saffron can lead to a decrease in fasting plasma glucose (FPG), Cholesterol, low-density lipoprotein (LDL), and LDL/HDL ratio in type 2 diabetic patients.[75] The benefits of Saffron for improving the life quality of type 2 diabetic patients have also been demonstrated by Suh et al., who reported that Saffron can reduce diabetic bone disorders via regulation of glyoxalase, oxidative stress, and mitochondrial function.[76] Saffron is also capable to improve anxiety and sleep disturbance in the patients with type 2 diabetes.[77] Sepahi and colleagues showed that Saffron treatment of the diabetic patients can be associated with reduction of HbA1c and central macular thickness (CMT) and improve best-corrected visual acuity (BCVA).[78] Saffron administration benefits, in long term therapy, for regulation of fasting blood glucose (FBG) in the type 2 diabetes have also been documented by Milajerdi and colleagues.[79] The modulatory effects of Saffron on the soluble intercellular adhesion molecule (sICAM-I), a pro-inflammatory molecule, in type 2 diabetic patients has also been reported by Azimi and colleagues.[80] Mohammadzadeh-Moghadam and colleagues have evaluated the effects of the Saffron gel on the erectile dysfunction in type 2 diabetic patients. Accordingly, they reported that the Saffron gel significantly improved erectile dysfunction in the patients.[68] Another study showed that 1 g of Saffron reduced FBG and pro-inflammatory cytokines in patients who suffered from type 2 diabetes.[81] It has been reported that antibody titers to heat-shock proteins (HSPs) 27, 60, 65 and 70 are elevated among patients with metabolic syndromes, such as diabetes.[82] Shemshian et al revealed that Saffron decreased anti-HSP 27, 70 levels in the patients.[83] However, some investigations using lower doses of Saffron were not associated with significant

effects. For instance, a study by Shahbazian et al revealed that consumption of 15 mg of Saffron had no effects on serum levels of homocysteine, antioxidant and pro/anti-inflammatory molecules, such as IL-6, TNF-α, C-reactive protein (CRP) and IL-10 in the patients with type 2 diabetes.[84] Another clinical trial showed similar results and demonstrated that Saffron (100 mg daily) had no effects on the BG, oxidative burden and inflammatory cytokines, but reduced systolic blood pressure (SBP).[85]Figure 4 illustrates the roles played by Saffron in diabetic patients.

Saffron and Its Effects on IBD

It has been documented that IBD is a disorder with various inflammatory responses in the mucosa.[86] So, it has been hypothesized that Saffron can be considered as a therapeutic agent to reduce the inflammation in the IBD. Accordingly, a study by Rezaei et al[87] on ulcerative colitis (UC), which is a chronic IBD,[88] revealed that Saffron significantly reduced the disease activity index, such as rectal bleeding, bodyweight loss, diarrhea, and colon shortening. Interestingly, Saffron attenuate the inflammatory responses in the UC rat model by targeting the Nrf2/HO-1 signaling,[89] which was documented regarding the regulatory mechanisms played by Saffron in the arthritis[19] and myocardial ischemia-reperfusion injury.[37] A study by Kawabata and colleagues demonstrated that 4 weeks crocin feeding leads to suppress dextran sulfate sodium (DSS)-induced colitis and reduced expression of IL-1β, IL-6, IFN-γ, TNF-α, NF-κB, COX-2, and iNOs via targeting Nrf2/HO-1 signaling.[90] Therefore, due to the results, it seems that Saffron attenuates inflammatory responses in similar mechanisms in the IBD suffering patients.[89] Figure 5 shows the roles of Saffron in IBD.

Saffron and Its Effects on Various Cancerous Diseases

Due to the anti-inflammatory and anti-oxidant effects of Saffron, several studies were performed to evaluate the anti-cancerous properties of Saffron. The experiments revealed that Saffron has cytotoxicity, suppressive cancer cell growth properties and inhibitory roles for resistance to anti-cancer drugs in the concentration and time-dependent fashions [91]. It appears that Saffron induces cytotoxicity via a reduction in the protein expression of lactate dehydrogenase A (LDHA) and consequent increasing cellular reactive oxygen species (ROS).[92] Accordingly, in vitro investigations on cancer cell lines revealed that Saffron extract inhibits cell proliferation and significantly decreases colony-forming and migration capabilities in several cancer cell lines, such as osteosarcoma,[93] AGS and HGC-27 gastric,[94] KYSE-150 epithelial,[95] Y79 and WERI-RB-1 retinoblastoma,[96] QGY-7703 liver,[97] A2780-RCIS ovarian cisplatin-resistant,[98] C6 glioma,[99] 4T1 Breast,[100] C2C12 myoblast and HCT116 colon,[101] Hep3B and HepG2 hepatocellular carcinoma[102] cancer cell lines. Interestingly, like other inflammatory-related diseases, Saffron plays its anti-cancerous properties via alteration in miR-320/Krüppel-like factor 5 (KLF5)/HIF-1α, PI3K, AKT, ERK1/2, p38, c-Jun NH-terminal kinase (JNK), Wnt/β-Catenin signaling, p53/p21, Signal transducer and activator of transcription 3 (STAT3) (via induction of Src homology region 2 domain-containing phosphatase-1 (SHP-1)) pathways[94,95,102-104]. Collectively, the alteration in the signaling pathways leads to the up-regulation of apoptotic molecules, such as BAX and Caspase-8, and down-regulation of anti-apoptotic molecules, like B-cell lymphoma 2 (BCL2) and mitochondrial membrane potential (MMP).[105] It also shows its anti-cancerous properties through activation of the mitotic checkpoint by binding to tubulin[106] and shift autophagic cell survival to death in the breast cancer cells, via alteringautophagy-related 1 (ATG1) and Beclin-1 expression.[107]

Moreover, saffron plays key roles against leukemia disorders. For instance, the anti-cancerous properties of Saffron against K-562 cells of chronic myelogenous leukemia have been demonstrated by Geromichalos and colleagues.[108] The cytotoxic effects of Saffron on the MOLT-4 and HL-60 human leukemia cells were also confirmed previously.[109-112] Moradzadeh et al reported that Saffron significantly decreases expressions of multidrug resistance (MDR) proteins and tyrosyl-DNA phosphodiesterase 1 (TDP1), while it increases the expressions of Caspase 3, Caspase 9, and BAX/BCL2 ratio in acute promyelocytic leukemia (APL) cells.[113] Figure 6 shows the main mechanisms used by Saffron to inhibit tumor development. Due to the scientific literature regarding the roles played by Saffron against various tumors, it appears that Saffron uses several mechanisms and alters various pathways to increase and decrease pro-apoptotic and anti-apoptotic molecules to overcome proliferation, metastasis, and resistance to anti-cancer drugs.

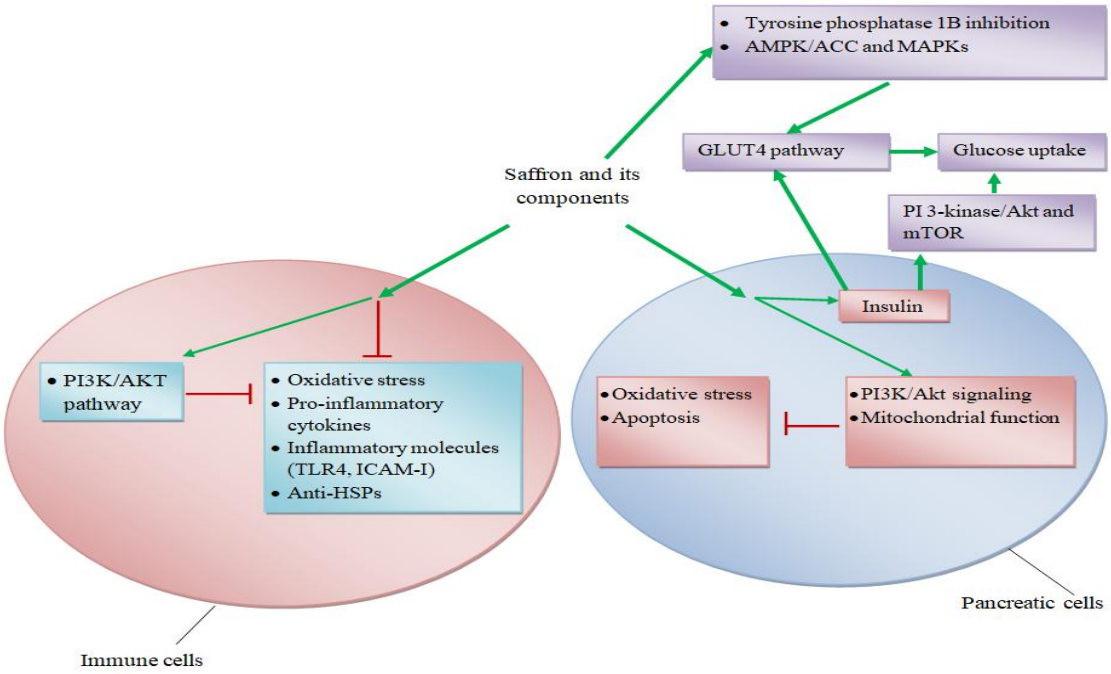

Figure 4: Saffron and diabetes. Saffron affects several cells, including immune and pancreatic cells. Saffron inhibits oxidative stress, apoptosis, pro-inflammatory cytokine, and molecules production and antibody against HSPs independent of PI3K/Akt signaling pathway. Saffron also increases glucose uptake via increased production of insulin, PI 3-kinase/Akt and mTOR pathway, and other mechanisms, such as inhibition of tyrosine phosphatase 1B and activation of AMPK/ACC and MAPKs pathways

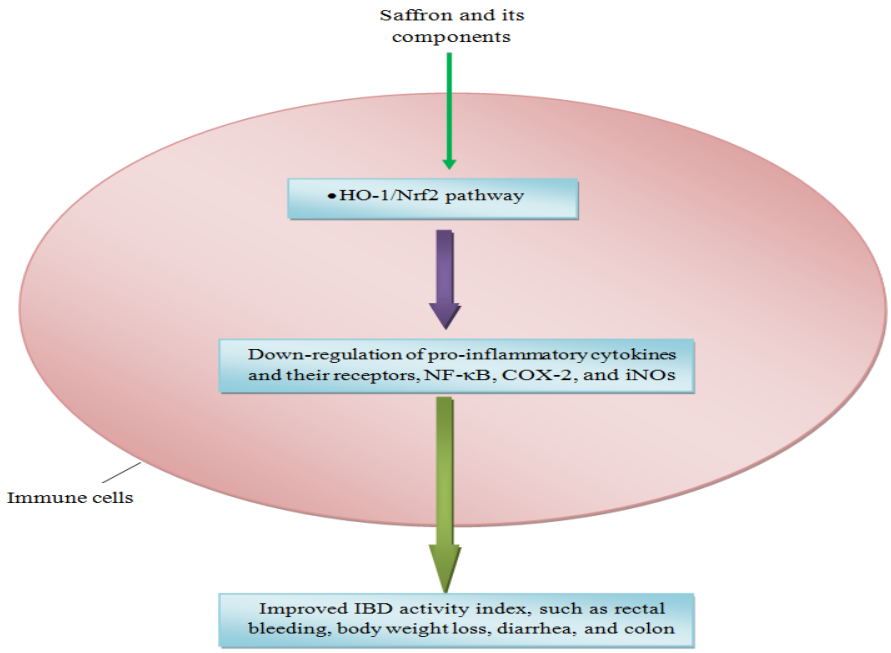

Figure 5. Saffron and inflammatory bowel disease. Saffron affects immune cells and inhibits oxidative stress, pro-inflammatory cytokine and molecules production independent onHO-1/Nrf2 signaling pathway. The effects result in decreased inflammatory bowel disease (IBD) activity index, including rectal bleeding, bodyweight loss, diarrhea, and colon shortening.

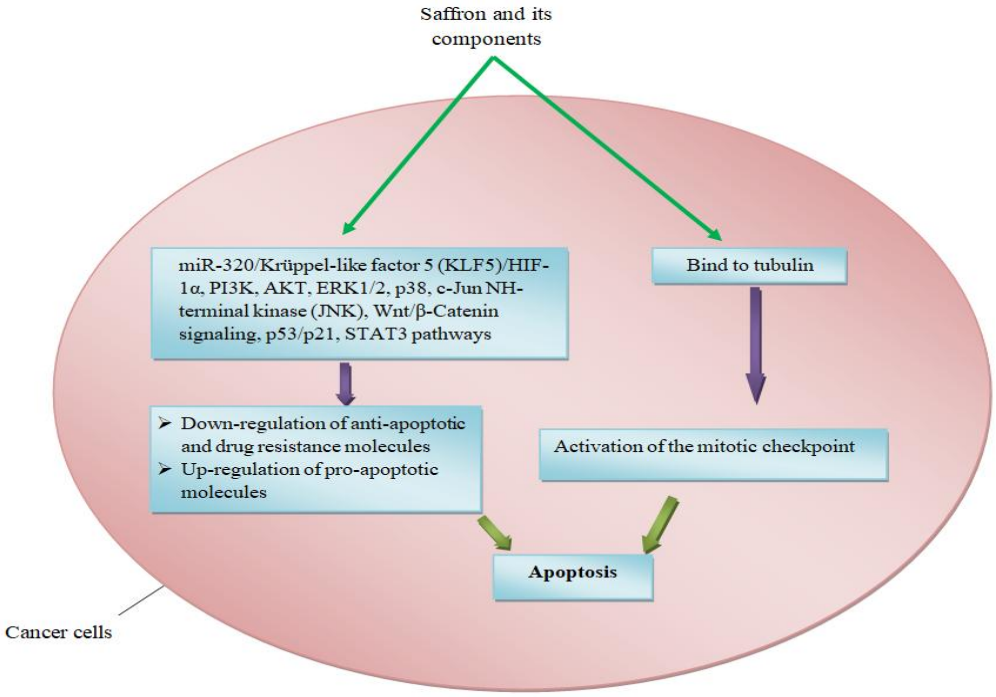

Figure 6. Saffron and various cancerous diseases. Investigations revealed that Saffron manipulates miR-320/Krüppel-like factor 5 (KLF5)/HIF-1α, PI3K, AKT, ERK1/2, p38, c-Jun NH-terminal kinase (JNK), Wnt/β-Catenin signaling, p53/p21, STAT3 pathways, which leads to down and up-regulation of anti-apoptotic and pro-apoptotic molecules, respectively. Additionally, Saffron binds to tubulin and then activates the mitotic checkpoint. Both mechanisms lead to increased apoptosis in the cancer cells

CONCLUSION

Saffron and its components can modulate several pathways, which lead to the down-regulation of pro-inflammatory cytokines, free radicals and the main molecules that participate in the pathogenesis of inflammatory based diseases. Many *in vitro* and *in vivo* studies regarding arthritis and osteoarthritis revealed that the production of inflammatory factors such as TNF-a, IL-1β, VEGF, their receptors, and molecules, such as PGE2, NO, and cartilage deteriorating enzymes can be suppressed by Saffron and its components via inhibition of NF-κB, COX-2, and iNOs. Activation of HO-1/Nrf2 and JNK pathways and also Iκκ molecule, which binds to NF-κB and inactivate this transcription factor was mediated by inhibitory effects of Saffron on the enzymes. Studies regardingthe cardiovascular diseases showed an ameliorated via decreasing in the production of inflammatory cytokines, tissue lysis enzymes and free radicals by treatment with Saffron. Decreasing in atherosclerosis plaque, smooth muscle cells proliferation and infarct size are other beneficial effects of Saffron. Moreover, HO-1/Nrf2, AMPK/mTOR and Akt/mTOR and AKT/P70S6K and ERK1/2 pathways were up-regulated by Saffron and its components and led to autophagy regulation, restore the expression of the contractile protein and decreased mitochondrial permeability transition pore and caspase-3 activity in the cardiovascular diseases. On the contrary, NF-κB, MDA, INOs, p38 pathway, and CK-MB were directly inhibited by Saffron and the production of pro-inflammatory cytokines, tissue lysis enzymes and free radicals were consequently decreased. Decreasing in atherosclerosis plaques, smooth muscle cell proliferation and infarct size reported being the beneficial effects of Saffron in cardiovascular disease via the above-mentioned mechanisms. Moreover, findings regarding the effects of Saffron and its components on brain ischemia showed a neuroprotective effect through the down-regulation of pro-inflammatory cytokines and free radicals. Additionally, gamma-glutamylcysteinyl synthase and ERK1/2 pathway can be up-regulated by Saffron which consequently leads to the reduction in

the infarcted areas and enhancement of antioxidant activities. Improvement in the spatial learning memory via down-regulation of oxidant factors was found to be important protective roles of Saffron against neuropathological alterations in the hippocampus following the brain ischemia. Studies regarding type 1 and 2 diabetes revealed important roles for Saffron and its components. FPG, cholesterol, LDL, and LDL/HDL ratio in type 2 diabetic patients were decreased by Saffron. Diabetic bone disorders were reduced by Saffron in patients with type 2 diabetes via regulation of glyoxalase, oxidative stress, and mitochondrial function. Anxiety, sleeping disturbance, and erectile dysfunction were improved by treatment with Saffron in type 2 diabetic patients. HbA1c, CMT, and SBP were reduced by Saffron in such patients, too. On the other hand, there are controversial reports with no effects of Saffron on the pro and anti-inflammatory cytokines such as IL-6, TNF-α, and IL-10 in type 2 diabetes. Blood glucose levels in the Type 1 diabetes (T1D) models were modulated by Saffron through three main mechanisms, including reduction of the oxidative burden, apoptotic pathway, and pancreatic inflammation. PI3K/Akt signaling pathway is induced by Saffron to decrease the production of inflammatory cytokines, free radicals and inflammatory molecules such as TLR-4 and ICAMin type one diabetes. Studies showed that glucose uptake was increased by Saffron via increased production of insulin, PI 3-kinase/Akt and mTOR pathway, and induction of other mechanisms, such as inhibition of tyrosine phosphatase 1B and activation of AMPK/ACC and MAPKs pathways. Studies regarding IBD and the role of Saffron on this disease are limited and not investigated extensively. Disease activity indexes such as rectal bleeding, bodyweight loss, diarrhea, and colon shortening in IBD were significantly suppressed by Saffron. Additionally, inflammatory responses and expression of IL-1β, IL-6, IFN-γ, TNF-α NF-κB, COX-2, and iNOs in the UC rat model were attenuated by Saffron through targeting the Nrf2-OH signaling. Asimilar mechanism might be suggested for Saffron to ameliorate IBD symptoms in humans. Investigations regarding various cancerous diseases revealed that miR-320/Krüppel-like factor 5 (KLF5)/HIF-1α, PI3K, AKT, ERK1/2, p38, c-Jun NH-terminal kinase (JNK), Wnt/β-Catenin signaling, p53/p21, STAT3 pathways were modulated with Saffron and lead to down and up-regulation of anti-apoptotic and pro-apoptotic molecules, respectively. Additionally, the mitotic checkpoints were activated via the binding of Saffron to tubulin. Moreover, apoptotic molecules, such as BAX and Caspase-8 were up-regulated following the alteration in the signaling pathways by Saffron. On the contrary, studies regarding cancerous diseases revealed that anti-apoptotic molecules, like BCL2 and mitochondrial membrane potential (MMP) were down-regulatedby Saffron. Moreover, studies showed that cytotoxicity was induced by Saffron via a reduction in the protein expression of lactate dehydrogenase A (LDHA) and the consequent increase of cellular reactive oxygen species (ROS).Additionally, the anti-cancerous properties of Saffron were shown against K-562 cells of chronic myelogenous leukemia. The cytotoxic effects of Saffron on the MOLT-4 and HL-60 human leukemia cells were reported by other investigators. Studies showed that expressions of MDR proteins and TDP1 were significantly decreased by Saffron. On the contrary, Saffron showed the effects on the up-regulation of Caspase 3, Caspase 9, and BAX/BCL2 ratio in APL cells. Similar mechanisms, which were mentioned for the effects of Saffron on the various cancerous diseases might be suggested for leukemia too. Conclusively, it appears that Saffron is a powerful anti-oxidant and anti-inflammatory factor, which can be considered for the treatment of the patient suffering from the diseases that are associated with inflammation. Additionally, it seems that Saffron can be used either as the predictor against cancers, by reducing the free radicals and chronic inflammation, or therapeutic factor, by reducing the anti-apoptotic molecules. However, since most investigations were performed in the *in vitro* conditions, further investigations using *in vivo* models and clinical trials need to be designed to get a better conclusion.

REFERENCES

1. Job KM, Kiang TKL, Constance JE, Sherwin CMT, Enioutina EY. Herbal medicines: challenges in the modern world. Part 4. Canada and United States. Expert Rev Clin Pharmacol. 2016;9(12):1597-609.
2. Ege B, Yumrutas O, Ege M, Pehlivan M, Bozgeyik I. Pharmacological properties and therapeutic potential of saffron (Crocus sativus L.) in osteosarcoma. J Pharm Pharmacol. 2019;72(1):56-67.
3. Srivastava R, Ahmed H, Dixit RK. Crocus sativus L.: a comprehensive review. Pharmacogn Rev.

2010;4(8):200-8.
4. Bhat JV, Broker R. Riboflavine and thiamine contents of saffron, Crocus sativus linn. Nature. 1953;172(4377):544.
5. Christodoulou E, Kadoglou NPE, Stasinopoulou M, Konstandi OA, Kenoutis C, Kakazanis ZI, et al. Crocus sativus L. aqueous extract reduces atherogenesis, increases atherosclerotic plaque stability and improves glucose control in diabetic atherosclerotic animals. Atherosclerosis. 2018;268:207-14.
6. Zengin G, Aumeeruddy MZ, Diuzheva A, Jekő J, Cziáky Z, Yıldıztugay A, et al. A comprehensive appraisal on Crocus chrysanthus (Herb.) Herb. flower extracts with HPLC–MS/MS profiles, antioxidant and enzyme inhibitory properties. J Pharm Biomed Anal. 2019;164:581-9.
7. Karimi-Nazari E, Nadjarzadeh A, Masoumi R, Marzban A, Mohajeri SA, et al. Effect of saffron (Crocus sativus L.) on lipid profile, glycemic indices and antioxidant status among overweight/obese prediabetic individuals: A double-blinded, randomized controlled trial. Clinical nutrition ESPEN. 2019;34:130-6.
8. Nikbakht-Jam I, Khademi M, Nosrati M, Eslami S, Foroutan-Tanha M, Sahebkar A, et al. Effect of crocin extracted from saffron on pro-oxidant–anti-oxidant balance in subjects with metabolic syndrome: a randomized, placebo-controlled clinical trial. European Journal of Integrative Medicine. 2016;8:307-12.
9. Safarinejad MR, Shafiei N, Safarinejad S. A prospective double- blind randomized placebo- controlled study of the effect of saffron (Crocus sativus Linn.) on semen parameters and seminal plasma antioxidant capacity in infertile men with idiopathic oligoasthenoteratozoospermia. Phytother Res. 2011;25(4):508-16.
10. Lopresti AL, Drummond PD. Saffron (Crocus sativus) for depression: a systematic review of clinical studies and examination of underlying antidepressant mechanisms of action. Human Psychopharmacology: Clinical and Experimental. 2014;29(6):517-27.
11. Tabeshpour J, Sobhani F, Sadjadi SA, Hosseinzadeh H, Mohajeri SA, Rajabi O, et al. A double-blind, randomized, placebo-controlled trial of saffron stigma (Crocus sativus L.) in mothers suffering from mild-to-moderate postpartum depression. Phytomedicine. 2017;36:145-52.
12. Khorasanchi Z, Shafiee M, Kermanshahi F, Khazaei M, Ryzhikov M, Parizadeh MR, et al. Crocus sativus a natural food coloring and flavoring has potent anti-tumor properties. Phytomedicine. 2018;43:21-7.
13. Alavizadeh SH, Hosseinzadeh H. Bioactivity assessment and toxicity of crocin: a comprehensive review. Food Chem Toxicol. 2014;64:65-80.
14. Khazdair MR, Boskabady MH, Hosseini M, Rezaee R, Tsatsakis AM. The effects of Crocus sativus (saffron) and its constituents on nervous system: A review. Avicenna journal of phytomedicine. 2015;5(5):376-391.
15. Sadeghnia HR, Shaterzadeh H, Forouzanfar F, Hosseinzadeh H. Neuroprotective effect of safranal, an active ingredient of Crocus sativus, in a rat model of transient cerebral ischemia. Folia Neuropathol. 2017;55(3):206-13.
16. Dehestani V, SHARIATI SZ, Mohiti S, Akhlaghi S. Liver toxicity in rheumatoid arthritis patients treated with methotrexate. 2015;4(3):102-105.
17. Sarabi ZS, Saeidi MG, Khodashahi M, Rezaie AE, Hashemzadeh K, Khodashahi R, et al. Evaluation of the anti-inflammatory effects of atorvastatin on patients with rheumatoid arthritis: a randomized clinical trial. Electronic physician. 2016;8(8):2700.
18. Ghoryani M, Shariati-Sarabi Z, Tavakkol-Afshari J, Ghasemi A, Poursamimi J, Mohammadi M. Amelioration of clinical symptoms of patients with refractory rheumatoid arthritis following treatment with autologous bone marrow-derived mesenchymal stem cells: A successful clinical trial in Iran. Biomed Pharmacother. 2019;109:1834-40.
19. Li Y, Kakkar R, Wang J. In vivo and in vitro approach to anti-arthritic and anti-inflammatory effect of crocetin by alteration of nuclear factor-E2-related factor 2/hem oxygenase (HO)-1 and NF-κB expression. Front Pharmacol. 2018;9:1341.
20. Li L, Zhang H, Jin S, Liu C. Effects of crocin on inflammatory activities in human fibroblast-like synoviocytes and collagen-induced arthritis in mice. Immunologic research. 2018;66(3):406-13.
21. Lei M, Guo C, Hua L, Xue S, Yu D, Zhang C, et al. Crocin Attenuates Joint Pain and Muscle Dysfunction in Osteoarthritis Rat. Inflammation. 2017;40(6):2086-93.
22. Hemshekhar M, Sebastin Santhosh M, Sunitha K, Thushara RM, Kemparaju K, Rangappa KS, et al. A dietary colorant crocin mitigates arthritis and associated secondary complications by modulating cartilage deteriorating enzymes, inflammatory mediators and antioxidant status. Biochimie. 2012;94(12):2723-33.
23. Ding Q, Zhong H, Qi Y, Cheng Y, Li W, Yan S, et al. Anti-arthritic effects of crocin in interleukin-1β-treated

articular chondrocytes and cartilage in a rabbit osteoarthritic model. Inflamm Res. 2013;62(1):17-25.
24. Poursamimi J, Shariati-Sarabi Z, Tavakkol-Afshari J, Mohajeri SA, Ghoryani M, Mohammadi M. Immunoregulatory effects of KrocinaTM, an herbal medicine made of crocin, on osteoarthritis patients: a successful clinical trial in Iran. Iranian Journal of Allergy, Asthma and Immunology. 2019; article in press.
25. Li J, Lei H-t, Cao L, Mi Y-N, Li S, Cao Y-X. Crocin alleviates coronary atherosclerosis via inhibiting lipid synthesis and inducing M2 macrophage polarization. Int Immunopharmacol. 2018;55:120-7.
26. Pan JX. LncRNA H19 promotes atherosclerosis by regulating MAPK and NF-kB signaling pathway. Eur Rev Med Pharmacol Sci. 2017;21(2):322-8.
27. Christodoulou E, Kadoglou NPE, Stasinopoulou M, Konstandi OA, Kenoutis C, Kakazanis ZI, et al. Crocus sativus L. aqueous extract reduces atherogenesis, increases atherosclerotic plaque stability and improves glucose control in diabetic atherosclerotic animals. Atherosclerosis. 2018;268:207-14.
28. Mahdavifard S, Bathaie SZ, Nakhjavani M, Taghikhani M. The synergistic effect of antiglycating agents (MB-92) on inhibition of protein glycation, misfolding and diabetic complications in diabetic-atherosclerotic rat. Eur J Med Chem. 2016;121:892-902.
29. Hemmati M, Zohoori E, Mehrpour O, Karamian M, Asghari S, Zarban A, et al. Anti-atherogenic potential of jujube, saffron and barberry: anti-diabetic and antioxidant actions. EXCLI journal. 2015;14:908.
30. Xu G-L, Yu S-Q, Gong Z-N, Zhang S-Q. Study of the effect of crocin on rat experimental hyperlipemia and the underlying mechanisms. Zhongguo Zhongyao Zazhi. 2005;30(5):369-72.
31. Abedimanesh N, Bathaie SZ, Abedimanesh S, Motlagh B, et al. Saffron and crocin improved appetite, dietary intakes and body composition in patients with coronary artery disease. Journal of cardiovascular and thoracic research. 2017;9(4):200.
32. Wang Y, Sun J, Liu C, Fang C. Protective effects of crocetin pretreatment on myocardial injury in an ischemia/reperfusion rat model. Eur J Pharmacol. 2014;741:290-6.
33. Joukar S, Ghasemipour-Afshar E, Sheibani M, Naghsh N, Bashiri A. Protective effects of saffron (Crocus sativus) against lethal ventricular arrhythmias induced by heart reperfusion in rat: a potential anti-arrhythmic agent. Pharm Biol. 2013;51(7):836-43.
34. Jahanbakhsh Z, Rasoulian B, Jafari M, Shekarforoush S, Esmailidehaj M, taghi Mohammadi M, et al. Protective effect of crocin against reperfusion-induced cardiac arrhythmias in anaesthetized rats. EXCLI journal. 2012;11:20-9.
35. Llorens S, Mancini A, Serrano-Díaz J, D'Alessandro A, Nava E, Alonso G, et al. Effects of crocetin esters and crocetin from Crocus sativus L. on aortic contractility in rat genetic hypertension. Molecules. 2015;20(9):17570-84.
36. Wang Y, Wang Q, Yu W, Du H. Crocin Attenuates Oxidative Stress and Myocardial Infarction Injury in Rats. Int Heart J. 2018;59(2):387-393.
37. Efentakis P, Rizakou A, Christodoulou E, Chatzianastasiou A, López MG, León R, et al. Saffron (Crocus sativus) intake provides nutritional preconditioning against myocardial ischemia–reperfusion injury in Wild Type and ApoE (−/−) mice: Involvement of Nrf2 activation. Nutrition, Metabolism and Cardiovascular Diseases. 2017;27(10):919-29.
38. Nader M, Chahine N, Salem C, Chahine R. Saffron (Crocus sativus) pretreatment confers cardioprotection against ischemia-reperfusion injuries in isolated rabbit heart. J Physiol Biochem. 2016;72(4):711-9.
39. Bharti S, Golechha M, Kumari S, Siddiqui KM, Arya DS. Akt/GSK-3β/eNOS phosphorylation arbitrates safranal-induced myocardial protection against ischemia–reperfusion injury in rats. Eur J Nutr. 2012;51(6):719-27.
40. Zeng C, Li H, Fan Z, Zhong L, Guo Z, Guo Y, et al. Crocin-elicited autophagy rescues myocardial ischemia/reperfusion injury via paradoxical mechanisms. The American journal of Chinese medicine. 2016;44(3):515-30.
41. Chahine N, Nader M, Duca L, Martiny L, Chahine R. Saffron extracts alleviate cardiomyocytes injury induced by doxorubicin and ischemia-reperfusion in vitro. Drug Chem Toxicol. 2016;39(1):87-96.
42. Chahine N, Makhlouf H, Duca L, Martiny L, Chahine R. Cardioprotective effect of saffron extracts against acute doxorubicin toxicity in isolated rabbit hearts submitted to ischemia-reperfusion injury. Zeitschrift für Naturforschung C. 2014;69(11-12):459-70.
43. Huang Z, Nan C, Wang H, Su Q, Xue W, Chen Y, et al. Crocetin ester improves myocardial ischemia via Rho/ROCK/NF-κB pathway. Int Immunopharmacol. 2016;38:186-93.
44. Ochiai T, Shimeno H, Mishima K-i, Iwasaki K, Fujiwara M, Tanaka H, et al. Protective effects of

carotenoids from saffron on neuronal injury in vitro and in vivo. Biochimica et Biophysica Acta (BBA)-General Subjects. 2007;1770(4):578-84.
45. Huang A, Jia L. Crocin enhances hypothermia therapy in hypoxic ischemia-induced brain injury in mice. Acta Neurol Belg. 2019:1-8.
46. Farjah GH, Salehi S, Ansari MH, Pourheidar B. Protective effect of Crocus sativus L.(Saffron) extract on spinal cord ischemia-reperfusion injury in rats. Iranian journal of basic medical sciences. 2017;20(3):334-37.
47. Chen L, Qi Y, Yang X. Neuroprotective effects of crocin against oxidative stress induced by ischemia/reperfusion injury in rat retina. Ophthalmic Res. 2015;54(3):157-68.
48. Vakili A, Einali MR, Bandegi AR. Protective effect of crocin against cerebral ischemia in a dose-dependent manner in a rat model of ischemic stroke. J Stroke Cerebrovasc Dis. 2014;23(1):106-13.
49. Zheng Y-Q, Liu J-X, Wang J-N, Xu L. Effects of crocin on reperfusion-induced oxidative/nitrative injury to cerebral microvessels after global cerebral ischemia. Brain Res. 2007;1138:86-94.
50. Saleem S, Ahmad M, Ahmad AS, Yousuf S, Ansari MA, et al. Effect of saffron (Crocus sativus) on neurobehavioral and neurochemical changes in cerebral ischemia in rats. J Med Food. 2006;9(2):246-53.
51. Hosseinzadeh H, Sadeghnia HR, Ghaeni FA, Motamedshariaty VS, Mohajeri SA. Effects of saffron (Crocus sativus L.) and its active constituent, crocin, on recognition and spatial memory after chronic cerebral hypoperfusion in rats. Phytother Res. 2012;26(3):381-6.
52. Hosseinzadeh H, Sadeghnia HR. Safranal, a constituent of Crocus sativus (saffron), attenuated cerebral ischemia induced oxidative damage in rat hippocampus. J Pharm Pharm Sci. 2005;8(3):394-9.
53. Asadollahi M, Nikdokht P, Hatef B, Sadr SS, Sahraei H, Assarzadegan F, et al. Protective properties of the aqueous extract of saffron (Crocus sativus L.) in ischemic stroke, randomized clinical trial. J Ethnopharmacol. 2019;238:111833.
54. Greene S. Perspectives of quality control in diabetes treatment at the end of the century: facts and visions. Horm Res Paediatr. 1998;50(1):103-5.
55. Arababadi MK, Naghavi N, Hassanshahi G, Mahmoodi M. Is CCR5-Δ32 mutation associated with diabetic nephropathy in type 2 diabetes? Ann Saudi Med. 2009;29(5):413.
56. Mohammadi M, Gozashti MH, Aghadavood M, Mehdizadeh MR, Hayatbakhsh MM. Clinical significance of serum IL-6 and TNF-α levels in patients with metabolic syndrome. Reports of biochemistry & molecular biology. 2017;6(1):74-79.
57. Samaha MM, Said E, Salem HA. A comparative study of the role of crocin and sitagliptin in attenuation of STZ-induced diabetes mellitus and the associated inflammatory and apoptotic changes in pancreatic β-islets. Environ Toxicol Pharmacol. 2019;72:103238.
58. Motamedrad M, Shokouhifar A, Hemmati M, Moossavi M. The regulatory effect of saffron stigma on the gene expression of the glucose metabolism key enzymes and stress proteins in streptozotocin-induced diabetic rats. Res Pharm Sci. 2019;14(3):255-262.
59. Faridi S, Delirezh N, Froushani SMA. Beneficial effects of hydroalcoholic extract of saffron in alleviating experimental autoimmune diabetes in C57bl/6 mice. Iranian Journal of Allergy, Asthma and Immunology. 2019;18(1).
60. Jiang SP, Shen Q, Lu Y, Yan YQ, Tong YP, Wang P. Effect of saffron aqueous extract on the level of blood glucose in experimental diabetes mice. Zhongguo ying yong sheng li xue za zhi= Zhongguo yingyong shenglixue zazhi= Chinese journal of applied physiology. 2018;34(2):173-76.
61. Roshankhah S, Jalili C, Salahshoor MR. Effects of Crocin on Sperm Parameters and Seminiferous Tubules in Diabetic Rats. Advanced biomedical research. 2019;8(4).
62. Delkhosh-Kasmaie F, Farshid AA, Tamaddonfard E, Imani M. The effects of safranal, a constitute of saffron, and metformin on spatial learning and memory impairments in type-1 diabetic rats: behavioral and hippocampal histopathological and biochemical evaluations. Biomed Pharmacother. 2018;107:203-11.
63. Abou-Hany HO, Atef H, Said E, Elkashef HA, Salem HA. Crocin mediated amelioration of oxidative burden and inflammatory cascade suppresses diabetic nephropathy progression in diabetic rats. Chem Biol Interact. 2018;284:90-100.
64. Asri-Rezaei S, Tamaddonfard E, Ghasemsoltani-Momtaz B, Erfanparast A, Gholamalipour S. Effects of crocin and zinc chloride on blood levels of zinc and metabolic and oxidative parameters in streptozotocin-induced diabetic rats. Avicenna journal of phytomedicine. 2015;5(5):403.
65. Altinoz E, Oner Z, Elbe H, Cigremis Y, Turkoz Y. Protective effects of saffron (its active constituent, crocin) on nephropathy in streptozotocin-induced

diabetic rats. Hum Exp Toxicol. 2015;34(2):127-34.
66. Ishizuka F, Shimazawa M, Umigai N, Ogishima H, Nakamura S, Tsuruma K, et al. Crocetin, a carotenoid derivative, inhibits retinal ischemic damage in mice. Eur J Pharmacol. 2013;703(1-3):1-10.
67. Xuan B, ZHOU Y-H, Li NA, MIN Z-D, CHIOU GCY. Effects of crocin analogs on ocular blood flow and retinal function. J Ocul Pharmacol Ther. 1999;15(2):143-52.
68. Mohammadzadeh-Moghadam H, Nazari SM, Shamsa A, Kamalinejad M, Esmaeeli H, Asadpour AA, et al. Effects of a topical saffron (Crocus sativus L) gel on erectile dysfunction in diabetics: A randomized, parallel-group, double-blind, placebo-controlled trial. J Evid Based Complementary Altern Med. 2015;20(4):283-6.
69. Abou-Hany HO, Atef H, Said E, Elkashef HA, Salem HA. Crocin reverses unilateral renal ischemia reperfusion injury-induced augmentation of oxidative stress and toll like receptor-4 activity. Environ Toxicol Pharmacol. 2018;59:182-9.
70. Qi Y, Chen L, Zhang L, Liu W-B, Chen X-Y, Yang X-G. Crocin prevents retinal ischaemia/reperfusion injury-induced apoptosis in retinal ganglion cells through the PI3K/AKT signalling pathway. Exp Eye Res. 2013;107:44-51.
71. Yang X, Huo F, Liu B, Liu J, Chen T, Li J, et al. Crocin inhibits oxidative stress and pro-inflammatory response of microglial cells associated with diabetic retinopathy through the activation of PI3K/Akt signaling pathway. J Mol Neurosci. 2017;61(4):581-9.
72. Dehghan F, Hajiaghaalipour F, Yusof A, Muniandy S, Hosseini SA, Heydari S, et al. Saffron with resistance exercise improves diabetic parameters through the GLUT4/AMPK pathway in-vitro and in-vivo. Sci Rep. 2016;6:25139.
73. Maeda A, Kai K, Ishii M, Ishii T, Akagawa M. Safranal, a novel protein tyrosine phosphatase 1 B inhibitor, activates insulin signaling in C 2 C 12 myotubes and improves glucose tolerance in diabetic KK- Ay mice. Mol Nutr Food Res. 2014;58(6):1177-89.
74. Kang C, Lee H, Jung E-S, Seyedian R, Jo M, Kim J, et al. Saffron (Crocus sativus L.) increases glucose uptake and insulin sensitivity in muscle cells via multipathway mechanisms. Food Chem. 2012;135(4):2350-8.
75. Moravej Aleali A, Amani R, Shahbazian H, Namjooyan F, Latifi SM, Cheraghian B. The effect of hydroalcoholic Saffron (Crocus sativus L.) extract on fasting plasma glucose, HbA1c, lipid profile, liver, and renal function tests in patients with type 2 diabetes mellitus: A randomized double- blind clinical trial. Phytother Res. 2019;33(6):1648-1657.
76. Suh KS, Chon S, Jung W-W, Choi EM. Crocin attenuates methylglyoxal-induced osteoclast dysfunction by regulating glyoxalase, oxidative stress, and mitochondrial function. Food Chem Toxicol. 2019;124:367-73.
77. Milajerdi A, Jazayeri S, Shirzadi E, Hashemzadeh N, Azizgol A, Djazayery A, et al. The effects of alcoholic extract of saffron (Crocus satious L.) on mild to moderate comorbid depression-anxiety, sleep quality, and life satisfaction in type 2 diabetes mellitus: A double-blind, randomized and placebo-controlled clinical trial. Complement Ther Med. 2018;41:196-202.
78. Sepahi S, Mohajeri SA, Hosseini SM, Khodaverdi E, Shoeibi N, Namdari M, et al. Effects of Crocin on diabetic maculopathy: a placebo-controlled randomized clinical trial. Am J Ophthalmol. 2018;190:89-98.
79. Milajerdi A, Jazayeri S, Hashemzadeh N, Shirzadi E, Derakhshan Z, Djazayeri A, et al. The effect of saffron (Crocus sativus L.) hydroalcoholic extract on metabolic control in type 2 diabetes mellitus: A triple-blinded randomized clinical trial. Journal of research in medical sciences: the official journal of Isfahan University of Medical Sciences. 2018;23(16):1-25.
80. Azimi P, Ghiasvand R, Feizi A, Hosseinzadeh J, Bahreynian M, et al. Effect of cinnamon, cardamom, saffron and ginger consumption on blood pressure and a marker of endothelial function in patients with type 2 diabetes mellitus: A randomized controlled clinical trial. Blood Press. 2016;25(3):133-40.
81. Azimi P, Ghiasvand R, Feizi A, Hariri M, Abbasi B. Effects of cinnamon, cardamom, saffron, and ginger consumption on markers of glycemic control, lipid profile, oxidative stress, and inflammation in type 2 diabetes patients. The review of diabetic studies: RDS. 2014;11(3-4):258-66.
82. Weitgasser R, Lechleitner M, Koch T, Galvan G, Mühlmann J, Steiner K, et al. Antibodies to heat-shock protein 65 and neopterin levels in patients with type 1 diabetes mellitus. Experimental and clinical endocrinology & diabetes. 2003;111(3):127-31.
83. Shemshian M, Mousavi SH, Norouzy A, Kermani T, Moghiman T, Sadeghi A, et al. Saffron in metabolic syndrome: its effects on antibody titers to heat-shock proteins 27, 60, 65 and 70. Journal of Complementary and Integrative Medicine. 2014;11(1):43-9.

84. Shahbazian H, Aleali AM, Amani R, Namjooyan F, Cheraghian B, Latifi SM, et al. Effects of saffron on homocysteine, and antioxidant and inflammatory biomarkers levels in patients with type 2 diabetes mellitus: a randomized double-blind clinical trial. Avicenna journal of phytomedicine. 2019;9(5):436-45.
85. Ebrahimi F, Aryaeian N, Pahlavani N, Abbasi D, Hosseini AF, Fallah S, et al. The effect of saffron (Crocus sativus L.) supplementation on blood pressure, and renal and liver function in patients with type 2 diabetes mellitus: A double-blinded, randomized clinical trial. Avicenna journal of phytomedicine. 2019;9(4):322-33.
86. Sairenji T, Collins KL, Evans DV. An Update on Inflammatory Bowel Disease. Primary care. 2017;44(4):673-92.
87. Rezaei N, Avan A, Pashirzad M, Rahmani F, Moradi Marjaneh R, Behnam-Rassouli R, et al. Crocin as a novel therapeutic agent against colitis. Drug Chem Toxicol. 2019;24:1-8.
88. Mohammadi M, Zahedi MJ, Nikpoor AR, Nazem M, et al. Determination of Vitamin D Serum Levels and Status of the C3435T Polymorphism of Multidrug Resistance 1 Gene in Southeastern Iranian Patients with Ulcerative Colitis. Middle East journal of digestive diseases. 2015;7(4):245-52.
89. Khodir AE, Said E, Atif H, ElKashef HA, Salem HA. Targeting Nrf2/HO-1 signaling by crocin: Role in attenuation of AA-induced ulcerative colitis in rats. Biomed Pharmacother. 2019;110:389-99.
90. Kawabata K, Tung NH, Shoyama Y, Sugie S, Mori T, Tanaka T. Dietary Crocin Inhibits Colitis and Colitis-Associated Colorectal Carcinogenesis in Male ICR Mice. 2012;2012.
91. Gezici S. Comparative anticancer activity analysis of saffron extracts and a principle component, crocetin for prevention and treatment of human malignancies. J Food Sci Technol. 2019;56(12):5435-43.
92. Kim SH, Lee JM, Kim SC, Park CB, Lee PC. Proposed cytotoxic mechanisms of the saffron carotenoids crocin and crocetin on cancer cell lines. Biochem Cell Biol. 2014;92(2):105-11.
93. Ege B, Yumrutas O, Ege M, Pehlivan M, Bozgeyik I. Pharmacological properties and therapeutic potential of saffron (Crocus sativus L.) in osteosarcoma. J Pharm Pharmacol. 2019;72(1):56-67.
94. Zhou Y, Xu Q, Shang J, Lu L, Chen G. Crocin inhibits the migration, invasion, and epithelial- mesenchymal transition of gastric cancer cells via miR- 320/KLF5/HIF- 1α signaling. Journal of cellular physiology. 2019;234(10):17876-885.
95. Li S, Qu Y, Shen X-Y, Ouyang T, Fu W-B, Luo T, et al. Multiple Signal Pathways Involved in Crocetin-Induced Apoptosis in KYSE-150 Cells. Pharmacology. 2019;103(5-6):263-72.
96. Deng L, Li J, Lu S, Su Y. Crocin inhibits proliferation and induces apoptosis through suppressing MYCN expression in retinoblastoma. J Biochem Mol Toxicol. 2019;33(5):e22292.
97. Liu T, Tian L, Fu X, Wei L, Li J, Wang T. Saffron inhibits the proliferation of hepatocellular carcinoma via inducing cell apoptosis. Panminerva Med. 2019.
98. Neyshaburinezhad N, Hashemi M, Ramezani M, Arabzadeh S, Behravan J, Kalalinia F. The effects of crocetin, extracted from saffron, in chemotherapy against the incidence of multiple drug resistance phenotype. Iranian journal of basic medical sciences. 2018;21(11):1192.
99. Giakoumettis D, Pourzitaki C, Vavilis T, Tsingotjidou A, Kyriakoudi A, Tsimidou M, et al. Crocus sativus L. Causes a Non Apoptotic Calpain Dependent Death in C6 Rat Glioma Cells, Exhibiting a Synergistic Effect with Temozolomide. Nutr Cancer. 2019;71(3):491-507.
100. Arzi L, Riazi G, Sadeghizadeh M, Hoshyar R, Jafarzadeh N. A Comparative Study on Anti-Invasion, Antimigration, and Antiadhesion Effects of the Bioactive Carotenoids of Saffron on 4T1 Breast Cancer Cells Through Their Effects on Wnt/β-Catenin Pathway Genes. DNA Cell Biol. 2018;37(8):697-707.
101. Menghini L, Leporini L, Vecchiotti G, Locatelli M, Carradori S, Ferrante C, et al. Crocus sativus L. stigmas and byproducts: qualitative fingerprint, antioxidant potentials and enzyme inhibitory activities. Food research international. 2018;109:91-8.
102. Kim B, Park B. Saffron carotenoids inhibit STAT3 activation and promote apoptotic progression in IL-6-stimulated liver cancer cells. Oncol Rep. 2018;39(4):1883-91.
103. Arzi L, Farahi A, Jafarzadeh N, Riazi G, Sadeghizadeh M, Hoshyar R. Inhibitory Effect of Crocin on Metastasis of Triple-Negative Breast Cancer by Interfering with Wnt/β-Catenin Pathway in Murine Model. DNA Cell Biol. 2018;37(12):1068-75.
104. Li S, Shen X-Y, Ouyang T, Qu Y, Luo T, Wang H-Q. Synergistic anticancer effect of combined crocetin and cisplatin on KYSE-150 cells via p53/p21 pathway. Cancer Cell Int. 2017;17(1):98.
105. Lu P, Lin H, Gu Y, Li L, Guo H, Wang F, et al.

Antitumor effects of crocin on human breast cancer cells. Int J Clin Exp Med. 2015;8(11):20316.
106. Sawant AV, Srivastava S, Prassanawar SS, Bhattacharyya B, Panda D. Crocin, a carotenoid, suppresses spindle microtubule dynamics and activates the mitotic checkpoint by binding to tubulin. Biochem Pharmacol. 2019;163:32-45.
107. Zhang A, Li J. Crocetin shifts autophagic cell survival to death of breast cancer cells in chemotherapy. Tumor Biology. 2017;39(3):1010428317694536.
108. Geromichalos GD, Papadopoulos T, Sahpazidou D, Sinakos Z. Safranal, a Crocus sativus L constituent suppresses the growth of K-562 cells of chronic myelogenous leukemia. In silico and in vitro study. Food Chem Toxicol. 2014;74:45-50.
109. Rezaee R, Mahmoudi M, Abnous K, Rabe SZT, Tabasi N, Hashemzaei M, et al. Cytotoxic effects of crocin on MOLT-4 human leukemia cells. Journal of Complementary and Integrative Medicine. 2013;10(1):105-12.
110. Sun Y, Xu H-J, Zhao Y-X, Wang L-Z, Sun L-R, Wang Z, et al. Crocin exhibits antitumor effects on human leukemia HL-60 cells in vitro and in vivo. Evid Based Complement Alternat Med. 2013;2013.
111. Bhandari PR. Crocus sativus L.(saffron) for cancer chemoprevention: a mini review. Journal of traditional and complementary medicine. 2015;5(2):81-7.
112. Babaei A, Arshami J, Haghparast A, Mesgaran MD. Effects of saffron (Crocus sativus) petal ethanolic extract on hematology, antibody response, and spleen histology in rats. Avicenna journal of phytomedicine. 2014;4(2):103.
113. Moradzadeh M, Ghorbani A, Erfanian S, Mohaddes ST, Rahimi H, Karimiani EG, et al. Study of the mechanisms of crocetin- induced differentiation and apoptosis in human acute promyelocytic leukemia cells. J Cell Biochem. 2019;120(2):1943-57.