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REVIEW

WILEY

The cardiovascular-protective properties of saffron and its potential pharmaceutical applications: A critical appraisal of the literature

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Saffron, the dried stigma of *Crocus sativus* L., is used in traditional medicine for its healing properties and the treatment of various pathological conditions. The present literature review aimed to summarize and evaluate the preclinical and clinical data regarding the protective effects and mechanisms of saffron and its main components (crocin, crocetin, safranal) on cardiovascular risk factors and diseases. Many in vitro and animal studies have been conducted implicating antioxidant, hypolipidemic, antidiabetic, and antiinflammatory impact of saffron and its constituents. Notably, there is evidence of direct atherosclerosis regression and stabilization in valid atherosclerosis-prone animal models. However, current clinical trials have shown mostly weak effects of saffron and its constituents on cardiovascular risk factors: (a) Modest lowering of fasting blood glucose, without significant reduction of HbA1c in type 2 diabetic patients, (b) moderate/controversial hypolipidemic effects, (c) negligible hypotensive effect, and (d) inconsistent modification of metabolic syndrome parameters. There are important drawbacks in clinical trial design, including the absence of pharmacokinetic/pharmacodynamic tests, the wide variance of doses and cohorts' characteristics, the small number of patients, the short duration.
Therefore, large, properly designed, high-quality clinical trials, focusing on specific conditions are required to evaluate the biological/pharmacological activities and firmly establish the clinical efficacy of saffron and its possible therapeutic uses in cardiovascular diseases.

KEYWORDS
cardiovascular diseases, crocetin, crocin, saffron, safranal

1 | INTRODUCTION

The manifestations of cardiovascular diseases (CVDs), such as coronary artery disease (CAD), myocardial infarction, ischemic stroke, and so forth, remain the first leading cause of morbidity and mortality worldwide (Cho et al., 2020). Nowadays, pharmaceutical and non-pharmaceutical interventions modifying cardiovascular risk factors (e.g., diabetes, hyperlipidemia, etc.) predisposing to CVDs are at the top priority of scientific research (Kadoglou et al., 2013, 2015). In the context of a healthier lifestyle, non-pharmaceutical measures

Abbreviations: ALT, alanine aminotransferase; AMPK, 5'-adenosine monophosphate-activated protein kinase; AST, aspartate aminotransferase; BMI, body mass index; BP, blood pressure; BW, body weight; CAD, coronary artery disease; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HOMA-IR, homeostatic model assessment for insulin resistance; LOX1, lectin-like oxidized LDL receptor 1; MS, metabolic syndrome; QUICKI, quantitative insulin sensitivity check index; SAE, saffron aqueous extract; SBP, systolic blood pressure; SD, sexual dysfunction; T2DM, type 2 diabetes mellitus; TC, total cholesterol; TG, triglycerides; WC, waist circumstance.

including herbal supplements have recently attracted the growing interest from research groups as a part of therapeutic strategies for CVDs primary or secondary prevention (Baumgartner, Bruckert, Gallo, & Plat, 2020; Christodoulou, Kadoglou, Kostomitsopoulos, & Valsami, 2015). A plethora of in vitro and in vivo experimental studies, where all modifiers are well-controlled, have supported the beneficial effects of some herb derivatives as beneficial supplements in cohorts of people at risk of CVDs. However, there are still too many uncertainties to precisely assess the impact of such dietary supplements on humans and hence extrapolate experimental promising results to clinical practice due to: heterogeneous doses, divergent constituents, absence of pharmacodynamic/pharmacokinetic analyses, appliance to populations with variable characteristics, overshadowing by concordant pharmaceutical treatment, and so forth. Thereby, the vast majority of findings in clinical trials are often inconclusive or with inadequate power, limiting their generalizability to the overall population (Venkatakrishnan, Chiu, & Wang, 2020; Watanabe et al., 2020).

Saffron (*Crocus sativus* L., Iridaceae), obtained from the dehydrated stigmas of the plant's flower, and its main constituents (safranal, crocin, crocetin) are known for many years for a variety of medicinal uses. In recent years, published data from in vitro, ex vivo, and in vivo animal studies have described the pharmacological properties of saffron and its constituents (e.g., Akbari, Ali Mard, & Veisi, 2018; Azami et al., 2021; Bukhari, Manzoor, & Dhar, 2018; Christodoulou et al., 2015; Ghaffari & Roshanravan, 2019; Hatziagapiou & Lambrou, 2018; Leone et al., 2018; Razavi & Hosseinzadeh, 2017; Soeda et al., 2007; Su et al., 2021; Vahedi et al., 2016). Experimental in vitro and pre-clinical animal data have indicated the cardioprotective effects of saffron and its constituents through modulation of oxidative stress, inflammation, blood pressure, lipid and glycaemic profile, or direct anti-atherogenic effects (Tables 1 and 2; Figure 2). On the other hand, an increasing body of observational or randomized, small-scale, clinical trials have examined the cardiovascular actions of saffron and its constituents (Tables 3 and 4).

Taken all together, the present literature review assessed the pharmacological properties of saffron (from saffron stigmas) and its constituents in relation to cardiovascular risk factors and the potential clinical applications in CVD prevention and therapy. Compared to recent meta-analyses and literature review (Ghaffari & Roshanravan, 2019; Nanda & Madan, 2021; Pourmasoumi et al., 2019; Roshanravan et al., 2020), the present review paper summarizes both experimental and clinical data about cardiovascular risk factors and diseases. From the bottom to the top, we initially assessed in each section of the main cardiovascular risk factors the pathophysiological therapeutic mechanisms of saffron and its constituents. In order to ensure an extrapolation of experimental findings to clinical studies, we mainly focused on in vivo animal studies, rather than in vitro experiments, with low reproducibility in live organisms. We then evaluated the clinical applicability of experimental results, along with a critical appraisal of limitations and potentialities. Overall, this is a more realistic approach, which tones down the clinical relevance of initially remarkable experimental findings since the current clinical findings are modest and have derived from widely heterogeneous cohorts.

2 | METHODS—LITERATURE SEARCH

A literature search in the English language was conducted for publications on MEDLINE and EMBASE, Web of Science, Cochrane, and Google Scholar databases from 2000 to August 1, 2021. The following search terms, in titles and abstracts, including Medical Subject Headings (MeSH), of saffron and its constituents (*C. sativus* or crocin or saffron or safranal) were used interchangeably. Those terms were combined with any of the following most known cardiovascular risk factors or CVDs: diabetes mellitus or hyperglycemia or hypertension or metabolic syndrome (MS) or dyslipidemia or hyperlipidemia or atherosclerosis or CAD or peripheral artery diseases or arrhythmias or cardiomyopathy or diabetic macro/micro-vascular complications or oxidative stress or antioxidant factors. Two investigators (Nikolaos Kadoglou and Eirini Christodoulou) independently performed the literature search. Regarding the experimental section, 931 studies were initially found fulfilling any of the above-mentioned inclusion criteria. We then focused mainly on in vivo or experimental studies for underlying mechanisms explanation rather than in vitro data. In the end, after limiting of in vitro and ex vivo studies, 25 experimental studies remained for extrapolation of the regulatory mechanisms of cardiovascular risk factors and CVDs by saffron and its constituents. Using the same list of cardiovascular risk factors and CVDs as in the experimental section, we searched among clinical studies, and we initially found 119 studies. We further limited our literature search by setting the following inclusion criteria: interventional, randomized and non-randomized, prospective studies, published only in the English language, enrolling at least 10 patients in each arm. We ended-up in 21, mostly randomized, clinical trials included in six systematic reviews and meta-analyses for diabetes mellitus, hyperlipidemia, and multiple cardiovascular risk factors. In addition, 11 most recent randomized clinical trials (RCTs) for CAD, hypertension, and MS were included in ours review. The reference lists of the identified articles were checked, and no additional relevant articles were found. Tables 1–4 summarize the results from both experimental/animal and clinical studies, while in Figure 1 are given schematically the most important cardiovascular protective properties of saffron and its active constituents.

3 | RESULTS AND DISCUSSION

3.1 | Description of Saffron's main components

The main active components present in the red stigmas of the plant's (*C. sativus* L.) flower are shown in Figure 2 (Alonso, Salinas, Garijo, & Sanches-Fernandez, 2001; Carmona, Zalacain, & Alonso, 2006; Fernandez, 2004; García-Blázquez, Moratalla-López, Lorenzo, Salinas, & Alonso, 2021; Winterhalter & Straubinger, 2000). Picrocrocin is the substance giving saffron its bitter taste and is present in the plant's stigmas in the amount of 4%. Picrocrocin ($C_{16}H_{26}O_7$, Figure 2) is a glycoside that, in the presence of acids and alkali, easily turns into safranal (Carmona et al., 2006; García-Blázquez

TABLE 1 Animal studies investigating the effects of saffron and its constituents on atherosclerosis and myocardial ischemia/reperfusion

Disease	Animal model—duration—saffron or individual compound	Intervention	Effect-mechanism of action	Reference
Atherosclerosis	Quails—9 weeks Crocin	Control: Normal diet; model: Hyperlipidamic diet; Crocin: Hyperlipidamic diet and crocin (25, 50, 100 mg kg^{-1} day^{-1}); Zhibituo (lovastatin): Hyperlipidamic diet and Zhibituo (1 g/kg)	Crocin reduced serum TC, TG, LDL-c and inhibit the formation of aortic plaque. Crocin reduced serum MDA and increased NO Crocin exhibited antiatherosclerotic effects through decreasing the level of Ox-LDL.	He et al. (2005)
	ApoE$^{-/-}$ C57BL/6 mice—4 weeks Saffron aqueous extract (SFE)	(a) Control (WFI gavage), (b) SFE low-dose (30 mg kg^{-1} day^{-1}), (c) medium-dose (60 mg kg^{-1} day^{-1}), and (d) high-dose (90 mg kg^{-1} day^{-1}).	SFE induced plaque regression and stabilization in a dose-dependent manner, via anti-inflammatory mechanisms (decreased TNFa, IL-6, MCP1, MMPs-2,3,9 and increased TIMP-2 aortic tissue levels). Glucose and triglycerides levels decreased in high-dose SFE	Christodoulou et al. (2018)
	Male Wistar rats—4 weeks Crocin	Controls: Normal chow and i.p. 2 ml/kg normal saline. Vit D3 group: High-fat diet and i.p. 2 ml/kg Vit D3 (600,000 IU/kg). Vit D3 + Crocin group: Half of Vit D3 group received 100 mg kg^{-1} day^{-1} Crocin (gavage)	Crocin decreased blood lipid levels, ET, TC, TG, LDL-c, increased HDL-c. inhibited lipogenesis and alleviated inflammation by promoting M2 macrophage polarization and inhibiting NF-κB p65 nuclear translocation.	Li et al. (2018)
Myocardial ischemia/reperfusion	Male Wistar albino rats—14 days Safranal	Safranal at different doses (0.1–0.5 ml kg^{-1} day^{-1}, i.p.) or saline administration followed I/R (transient coronary artery ligation)	Decrease of infarct size, improved left ventricular function and myocardium hemodynamic status. Akt/GSK-3b/eNOS phosphorylation, increased NO bioavailability and suppressed of IKK-β/NF-κB pathway.	Bharti, Golechha, Kumari, Siddiqui, and Arya (2011)
	Male Wistar albino rats—21 days Crocin	Eight groups: (a) vehicle-control, (b) crocin per os (5, 10, and 20 mg kg^{-1} day^{-1}) for 21 days, (c) isoproterenol-control per os, and (d) crocin + isoproterenol: Per os crocin (5, 10, and 20 mg kg^{-1} day^{-1}) with isoproterenol	Crocin pretreatment improved cardiac function by via restoration of endogenous antioxidants, controlling lipid peroxide formation, and preserving activities of CK-MB, LDH enzymes. Preservation of histoarchitecture of myocyte.	Goyal et al. (2010)
	WT and ApoE$^{-/-}$ C57BL/6 mice—4 weeks Saffron aqueous extract	30 min, 1 and 2 hr I/R, after oral interventions: (a) WT control: WFI, (b) WT *crocus*: SFE 60 mg kg^{-1} day^{-1}, (c) WT *crocus* + wortmannin: SFE 60 mg kg^{-1} day^{-1}, wortmannin 60 μg/kg bolus 15 min before R, (d) ApoE$^{-/-}$ control: WFI, (e) ApoE$^{-/-}$ *crocus*: SFE 60 mg kg^{-1} day^{-1}, and (f) ApoE$^{-/-}$ *crocus*	SFE reduced infarct size in WT and ApoE$^{-/-}$ mice. Bolus wortmannin partially inhibited the efficacy of SFE (in both WT and ApoE$^{-/-}$ mice). SFE limits myocardial infarction in WT and ApoE$^{-/-}$ mice activating Akt/eNOS/ERK1/2/GSK3-β and through Nrf2 pathway, bestowing	Efentakis et al. (2017)

(Continues)

TABLE 1 (Continued)

Disease	Animal model—duration—saffron or individual compound	Intervention	Effect-mechanism of action	Reference
		+ wortmannin: SFE 60 mg kg^{-1} day^{-1}, wortmannin 60 μg/kg bolus, 15 min before R.	antioxidant protection against I/R.	
	Male Wistar rats—7 days SFE	(a) Control (CTL) (tap water), (b) Saf50, Saf100, Saf200 (SFE—50, 100, and 200 mg kg^{-1} day^{-1}), and (c) Amiodarone (amiodarone 30 mg kg^{-1} day^{-1}). Day 8, heart I/R	Saffron attenuates susceptibility and incidence of fatal ventricular I/R arrhythmia. Effect mediated through reduction of electrical conductivity and prolonging the action potential duration. The PR and QTcn intervals of ECG were significantly longer in the Saf200 group.	Joukar, Ghasemipour-Afshar, Sheibani, Naghsh, and Bashiri (2013)
	Male Wistar rats—21 days Crocin	Four groups: Crocin (20 mg kg^{-1} day^{-1}) or vehicle (i.p.) with or without cardiac I/R.	Protective role of crocin on cardiac I/R arrhythmias probably related to stability/amplification of antioxidant systems. Crocin decreased SOD activity and GSH level and increased MDA level and catalase activity of heart muscle.	Jahanbakhsh et al. (2012)
	Wistar rats, (ISO)-induced MI—9 days SFE	SFE (20, 40, 80, and 160 mg kg^{-1} day^{-1} i.p.) or safranal (0.025, 0.050, and 0.075 ml/kg i.p.) or control with isoproterenol (85 mg/kg, SC, at 24 hr interval) on Days 8 and 9	Saffron and safranal exhibited cardioprotective effect in isoproterenol-induced myocardial infarction, modulating of oxidative stress and maintaining cell redox status. Saffron or safranal pretreatment decreased serum LDH, CK-MB and myocardial lipid peroxidation.	Mehdizadeh, Parizadeh, Khoeei, Mehri, and Hosseinzadeh (2013)

Abbreviations: Akt, protein kinase A,B; $ApoE^{-/-}$, *Apolipoprotein E*-deficient; BGL, blood glucose levels; CK-MB, creatine kinase-muscle brain; eNOS, endothelial nitric oxide synthetase; ERK1/2, extracellular signal-regulated kinase 1/2; ET, endothelin; GSK-3b, glycogen synthase kinase-3b; HDL-c, high-density lipoprotein cholesterin; i.p., intra-peritoneal; I/R, ischemia/reperfusion; IKK-β, inhibitor of nuclear factor kappa-B kinase subunit beta; IL-6, interleucin-6; ISO, isoproterenol; LDL-c, low-density lipoprotein cholesterol; MCP1, monocyte chemoattractant protein-1; MI, myocardial infarction; MMPs, matrix metalloproteinases; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; Nrf2, nuclear factor E2-related factor 2; SFE, saffron extract; STZ, streptozotocin; TIMP-2: MMP-2 inhibitor; TNFa, tumor necrosis factor-a; WFI, water for injection; WT, wild type.

et al., 2021). Safranal is present as 70% in the volatile oil giving saffron the characteristic aroma. It is a cyclical terpenic aldehyde (2,6,6-trimethyl-1,3-cyclohexadien-1-carboxaldehyde). Crocins are water-soluble carotenoids with high glycosyl contents and saffron's colored compounds. Crocin is the most intensively studied saffron constituent (García-Blázquez et al., 2021; García-Rodríguez et al., 2017). Crocetin is the active metabolite of crocin and also belongs to carotenoids (Carmona et al., 2006; Fernandez, 2004; García-Blázquez et al., 2021). Crocetin is mainly referred to for its antioxidant properties (due to its chemical structure) and is the most up-to-date saffron constituent under continuous study, as the metabolite of crocin.

3.2 | Effects of saffron on atherosclerosis and cardiovascular risk factors

3.2.1 | Atherosclerosis

It is a chronic disease of the arterial wall characterized by inflammatory infiltration, endothelial dysfunction, intimal lipid deposition, smooth muscle cell proliferation, cell apoptosis and necrosis, and local and systemic inflammation, involving key contributions to innate and adaptive immunity (Gistera & Hansson, 2017; Kadoglou et al., 2012; Park & Oh, 2019). Scarce experimental data support the anti-atherosclerotic effects of saffron and its derivatives and report the

TABLE 2 Animal studies investigating the effects of saffron and its constituents on cardiovascular risk factors

Risk Factor	Animal model—duration—saffron or individual compound	Intervention	Effect-mechanism of action	Reference
Type 2 diabetes and oxidative stress	Normal and diabetic rats—14 days Saffron ethanolic extract	Ethanolic extract of *Crocus sativus L. stigma* (doses: 20, 40, and 80 mg/kg). IP	IP saffron decreased FPG in rats, 33.9% (normal) and 41.4% (mild diabetic) Hypoglycaemic/anti-hyperglycaemic effect, regeneration of damaged pancreas in experimental diabetes.	Mohajeri et al. (2008)
	Diabetic (STZ) rats—6 weeks Saffron, Crocin, Safranal	Saffron methanolic extract (80 and 240 mg/kg); Crocin (15, 30, and 60 mg/kg); safranal (0.25 and 0.5 ml/kg); i.p. administration	Saffron, crocin and safranal significantly reduced the FPG and HbA1c, increased INL, had no effect on blood SGOT, SGPT, Cr levels. Saffron may have anti-hyperglycemic and blood insulin level elevating effects without hepatic and renal toxicities; crocin, crocetin and safranal may be involved in these effects of saffron	Kianbakht and Hajiaghaee (2011)
	Diabetic (STZ) rats—8 weeks Crocin, Safranal	Crocin (30 mg/kg, i.p.), safranal (1 mg/kg, i.p.) (alone or in combination with insulin) and insulin (5 IU/kg, s.c.)	Crocin, safranal and insulin improved STZ-induced behavioral, histopathological, and biochemical changes. Combination treatments crocin-safranal-insulin significantly decreased the STZ-increased levels of MDA.Neuroprotective effects of crocin, safranal and insulin, enhanced by combined treatment.	Farshid and Tamaddonfard (2015)
	STZ-induced diabetic rats—21 days Saffron aqueous (SFE) and alcoholic extracts	Two doses (25 and 100 mg/kg) of aqueous and alcoholic extracts of three medicinal plants, *Ziziphus jujuba*, *B. vulgaris* and *C. sativus*	Decreased serum FPG, TG and VLDL; increased serum adiponectin. Extracts regulated glucose and lipid metabolism by changes in adiponectin level	Hemmati et al. (2015)
	Wistar albino rats STZ-induced diabetic—4 weeks Safranal	Control, untreated diabetic & three diabetic groups safranal-treated (0.25, 0.50, 0.75 mg kg^{-1} day^{-1}).	Decreased BGL, MDA, NO, total lipids, TG, TC; increased glutathione and CAT, SOD activity. Safranal improved STZ-induced diabetes and its complications by modulation of oxidative stress.	Samarghandian et al. (2013)
	STZ-induced type 2 diabetic male Wistar rats—5 months Crocin	Control (i.p vehicle); Crocin (i.p crocin 50 or 100 mg kg)	Crocin significantly decreased FPG, advanced glycation end-products, TG, TC, and LDL and increased HDL in the diabetic rats	Shirali et al. (2013)

(Continues)

TABLE 2 (Continued)

Risk Factor	Animal model—duration—saffron or individual compound	Intervention	Effect-mechanism of action	Reference
	Male type 2 diabetic KK-Ay/Kwl (KK-Ay) mice—14 days Safranal	Groups: Control, 0.5% MC (vehicle); Safranal, 2.0 mg/ml safranal-0.5% MC (20 mg kg^{-1} day^{-1}-10 ml/kg body weight) once a day, oral gavage	Safranal, is a principal PTP1B inhibitor inducing a ligand-independent activation of insulin signaling in cultured myotubes. Safranal significantly enhanced glucose uptake through the translocation of glucose transporter 4. 2-wk oral administration improved impaired glucose tolerance in type 2 diabetic mice	Maeda et al. (2014)
	Female STZ-induced diabetic Wistar rats—21 days Crocin	Groups: Control (non-DM rats); DM (STZ-induced untreated diabetic rats); DM + crocin (STZ-induced diabetic rats treated with crocin, 20 mg kg^{-1} day^{-1})	Crocin decreased serum MDA TC, TG, VLDL; increased GSH. Histopathological changes markedly regressed. Crocin reduced oxidative stress and dyslipidemia and prevented diabetes-induced cardiovascular complications	Altinoz et al. (2015)
	Male, STZ-induced diabetic Sprague–Dawely rats—8 weeks Crocin	Groups: Normal control and diabetic nephropathy (DN) control received the vehicle once daily; diabetic crocin treated, 20 mg/kg, orally, daily.	Crocin significantly reduced BGL, increased INL, improved impaired kidney functions, by free radicals scavenging properties, host antioxidant defense system enhancement, inhibition of inflammatory and fibrotic cascades activation.	Abou-Hany et al. (2018)
	STZ-induced diabetic rats—6 weeks Crocin	Groups: Control (citrate buffer vehicle); diabetic animals, (STZ); diabetic animals (crocin 10 mg/kg); diabetic animals (crocin 10 mg/kg)	Crocin normalized BGL; inhibited cardiac hypertrophy and fibrosis; improved cardiac contractile function; enhanced heat shock response; increased myocardial AMPK phosphorylation; normalized autophagy marker proteins (LC3BII/LC3BI ratio, SQSTM1/p62, Beclin-1); decreased diabetes-induced myocardial apoptosis. Crocin enhanced the heat shock response, inhibited apoptosis and normalized autophagy in cardiac myocytes, improving deteriorated cardiac function in diabetic animals.	Feidantsis et al. (2018)

TABLE 2 (Continued)

Risk Factor	Animal model—duration—saffron or individual compound	Intervention	Effect-mechanism of action	Reference
	Wistar rats, normal and diabetic—8 weeks Crocin	(a,b) Control (normal and diabetic) and (c,d) Crocin (normal and diabetic) 40 mg kg^{-1} day^{-1}	Crocin: (a) increased SOD and CAT enzymes activities and strengthened the anti-oxidant defense system, (b) decreased nitrate content and MDA production, and (c) suppressed hepatic oxidative damage in diabetic animals	Yaribeygi et al. (2018)
	Sprague–Dawley rats—2 months Crocin	(a) Control (fresh air), (b) cigarette smoke exposure (CS) (nicotine 1 mg), (c) Crocin, 50 mg/kg, i.p, three times per week, once a day for 2 months, and (d) Crocin co-treatment CS	Crocin treatment decreased serum cotinine and inflammatory parameters and increased PO_2 and expression of PKC, PI3K, MAPK, Nrf2, and GCLc genes, antioxidant activity, and finally cardiac abnormalities in electrocardiogram and hemodynamic parameters, to normal levels. Crocin co-treatment protected lung injury caused by COPD and related cardiac dysfunction, by modulating of Nrf2 pathway.	Dianat et al. (2018)
Hyperlipidemia	Male Sprague–Dawley rats—10 days Crocin	Normal diet; control (HFD); Crocin (HFD + crocin 25, 50, and 100 mg kg^{-1} day^{-1})	Crocin significantly reduced serum TG, TC, LDL, and VLDL, and decreased fat and cholesterol absorption by pancreatic lipase inhibition.	Sheng et al. (2006)
	Albino Wistar rats of either sex—5 days Saffron, Crocin	After NFD or HFD groups received either saffron orally (25, 50, and 100 mg/kg), or crocin (4.84, 9.69, and 19.38 mg/kg), or vehicle	Both saffron and crocin decreased serum TG, TC, ALP, AST, ALT, MDA, GSHPx, GSH, GSSG and increased SOD, CAT, FRAP, SH in liver tissue; no reduction in TBARS. Saffron, was found superior to crocin; possible involvement of other saffron's constituents on quenching free radicals and ameliorating damages of hyperlipidemia	Asdaq & Inamdar (2010)
Hypertension	Wistar rats—single dose Saffron	Ten groups: (a) control, (b) AngII (50 ng/kg, i.v.), (c) losartan (10 mg/kg, i.p.) + AngII, (d) L-NAME (10 mg/kg, i.v.), (e) sodium nitroprusside (SNP) (50 mg/kg, i.p.) + L-NAME, (f,g) saffron stamen extract (SS) (100 and 200 mg/kg, i.p.) + AngII and 8, 9) SS	SS extract attenuated the pressor effect induced by AngII, and increased MAP and SBP induced by L-NAME. Higher effect on L-NAME than that of AngII BRS improvement Anti-hypertensive effects probably mediated by an	Mohebbati et al. (2020)

(Continues)

TABLE 2 (Continued)

Risk Factor	Animal model—duration—saffron or individual compound	Intervention	Effect-mechanism of action	Reference
		(100 and 200 mg/kg) + L-NAME, and (h) SS (200 mg/kg) + phenylephrine (Phen, i.v.).	inhibitory effect on AngII, increasing nitric oxide production, or improving baroreflex sensitivity	
	Wistar rats—single dose Saffron	Eleven groups: (a) control, (b) angiotensin II (AngII) (50 ng/kg, i.v.), (c) losartan (10 mg/kg, i.p.) + AngII, (d) L-NAME (10 mg/kg, i.v.), (e) sodium nitroprusside (SNP) (50 mg/kg, i.p.) + L-NAME, (f,g) saffron petal extract (SPE i.p. 100 and 200 mg/kg), (h,i) saffron petals (i.p. 100 and 200 mg/kg) + (AngII), and (j,k) saffron petals (i.p. 100 and 200 mg/kg) + L-NAME	Pre-treatment with saffron petals, at both doses studied, significantly attenuated the hypertensive effects of both AngII and L-NAME. Results indicate the anti-hypertensive effects of the saffron petals via renin-angiotensin as well as NO system	Mohebbati et al. (2021)
Cardiac hypertrophy	Wistar rats—30 days Crocetin	Groups: Sham-operation (0.5% CMC-Na); model (operation +0.5% CMC-Na); captopril (operation +50 mg/kg; crocetin I (100 mg/kg); crocetin II (50 mg/kg); ig administration.	Crocetin reduced cardiac indexes and content of hydroxyproline; increased Na+, K+ -ATPase, Ca2+, Mg2+ -ATPase activity; inhibited MMPs activity; prevented remodeling of overloading pressure induced cardiac hypertrophy	Shen and Qian (2004)
	Female Sprague–Dawley rats—15 days Crocetin	Norepinephrine (NE) induced cardiac hypertrophy, five groups: (a) control (normal saline), (b) NE, (c) NE + crocelin (50 mg kg^{-1} day^{-1}), (d) NE-I-crocetin (25 mg kg^{-1} day^{-1}), and (e) NE + captopril (60 mg kg^{-1} day^{-1}). i.g administration.	Crocetin blocked the development of NE induced left ventricular; decreased myocardial collagen; enhanced cardiac Na^{+}–K^{+} ATPase and mitochondrial Ca^{2+}–Mg^{2+} ATPase activity; inhibited MMP-2 activity and MMP-2, MMP-9 expressions; may prevent NE induced cardiac hypertrophy in rats	Shen and Qian (2004)

Abbreviations: Akt, protein kinase A,B; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AMPK, adenosine monophosphate-activated protein kinase; AngII, angiotensin II; $ApoE^{-/-}$, *Apolipoprotein E*-deficient; AST, aspartate transaminase; Beclin 1, Bcl-2-homology (BH)-3 domain only protein; BGL, blood glucose levels; CAT, catalase; COPD, chronic obstructive pulmonary disease; CrCL, creatinine clearance; DM, diabetes mellitus; FPG, fasting plasma glucose; GSH: total glutathione; GSHPx, glutathione peroxidase enzyme activity; GSSG: oxidized glutathione; HDL: high-density lipoprotein; HFD: high-fat diet; HR, heart rate; i.p., intra-peritoneal; INL, insulin levels; LC3, microtubule-associated protein light chain 3; LDL, low-density lipoprotein; L-NAME, NG-nitro-L-arginine methyl ester; MAP, mean arterial pressure; MAPKs, mitogen-activated protein kinases; MDA, malondialdehyde; MMPs, matrix metalloproteinases; ND, normal diet; Nrf2, nuclear factor E2-related factor 2; PKC, PI3K and MAPK, upstream Nrf2 regulator genes; PTP1B, protein tyrosine phosphatase 1B; SBP, systolic blood pressure; SFE, saffron extract; SOD, superoxide dismutase; SQSTM1/p62, sequestosome 1 or p62 protein; STZ, streptozotocin; TC, total cholesterol; TG, triglycerides; VLDL, very low-density lipoprotein; WT, wild type.

involvement of a wide spectrum of underlying mechanisms (Table 1). Among them, the antiinflammatory properties of saffron and its main constituents, crocin, crocetin, and safranal, emerge as a prominent therapeutic mechanism.

In particular, crocin effectively suppresses the expression of pro-inflammatory cytokines, while it stimulates antiinflammatory cytokines, associated with alleviated coronary atherosclerosis (Li et al., 2018). Besides this, adhesion and migration of the leukocyte to

TABLE 3 Clinical data from randomized clinical trials for the application of saffron and its constituents in coronary artery disease and cardiovascular risk factors

Disease/risk factor	Cohort—saffron or individual compound	Protocol design—intervention	Results	Reference
Coronary artery disease	84 CAD patients Crocin	RCTs double-blind; 8 weeks treatment: Crocin (30 mg/day) or SAE (30 mg/day) versus placebo.	SAE decreased BMI, waist circumference and fat mass more than crocin. Non-significant differences in lipid profile.	Abedimanesh et al. (2017)
	84 CAD patients Crocin	RCTs double-blind; 8 weeks treatment: Crocin (30 mg/day) or SAE (30 mg/day) versus placebo.	Crocin increased the gene expression of Sirtuin 1 and AMPK and decreased the expression of LOX1 and NF-κB.	Abedimanesh et al. (2020)
Diabetes mellitus	80 T2D patients Saffron	RCT: 12 weeks treatment: *C. sativus* powder (100 mg/day) versus placebo.	Slight but significant reduction of SBP. No effect on DBP.	Ebrahimi et al. (2019)
	81 T2D patients Saffron	RCT; 8 weeks treatment: Saffron (1 g/day) versus placebo.	No effect on BP at all.	Azimi et al. (2016)
	70 overweight/obese patients With T2D 8 weeks	RCT, double-blind; saffron 100 mg/day versus placebo	Saffron supplementation significantly ameliorated: (a) FPG, TG, insulin, AST and ALT, and (b) sleep and total quality of life.	Tajaddini et al. (2021)
Metabolic syndrome	44 MS patients Crocin	RCTs double-blind; 8 weeks treatment; crocin (30 mg/day) versus placebo.	Increase in serum cholesteryl ester transfer protein. Non-significant differences in lipid and glycemic profiles between groups.	Javandoost et al. (2017)
	48 MS patients Crocin	RCTs double-blind; 6 weeks treatment: Crocin (100 mg/day) versus placebo.	No effect on MS parameters.	Kermani et al. (2017)
	76 MS patients Saffron	RCTs double-blind; 12 weeks treatment; saffron dried stigma (100 mg/day) versus placebo.	A marginally significant increase in serum leptin and a significant decrease in LDL levels in saffron than placebo group.	Zilaee et al. (2018)
Hypertension	30 healthy adults 7 days Saffron	RCT double-blind, placebo-controlled; three groups of 10 each (five males, five females) 200, 400 mg saffron (stigmas) tablets	Saffron (400 mg): (a) decreased standing systolic blood pressure, mean arterial pressure, red blood cells, hemoglobin, hematocrit, platelets and (b) increased sodium, blood urea nitrogen, creatinine	Modaghegh, Shahabian, Esmaeili, Rajbai, and Hosseinzadeh (2008)
	Hypertensive men Aged 60–70 years 12 weeks Saffron	Control group (CO); three experimental groups; resistance training (RT); saffron (S) (one tablet 200 mg); and resistance training + saffron (RTS) (one tablet 200 mg)	Compared to CO group: (a) all three groups significantly reduced BP, but the most pronounced reduction was in RTS, (b) S increased adiponectin, (c) RTS increased nitric oxide, and (d) no difference between groups for atrial natriuretic peptide.	Hooshmand-Moghadam, Eskandari, Fatemeh Shabkhiz, Mojtahedi, and Mahmoudi (2021)

Abbreviations: ALT, alanine aminotransferase; AMPK, 5'-adenosine monophosphate-activated protein kinase; AST, aspartate aminotransferase; BMI, body mass index; BP, blood pressure; BW, body weight; CAD, coronary artery disease; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HOMA-IR, homeostatic model assessment for insulin resistance; LOX1, lectin-like oxidized LDL receptor 1; MS, metabolic syndrome; QUICKI, quantitative insulin sensitivity check index; SAE, saffron aqueous extract; SBP, systolic blood pressure; SD, sexual dysfunction; T2DM, type 2 diabetes mellitus; TC, total cholesterol; TG, triglycerides; WC, waist circumstance.

TABLE 4 Clinical data from systematic reviews & meta-analyses of randomized clinical trials for the application of saffron and its constituents in cardiovascular risk factors

Risk factor	Cohort—saffron or individual compound	Protocol design—intervention	Results	Reference
T2DM	Nine RCTs; CAD, T2DM, MS—Saffron	6–12 weeks treatment: 301 patients in saffron groups (doses: 5, 15, 30, 100, and 1,000 mg/day) versus 294 patients in placebo groups	Saffron supplementation reduced WC and FPG, but not HbA1c levels.	Rahmani, Bazmi, Clark, and Hashemi Nazari (2020)
	14 RCTS; T2DM, MS, prediabetes, CAD—Saffron, Crocin	6–12 weeks treatment of 859 patients with: Saffron (doses: 15, 30, 100, and 1,000 mg/day) or crocin (doses: 5, 15 30, and 100 mg/day) versus placebo / black tea	Three out of 10 studies revealed significant reduction in FPG after saffron administration. No effect of saffron or crocin on HbA1c.	Giannoulaki, Kotzakioulafi, Chourdakis, Hatzitolios, and Didangelos (2020)
Hyperlipidemia	10 RCTs, double-blind studies; CAD, T2DM, hypertension - Saffron, Crocin	8–12 weeks treatment: 205 patients in saffron groups (doses: 30, 100, and 1,000 mg/day), 136 patients in crocin groups (doses: 5, 15, and 30 mg/day) versus 244 patients in placebo groups.	Saffron supplementation ameliorated all lipid parameters (TG, TC, LDL-c HDL-c levels), however only LDL changes achieved statistical significance. Crocin supplementation did not affect lipid profile.	Roshanravan et al. (2020)
	6 RCTs; CAD, T2DM, MS, SD, schizophrenia - Saffron	4–12 weeks treatment: 127 patients in saffron groups (doses: 30, 100, and 1,000 mg/day) versus 126 patients in placebo groups.	Significant amelioration in TC, TG and HDL serum levels. No effect on serum LDL levels.	Asbaghi et al. (2019)
Multiple CV risk factors	11 RCTs; CAD, T2DM, MS, schizophrenia, depression, overweight, diabetic macular edema - Saffron, Crocin	28–90 days treatment of 622 patients with: Saffron (doses: 30, 353, and 1,000 mg/day) or crocin (5, 15, 20, 30, and 100 mg/day) versus placebo/black tea without herbals.	Significant reduction in DBP, BW, WC after saffron treatment. Significant reduction in FPG levels in a subgroup with high-quality studies of saffron. No significant change in lipid profile, fasting insulin, SBP and BMI.	Pourmasoumi, Hadi, Najafgholizadeh, Kafeshani, and Sahebkar (2019)
	15 RCTs Metabolic syndrome, psychosocial disorders, T2DM, prediabetes, healthy - Saffron, Crocin	4 to 12 weeks treatment: 1,139 patients, in saffron groups (15 to 1,000 mg/day) or crocin groups (5 to 30 mg/day), with (eight studies) or without (seven studies) hypoglycemic medications	Saffron supplementation: (a) decreased FPG, fasting insulin, HbA1c; increased QUICKI and (b) no effect on HOMA-IR. Considered as valuable adjuvant therapy regarding glycemic control of patients	Sohaei, Hadi, Karimi, and Arab (2020)

Abbreviations: ALT, alanine aminotransferase; AMPK, 5′-adenosine monophosphate-activated protein kinase; AST, aspartate aminotransferase; BMI, body mass index; BP, blood pressure; BW, body weight; CAD, coronary artery disease; DBP, diastolic blood pressure; FPG, fasting plasma glucose; HOMA-IR, homeostatic model assessment for insulin resistance; LOX1, lectin-like oxidized LDL receptor 1; MS, metabolic syndrome; QUICKI, quantitative insulin sensitivity check index; SAE, saffron aqueous extract; SBP, systolic blood pressure; SD, sexual dysfunction; T2DM, type 2 diabetes mellitus; TC, total cholesterol; TG, triglycerides; WC, waist circumstance.

endothelial cells is one of the early key steps in atherogenesis. Advance glycation-end products (AGEs) may promote this migration possibly by the expression of the intercellular adhesion molecule-1 (ICAM-1) protein. Crocetin (the major metabolite of crocin) was found to inhibit the AGE-induced growth of bovine endothelial cells (BECs) and significantly reduce the adhesion rate of leukocyte to BEC, in parallel to the downregulation of ICAM-1 expression (Alavizadeh & Hosseinzadeh, 2014; Xu et al., 2007). Crocin also decreases cholesteryl ester deposition in macrophages and the uptake of oxidized low-density lipoprotein (Ox-LDL). Thereby, crocin may slow down the formation of foam cells, which constitute an essential element of atherosclerotic plaque formation (He et al., 2005; Singla & Bhat, 2011). Another proposed atheroprotective mechanism of crocin and crocetin may be illustrated by their ability to increase the plasma

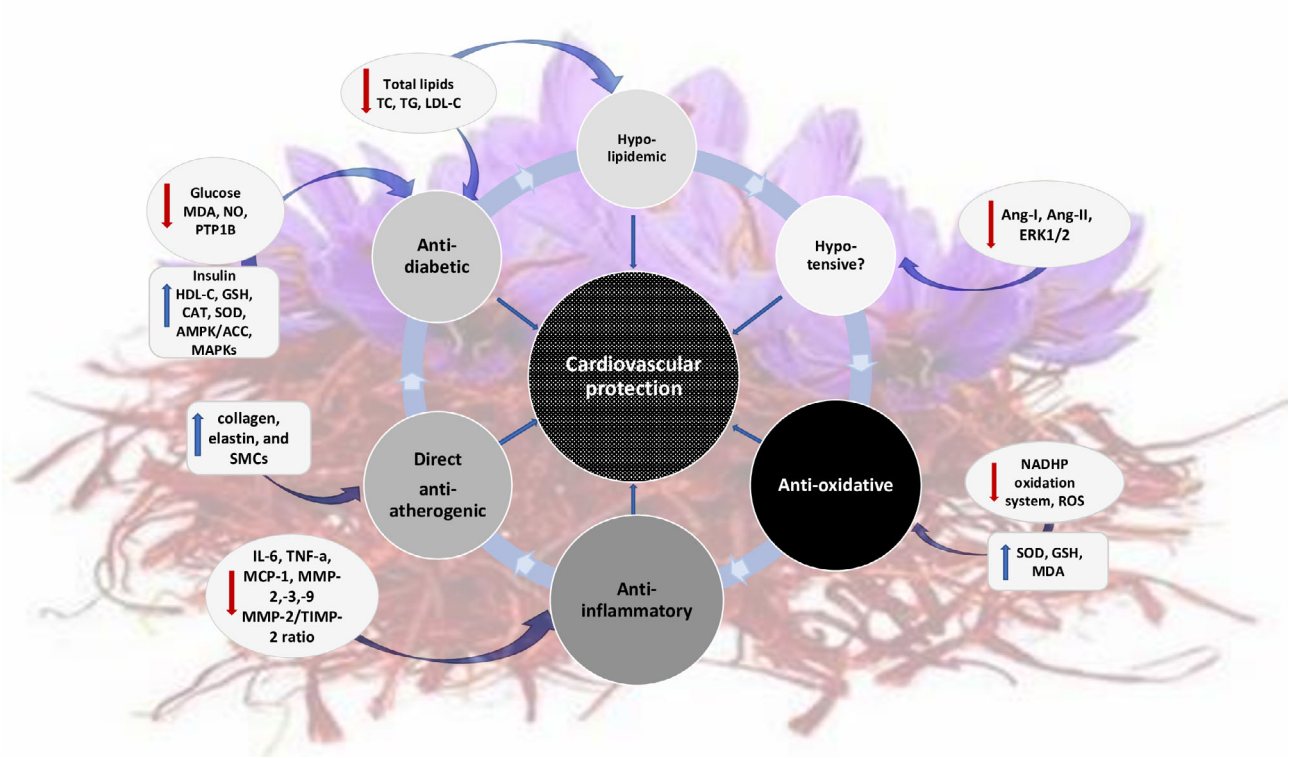

FIGURE 1 Schematic representation of the most important cardiovascular protective properties of saffron and its main active constituents. The ascending strength of evidence for each factor is graded by the color (from white to black) and the size of circles. Abbreviations: AMPK, AMPactivated protein kinase; AMP, adenosine monophosphate; ACC, acetyl-CoA carboxylase; Ang, angiotensin; ERK1/2, extracellular signal-regulated kinase ½; GSH, glutathione; HDL, high density lipoproteins; IL, interleukin; LDL, low density lipoproteins; MAPKs, mitogen-activated protein kinases; MCP-1, monocyte chemoattractant protein-1; MDA, malondialdehyde; MMP, matrix metalloproteinase; NADPH: nicotinamide adenine dinucleotide phosphate; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; PTP1B, tyrosine phosphatase 1B; ROS, reactive oxygen species; SOD, superoxide dismutase; TC, total cholesterol; TG, triglycerides; TNFa: Tumor necrosis factor-a [Colour figure can be viewed at wileyonlinelibrary.com]

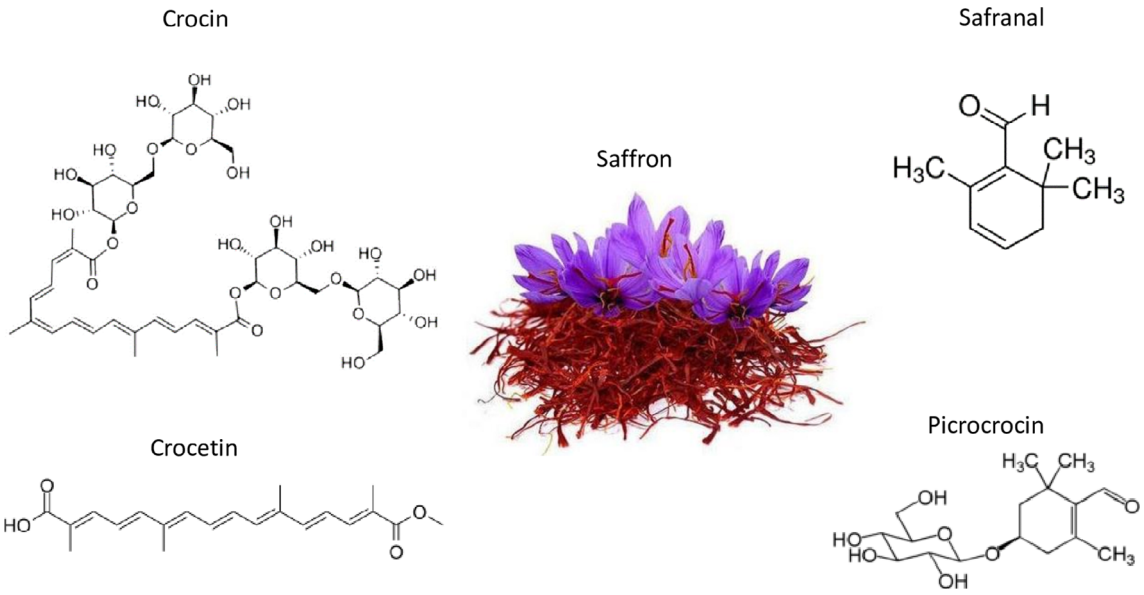

FIGURE 2 Saffron and its main active components [Colour figure can be viewed at wileyonlinelibrary.com]

oxygen diffusivity (Gainer, Rudolph, & Caraway, 1993), which could attenuate the artery damage and cholesterol insertion, which is of clinical relevance (Kamalipour & Akhondzadeh, 2011).

Considering saffron extract (SFE), in a recent in vivo study (Christodoulou et al., 2018), the administration of aqueous SFE in atherosclerosis-prone Apo $E^{-/-}$ transgenic mice significantly reduced

the mean area of the aortic atherosclerotic plaques and beneficially changed their composition. By measuring collagen and elastin concentrations within atherosclerotic plaques, saffron (and especially crocin) treatment enhanced plaque stability and attenuated the risk for plaque rupture. It is important to note that this is the only, to our knowledge, experimental study where the selection of the administered doses of SFE was based on the results of a pilot pharmacokinetic study and the measured plasma crocetin levels. Gathering the above experimental data, there is emerging evidence that saffron and its constituents may attenuate atherosclerosis progression and destabilization. Treatment with SFE before the ischemic period could have a protective effect as a membrane-stabilizing agent, whereas, during reperfusion, saffron maintained or bolstered the endogenous antioxidant defense system at near-normal levels, decreasing reactive oxygen species (ROS) accumulation and Ca^{2+} influx (Nader, Chahine, Salem, & Chahine, 2016). The impact of aqueous extracts of saffron, on myocardial "electrical stability" was examined in a rat model of myocardial ischemia/reperfusion. Pretreatment with saffron, especially at the dosage of 100 mg kg^{-1} day^{-1}, attenuated the susceptibility and incidence of fatal ventricular arrhythmia during the reperfusion period in that animal model (Joukar et al., 2013). This was at least partially be related to stability or even amplification of antioxidant systems (such as catalase (CAT), superoxide dismutase—SOD, glutathione—GSH, and malondialdehyde—MDA, as a marker of lipid peroxidation levels; Jahanbakhsh et al., 2012). Furthermore, in a recent study in mice (Efentakis et al., 2017), saffron (*C. sativus* L.) aqueous extract (SAE) was found to limit myocardial infarction not only in healthy animals but, more importantly, in animals with atherosclerosis.

Safranal has also shown interesting pharmacological properties concerning cardiac ischemia and reperfusion injury in animal models. Like SFE, safranal pre-treatment has significantly decreased the myocardial infarction area (Mehdizadeh et al., 2013). In addition to infarct protection, safranal has the potential to increase phosphorylation of protein kinase A,B (Akt)/glycogen synthase kinase-3b (GSK-3b)/endothelial nitric oxide synthetase (eNOS) and decrease inhibitor of nuclear factor kappa-B kinase subunit beta (IKK-β)/nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) protein expressions (Bharti et al., 2011; Rezaee & Hosseinzadeh, 2013). Those cardioprotective mechanisms may counter-regulate the ischemia–reperfusion injury.

All the aforementioned results implicate the antioxidant potential of saffron and its constituents as a promising nutraceutical cardioprotective agent in patients with endothelial dysfunction-related diseases, such as atherosclerosis, diabetes, and so forth.

3.2.2 | Oxidative stress

Most studies investigating the possible therapeutic properties of saffron and its main constituents are mainly discussed in conjunction with its strong antioxidant activity, mostly attributed to the presence of unique carotenoids (Kanakis et al., 2009). The antioxidant activity of saffron carotenoids is more potent in crocin than in safranal. However, saffron spice seems to exert the synergistic antioxidant effect of all bioactive constituents. In particular, these constituents may prevent the chemical transformation of DNA and tRNA through the formation of ligand–polynucleotide complexes (Kanakis et al., 2009; Kumar et al., 2011). Saffron's components can bind to proteins, nucleic acids (DNA, tRNA), and lipids (linoleic acid) protecting from free radicals such as ROS (Kanakis, Tarantilis, Tajmir-Riahi, & Polissiou, 2007). The methanolic solution of Saffron's crocin was found to exhibit high radical scavenging activity (Assimopoulou, Sinakos, & Papageorgiou, 2005), through a GSH-dependent mechanism protecting against stress-induced cell death (Soeda et al., 2007). Similarly, pretreatment of PC12 rat pheochromocytoma cells in glucose-induced neurotoxicity reduced ROS production (Mousavi, Tayarani, & Parsaee, 2010), confirming previous results in human monocytes (Ordoudi, Befani, Nenadis, Koliakos, & Tsimidou, 2009). By considering the multifactorial regulatory role of oxidative stress in clinical pathological conditions, the potential therapeutic applications of saffron and its active constituents may be expanded. For example, chronic treatment with crocin derived from *C. sativus* L. in the rat model induced significant alterations in the function and structure of the pulmonary and cardiac system by increasing the antioxidant enzymes (Dianat et al., 2018). Treatment of diabetic rats with crocin improved CAT and SOD enzyme activities and antioxidant defense system capacity, though no differentiations on glutathione (GSH) content were observed (Yaribeygi, Taghi Mohammadic, & Sahebkard, 2018). Un-controlled hyperglycemia induces oxidative stress in hepatic tissue enhancing free-radicals production. Treatment with crocin restored the physiological condition and prevented oxidative damages.

Although a large number of antioxidant substances have been tested for their therapeutic potential in a wide spectrum of diseases, all of them, unfortunately, have failed to show clinical efficacy, despite the promising results in the initial in vitro and in vivo in animals, experimental studies. In parallel, the multidisciplinary regulation of oxidative stress raises many obstacles in oxidative stress monitoring. An alternative scientific research supports the notion of stimulating the endogenous antioxidant pathways by dietary substances (Alfa & Arroo, 2019). However, more data are required. Accordingly, additional investigation is required to elucidate the antioxidant activity of saffron and its constituents in the context of the complex regulation of oxidative stress via exogenous administration or endogenous antioxidants triggering.

3.2.3 | Diabetes mellitus

Diabetes is a multi-metabolic disorder characterized by hyperglycemia and an atherogenesis predisposition by clustering several atherogenetic mechanisms such as: deposition of glycosylated end-products, hyperlipidemia, oxidative stress (imbalance between ROS and antioxidant defenses), insulin resistance, low-grade inflammation, and so forth (Kadoglou et al., 2013; Lambadiari, Dimitriadis, & Kadoglou, 2018).

Moreover, hyperglycemia has been predominantly related to microvascular and to a lesser extent to macrovascular disease and cardiomyopathy (Valensi, Prévost, Schnell, Standl, & Ceriello, 2019). There are accumulating indications that crocin is effective in blood-glucose-lowering through two major mechanisms: (a) the stimulation of Langerhans islets to secrete insulin or (b) the insulin-sensitizing of peripheral muscles (Arasteh et al., 2010; Razavi & Hosseinzadeh, 2017).

Regarding the former mechanism, it has been suggested that the strong antioxidant properties of crocin by scavenging ROS (Kianbakht & Hajiaghaee, 2011) may help β-pancreatic cells to increase insulin secretion and reduce elevated blood glucose levels (Assimopoulou et al., 2005; Shirali, Bathaie, & Nakhjavani, 2013). In addition to this, safranal has been demonstrated to reduce TNF-α and interleukin 1 beta (IL-1β) levels and oxidative stress in both plasma and pancreas tissue in diabetic animals (Farshid & Tamaddonfard, 2015; Hazman & Ovali, 2015). Those effects may explain the hypoglycemic results of ethanolic extract (40 mg/kg) of saffron by reversing the damage in the pancreas (Mohajeri, Tabrizi, Mousavi, & Abbasi, 2008). In addition, the number of immunoreactive beta-cells was significantly increased in saffron-treated diabetic rats (Kianbakht & Hajiaghaee, 2011). Daily oral administration of the saffron methanolic extract (doses: 80 and 240 mg kg^{-1} day^{-1}), crocin alone (doses: 50 and 150 mg kg^{-1} day^{-1}), or safranal (doses: 0.25 and 0.5 ml/kg) for 6 weeks have been also reported to significantly improve the glycemic profile and insulin secretion as compared to untreated diabetic rats.

Regarding the second mechanism, insulin-sensitizing effects of saffron, recently, Kang et al. (2012) investigated the hypoglycemic actions of saffron in C2C12 mouse skeletal muscle cells. Saffron strongly enhanced glucose uptake and the phosphorylation of AMP-activated protein kinase (AMPK)/acetyl-CoA carboxylase (ACC) and mitogen-activated protein kinases (MAPKs), but not phosphatidylinositol 3-kinase (PI 3-kinase)/Akt. Interestingly, the co-treatment of the C2C12 skeletal muscle cells line with saffron and insulin further improved their insulin sensitivity via both insulin-independent (AMPK/ACC and MAPKs) and insulin-dependent (PI 3-kinase/Akt and mammalian target of rapamycin) pathways. The authors proposed the mediating role of AMPK in saffron-induced glucose uptaking and insulin sensitization of skeletal muscle cells. In parallel, saffron alcoholic extract and SAE may regulate glucose and lipid metabolism by changes in adiponectin levels (Hemmati, Asghari, & Zohoori, 2015).

The antioxidant effects of crocin may also explain a significant decrease of serum glucose and AGEs in streptozotocin (STZ)-induced diabetic rats (Asri-Rezaei, Tamaddonfard, Ghasemsoltani-Momtaz, Erfanparast, & Gholamalipour, 2015; Shirali et al., 2013). In addition, crocetin, the active hydrolyzed form of crocin, may also favorably modulate adipokines expression (adiponectin, tumor necrosis factor-alpha [TNF-α] and leptin) in white adipose tissue of rats leading to improved insulin sensitivity (Xi et al., 2007).

Regarding the activity of the aroma constituent of saffron safranal, it is established that increased activity and expression of protein tyrosine phosphatase 1B (PTP1B) is implicated in the pathogenesis of insulin resistance. According to a study by Maeda, Kai, Ishii, Ishii, and Akagava, (2014), safranal managed to inhibit significantly the PTP1B activity and induced a ligand-independent activation of insulin signaling in C2C12 myotubes. Moreover, safranal may lead to an increased glucose uptake via the translocation of glucose transporter (Maeda et al., 2014).

Beyond glucose regulation, it is postulated that saffron and its active components may alleviate micro/macro-vascular diabetic complications according to in vivo and in vitro studies (Altinoz, Taskin, Oner, Elbe, & Arslan, 2015; Mousavi et al., 2010). Previous researchers (Abou-Hany, Atef, Saida, Elkashefa, & Salema, 2018; Altinoz et al., 2015) reported the beneficial effect of crocin in reducing diabetes-induced nephropathy in animal models. A wide spectrum of kidney histopathological changes such as congestion, severe inflammation, tubular desquamation, tubular necrosis, fewer fibrotic lesions, and hydropic degeneration in tubular cells were markedly reduced in crocin-treated rats. To summarize, Abou-Hany et al. (2018) proposed that the ameliorative impact of crocin on diabetic nephropathy may be attributed to its free radicals scavenging properties, its ability to enhance the host antioxidant defense system and to inhibit inflammatory, and fibrotic cascade activation.

Regarding diabetic cardiomyopathy, there are limited data from a recent animal study again (Feidantsis, Mellidis, Gallatou, Sinakos, & Lazou, 2018). Six-week treatment of STZ-diabetic rats and isolated cardiac myocytes with crocin led to normalization of plasma glucose levels, inhibition of cardiac hypertrophy and fibrosis, and improvement of cardiac contractile function. Generally, the results suggested that crocin has the potency to improve cardiac function in diabetic animals through the enhancement of heat shock response, inhibition of apoptosis, and normalization of the phenomenon of autophagy in cardiac myocytes.

All aforementioned studies may suggest saffron as a preventive or therapeutic agent against diabetes mellitus and its complications through a plethora of mechanisms.

3.2.4 | Hyperlipidemia

It represents one of the main aspects of MS, and it is among the most important risk factor for CVDs (Galassi, 2006), which has been widely studied as a therapeutic target.

Sheng's study (Sheng, Qian, Zheng, & Xi, 2006) first investigated the possible hypolipidemic mechanism of crocin, in rats receiving a high-fat diet. It was shown that crocin inhibited the malabsorption of fat and cholesterol and that effect was mediated by pancreatic lipase inhibition and fat hydrolysis. In another animal study of hyperlipidemic rats (Asdaq & Inamdar, 2010), both saffron and crocin were effective in decreasing the elevated levels of triglycerides, total cholesterol, alkaline phosphatase, aspartate transaminase, and other enzymes connected to oxidative stress along with the increase of SOD, CAT, and so forth, with saffron being superior to crocin, indicating the possible involvement of other saffron's active constituents apart from crocin. Indirectly, the SAE may improve lipid profile by decreasing appetite and improving body composition in patients with already known CAD

(Abedimanesh et al., 2017). Besides this, the other constituent, safranal, has shown hypolipidemic effects through the potentially protective activities on antioxidant enzymes (SOD, glutathione S-transferase, CAT), lipid peroxidation levels, and serum NO content in a rat model combining diabetes and hyperlipidemia (Samarghandian, Borji, Delkhosh, & Samini, 2013).

The results from all the above-mentioned studies indicate that the hypolipidemic effects of saffron seem modest, while the underlying mechanisms are still obscure.

3.2.5 | Hypertension and other cardioprotective properties

Previous research works have documented a vasorelaxant effect of saffron and its compounds, especially crocetin (Mancini et al., 2014). Those effects in combination with angiotensin II inhibition may mediate the hypotensive action of saffron stigma (Mohebbati, Kamkar-Del, & Shafei, 2020; Mohebbati, Kamkar-Del, Shafei, Rakhshandeh, & Aghaei, 2021). In the context of hypertension, cardiac hypertrophy is a common consequence contributing to cardiac remodeling. Animal in vivo studies have reported the amelioration of cardiac hypertrophy after crocetin administration in pressure overloaded rats (Shen, Lu, & Qian, 2006; Shen & Qian, 2004). Favorable alterations in matrix metalloproteinases, Na-K-ATPase activity, and myocardial collagen content have been proposed as the underlying mechanisms of those beneficial effects. The cardioprotective impact of saffron is supported as well by several experimental studies documenting less cardiac dysfunction in animals receiving SFEs and cardiotoxic agents, such as doxorubicin (Chahine et al., 2013) and diazinon (Razavi, Hosseinzadeh, Movassaghi, Imenshahidi, & Abnous, 2013). Moreover, saffron may promote cardiac electrical stability exerting anti-arrhythmic effects (Joukar et al., 2013; Joukar & Dehesh, 2015).

3.3 | Potential clinical applications of saffron in CAD and cardiovascular risk factors

3.3.1 | Coronary artery disease

Nowadays, there is increasing interest in the application of herbs in therapeutic strategies of chronic diseases. Although none of the herbs can substitute pharmaceutical regimens, they have been considered as a part of a holistic therapeutic approach. Clinical data about the potential application of saffron and its derivatives in atherosclerotic CVDs are still limited (Tables 3 and 4). Well-controlled clinical trials are required to validate the aforementioned anti-atherogenic in vitro and experimental animal data to human beings. The first clinical trial investigating an effect of SAE and crocin was recently carried out by Abedimanesh et al. (2020) in patients with CAD. The results of this placebo-controlled clinical trial revealed that crocin therapy increased the gene expression of Sirtuin 1 (SIRT1) and AMPK and decreased Lectin-like Ox-LDL receptor 1 (LOX1) and NF-κB in peripheral blood mononuclear cells along with beneficial effects on serum Ox-LDL and monocyte chemoattractant protein-1 (MCP-1) levels and anthropometric indices. However, as the authors stated, more studies are needed to reveal the exact mechanisms and their impact on CAD progression or peripheral arterial disease in humans, despite the related reports in atherosclerotic animals.

3.3.2 | Diabetes mellitus

It is well known that diabetes is associated 2–4-fold higher risk of CAD or stroke (Kadoglou et al., 2011). Current, anti-diabetic therapeutic approaches have targeted both optimal glycemic control and reduced cardiovascular risk (Siasos et al., 2020). Contrary to a plethora of animal studies, robust evidence supporting the hypoglycemic effects of saffron is lacking in humans, since this is documented only in a few randomized clinical studies (Milajerdi et al., 2018; Aleali et al., 2019; Tajaddini et al., 2021). Recently, a meta-analysis enrolled nine randomized trials assessing the hypoglycemic effects of saffron or crocin (Rahmani et al., 2020). The authors observed a significant reduction in fasting plasma glucose (FPG) after >12 weeks of therapy, but a non-significant decrease in HbA1c. Among limitations, the authors admitted the small sample sizes, the different types of participants' diseases, the usage of either saffron or crocin, and the improper design of trials. A concomitantly published meta-analysis by Giannoulaki et al. (2020) concluded that it was implausible to get a firm conclusion about the glucose-lowering effects of saffron, since in only three out of 10 studies, a significant reduction in FPG was observed, while a positive effect on HbA1c was not detected (Giannoulaki et al., 2020). The existence of low quality of studies was a major reason for results inconsistency. Pourmasoumi et al. (2019) had observed in their meta-analysis a significant alteration in FPG only within high-quality studies (−10.14 mg/dl; 95% CI: −13.80 to −6.48). In the case of overweight patients with pre-diabetes, saffron administration may improve the glycemic profile (Karimi-Nazari et al., 2019). In the future, well-designed and properly conducted studies in diabetic patients are warranted.

3.3.3 | Hyperlipidemia

In the absence of clinical data about the direct anti-atherogenic effects of saffron or its constituents, we searched for their modulating effect on individual atherogenic factors. Among others, the potential hypolipidemic effects of saffron may constitute a candidate atheroprotective mechanism. The most recent meta-analysis of 11 randomized, double-blind, placebo-controlled clinical trials reported the favorable effects of saffron treatment, on lipid parameters (Roshanravan et al., 2020). Although saffron seemed to ameliorate all lipid parameters, only LDL changes achieved statistical significance in the pooled analysis. In the same meta-analysis, crocin administration did not change all lipid profiles. Several aspects of that meta-analysis should be considered before results interpretation. First of all, the included studies were inhomogeneous examining a variety of doses

and duration of treatment. Secondly, the lipid profile improvement was inconsistent along with studies and longer therapy than 12 weeks was required to achieve significant effects. Thirdly, the authors attempted a comparison between groups of subjects with various diseases (e.g., diabetes, CAD, schizophrenia, MS, asthma), each at a different stage. Finally, most participants were not hyperlipidemic, which might have compromised the lipid-lowering effects of saffron. In contrast to those findings, another meta-analysis in a smaller group of randomized clinical studies ($N = 6$), showed the opposite results: significant amelioration of total cholesterol, triglycerides and high-density lipoprotein, but non-significant change in LDL levels after saffron supplementation in a still heterogeneous group of studies (Asbaghi et al., 2019).

Although the latter meta-analysis had the vast majority of studies in common with the previous one (five out of six studies), it focused on less randomized studies, exclusively using saffron, which may explain their discrepancy. Therefore, based on small clinical trials of a wide spectrum of diseases the impact of saffron on lipid profile remains controversial. High-quality studies including predominantly hyperlipidemic subjects without hypolipidemic therapy or with controlled hypolipidemic treatment will shed more light.

3.3.4 | Hypertension

Similar to previously described CVDs, few clinical studies are investigating whether the observed hypotensive properties of saffron and its derivatives on animals (Imenshaidi, Hosseinzadeh, & Javadpour, 2010), apply to humans. Apart from two recent studies (Ebrahimi et al., 2019; Hooshmand-Moghadam et al., 2021), reporting a significant reduction in either systolic blood pressure or in blood pressure in elderly hypertensive patients, the rest of them (Azimi et al., 2016; Kermani et al., 2017; Pourmasoumi et al., 2019) presented the neutral impact of saffron and its constituents on blood pressure levels in humans.

Up to now, the overall hypotensive action of saffron supplementation is not confirmed in clinical practice. However, those findings should be considered with caution due to the remarkable defects in methodology, such as the inclusion of normotensive subjects, the unknown anti-hypertensive regimen in the case of hypertensive patients and the small sample sizes.

3.3.5 | Metabolic syndrome

MS does not constitute a distinguished nosology, but a cluster of metabolic disorders predisposing to the high prevalence of CVDs (Nikolopoulou & Kadoglou, 2012). This term is mainly used to identify patients requiring multifaceted therapy to prevent cardiovascular complications. To our knowledge, four published RCTs are evaluating the impact of crocin (three studies, dose: 30 mg/day or 100 mg/day, duration: 6–8 weeks) (Javandoost et al., 2017; Kermani et al., 2017; Nikbakht-Jam et al., 2016) and saffron (one study; dose: 100 mg/day dried stigma; duration 12 weeks; Zilaee et al., 2018) on the parameters of MS. Except for one study which demonstrated only a significant reduction in LDL levels in saffron-treated patients, the rest of them failed to show any significant change in anthropometrical parameters, glycemic, and lipid profile.

Summarizing the aforementioned studies, saffron or its constituent, crocin, seem at first sight to have no effect on middle-aged cohorts with modestly disturbed MS parameters. Nevertheless, several factors might have confounded the clinical results regarding the dose, the small samples (up to 30 participants in the therapeutic arm), and the short duration of the study. Notably, Zilaee et al. (2018) formulated capsules of 100 mg of dried saffron stigma, which is far away from quantifying bioactive molecules. All those limitations are mentioned in the following section.

3.4 | Limitations of clinical studies

The aforementioned inconsistent randomized trials have raised several concerns, when the vast majority of animal studies strongly indicate beneficial metabolic (e.g., glucose-lowering, hypolipidemic, etc.) and direct atheroprotective effects of saffron and its constituents. The first among limitations is that the included clinical studies are characterized by high heterogeneity in the dose and the form of the used supplement, due to lack of pharmacokinetic and bioavailability data. There are limited studies reporting the chemical characterization, composition, and standardization of the SFE administered (e.g., Almodovar, Briskey, Rao, Prodanov, & Inarejos-Garcıa, 2020; Christodoulou et al., 2019), as well as information on the stability of the prepared extract (e.g., Christodoulou et al., 2019). The pharmacokinetic/pharmacodynamic parameters are the most crucial regarding the nutraceutical supplements efficacy. The stability of saffron and its constituents as well as their absorption from the gastro-intestinal tract have not been objectively and extensively studied in previous human cohorts. Probably, the underlying constraints in bioavailability may question the calculated doses and how they apply to either normal subjects or patients with underlying pathology, likely metabolic disorders. The completely different behavior of antioxidant substances between in vitro and in vivo studies has been demonstrated. For those reasons, the antioxidant molecules have failed, to our knowledge, to prove adequate clinical effectiveness, despite the quite promising experimental results (Bjelakovic, Nikolova, Gluud, Simonetti, & Gluud, 2008). In this context, the antioxidant actions of saffron may be unstable or even attenuated after oral administration. Pharmacokinetic studies are deemed necessary in order to quantify the bioavailability and effectiveness of each constituent and precisely set the "ideal" dose. Up to now, very limited pharmacokinetic studies after SAE administration have been published in literature (Almodovar et al., 2020; Christodoulou et al., 2019). They have report basic plasma pharmacokinetic parameters of crocetin (the active metabolite of saffron's main constituent, crocin), such as C_{max}, T_{max}, Area Under the concentration versus time Curve, elimination half-life ($t_{1/2}$), clearance (CL), and volume of distribution (V_d). Among these, the study of Christodoulou et al. (2019) was performed in mice administrating

orally lyophilized SAE at a dose of 60 mg/kg, while in the more recent study of Almodovar et al. (2020), SFE tablets were administered to 13 healthy volunteers at doses of 56 and 84 mg. Secondly, other beneficial actions of saffron (e.g., antiinflammatory) have not been investigated and may be of clinical relevance. Based on experimental data (Christodoulou et al., 2018; Efentakis et al., 2017), saffron may exert "pleiotropic" actions, which have not yet been evaluated in clinical trials. Thus, the absence of remarkable changes in classical cardiovascular risk factors does not preclude the cardiovascular protection of saffron. Presumably, those parameters should be assayed in future clinical studies. From the methodological point of view, clinical studies have enrolled patients with wide heterogeneity of clinical characteristics leading to inconsistent results. For instance, the baseline glucose levels varied across diabetic groups with the mostly unknown pathophysiological background (either insulin resistance or diminished insulin secretion). It would be wiser to carry out patients' grouping according to similar characteristics and already pharmaceutical regimen, in order to independently assess the therapeutic response to saffron. Finally, almost all clinical studies are of short duration to get significant clinical outcomes (6–12 weeks), have enrolled small samples (up to 40 participants in the therapeutic arm), mostly without previous power analysis, and have been conducted in a single country (Iran) and not in other ethnic populations.

Due to all aforementioned limitations, previous meta-analyses of clinical trials lack robust evidence and more well-designed trials are required.

4 | CONCLUSION

Saffron and its major components, crocin, crocetin, and safranal are generally reported to exhibit significant cardiovascular protective actions such as antioxidant, antiinflammatory, hypoglycaemic, and hypolipidemic in animal models. However, the possibility that most of the studies discussed in the present review article have been not performed in accordance with a recent consensus document providing a perspective on best practice in pharmacological research on bioactive preparations from plants (Izzo et al., 2020) should be taken into account. In addition, in clinical studies, the modification of CVDs or cardiovascular risk factors is absent or at best is weak. Further, larger, properly designed clinical trials, focusing on specific conditions are required to evaluate saffron as a natural supplement to conventional drug therapies for CVDs. In addition, the conduction of properly designed pharmacokinetic/pharmacodynamic studies both in healthy volunteers and patients will enable the establishment of the relationship between dose and clinical effect and to distinguish the role of saffron and its major constituents. Needless to say, that the requirement for proper experimental design is also applied to pre-clinical research, while attention should be paid to clearly distinct identity between plant, plant part, plant extracts, and purified plant components. Properly designed pre-clinical animal pharmacokinetic/pharmacodynamic studies would be also helpful for the selection of the optimum dose selection based on pharmacokinetic parameters.

CONFLICT OF INTEREST

All authors declare no financial/commercial conflicts of interest.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study

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