CHAPTER 17

Crocus sativus L. (Saffron) and Its Components Relaxant Effect on Smooth Muscles and Clinical Applications of This Effect

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INTRODUCTION

Crocus sativus L. (*C. sativus*) or saffron belongs to the family of Iridaceae, from the Liliaceae, is growing up to 8- to 30-cm high. The leaves are right and high with a ciliate margin and keel. This plant grows in dry climate especially in Iran as well as another region of the world such as Spain, Italia, France, Turkey, Greece, India, Egypt, Switzerland, United Arab Emirate, Morocco, Azerbaijan, Pakistan, Chania, New Zealand, and Japan (Fernández, 2004). Saffron is used as an herbal medicine in addition to its usage as a food additive in cooking because of its remarkable color and odor (Nilakshi et al., 2011).

The major components of *C. sativus* are picrocrocin, crocetin, and safranal. All saffron properties such as color, taste, odor, and aroma are dependent on these components. In addition, the plant contained volatile aroma and nonvolatile active fractions including lycopene, zeaxanthin, alpha- and beta-carotenes, as well as carotenoids (Srivastava et al., 2010). Chemical studies also showed the presence of glycoside components such as kaempferol and quercetin in the petals of *C. sativus* (Hosseinzadeh and Younesi, 2002).

This plant has been used in herbal medicine for the treatment of different diseases. The therapeutic effects of saffron have been demonstrated by pharmacological and clinical studies. The findings have shown that the plant is effective for neurodegenerative disorders such as multiple sclerosis (MS) (Hosseinzadeh and Younesi, 2002), coronary artery disorders (Xu et al., 2005), respiratory disease (Boskabady and Aslani, 2006), and gastrointestinal (GI) disease (Rios et al., 1996). Several studies also showed various pharmacological effects for saffron including antiinflammatory, antiantigenotoxic, antiatherosclerotic (Premkumar et al., 2001), antioxidant (Hosseinzadeh et al., 2009), anticancer (Abdullaev, 2002; Aung et al., 2007; Mousavi et al., 2011), memory improvement (Abe and Saito, 2000; Ghadrdoost et al., 2011), antidepressant (Hosseinzadeh and Noraei, 2009), genoprotective (Hosseinzadeh and Sadeghnia, 2007), and antitussive effects (Hosseinzadeh and Ghenaati, 2006).

The lowering blood pressure properties of the plant and its components, crocin and safranal, were also demonstrated in previous research (Fatehi et al., 2003). Crocin also showed an improving effect on cardiac arrhythmia (Jahanbakhsh et al., 2012), and it can reduce contractility and heart rate (Boskabady et al., 2008).

The therapeutic effects of the *C. sativus* extract and its constituent, safranal, on an animal model of asthma (sensitized guinea pig) including their effects on pathological changes, tracheal responsiveness, inflammatory cells, and mediators and Th1/Th2 balance were shown in previous studies (Bayrami and Boskabady, 2012; Byrami et al., 2013; Gholamnezhad et al., 2013). The effects of the saffron extract and safranal on Th1/Th2 balance in PHA-stimulated human peripheral

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mononuclear cells were also demonstrated (Boskabady et al., 2011b; Feyzi et al., 2016). Antiinflammatory, immunomodulatory, and antioxidant properties of *C sativus* and its components were also reviewed previously (Boskabady and Farkhondeh, 2016). The purpose of this chapter is reviewing the saffron and its component relaxant effect on smooth muscles and clinical application of this effect.

METHOD

Various databases including Medline/PubMed, Science Direct, ISI Web of Knowledge, Scopus, Embase, Google Scholar, Chemical Abstracts, and Biological Abstracts were searched to find available articles on the relaxant effect of saffron and its constituent on various types of smooth muscles until the end of December 2018. Keywords such as saffron, *Crocus sativus*, crocin, safranal, crocetin, relaxant effect, cardiovascular, GI, respiratory, and urogenital were used for this purpose.

SAFFRON AND ITS COMPONENTS RELAXANT EFFECT ON SMOOTH MUSCLES

Saffron and Its Components Relaxant Effect on Tracheal Smooth Muscle

Saffron aqueous ethanolic extract relaxant effect comparable to the effect of theophylline was reported. The plant extracts (0.15, 0.3, 0.45, and 0.60 g %) and theophylline (0.15, 0.30, 0.45, and 0.60 mM) exhibited relaxant action on guinea-pig tracheal smooth muscle (TSM) precontracted by 10-μM methacholine compared to saline. Also, there was a positive correlation between increasing concentrations of the extract and its relaxant effects (Boskabady and Aslani, 2006). Furthermore, administration of safranal (0.15, 0.30, 0.45, and 0.60 mL of 0.2 mg/mL solution) also showed a relaxant effect in a dose-dependent manner on TSM. The results also showed lower relaxant effect for safranal than the extract and theophylline effect (Boskabady and Aslani, 2006).

Decreased responsiveness of TSM to methacholine and ovalbumin (OVA) was also reported due to long-term oral administration of *C. sativus* extracts and safranal in sensitized guinea pigs (Byrami et al., 2013). Quercetin (20 mg/kg) showed significant bronchodilatory effects in both in vivo and in vitro studies (Joskova et al., 2011). These effects could be produced from the relaxant effect of the plant extract on TSM.

The antitussive effect of the saffron ethanolic extract (100–800 mg/kg, intraperitoneally [i.p.]) and safranal (0.25–0.75 mL/kg, i.p.) in guinea pigs was also reported that it may be attributed to their relaxant action on TSM (Hosseinzadeh and Ghenaati, 2006).

Saffron and Its Components Relaxant Effect on Vascular Smooth Muscle

Intravenous administration of aqueous extract of *C. sativus* (2.5, 5, and 10 mg/kg), safranal (0.25, 0.5, and 1 mg/kg), and crocin (50, 100, and 200 mg/kg) in normotensive and hypertensive animals reduced the mean arterial blood pressure and heart rate in a dose-dependent manner. Administration of 10 mg/kg of saffron, 200 mg/kg of crocin, and 1 mg/kg of safranal lowered mean systolic blood pressure (MSBP) by 60 ± 8.7, 51 ± 3.8, and 50 ± 5.2 mmHg, respectively. These results suggested that *C. sativus* extract and its two active components showed hypotensive properties in the rats. Moreover, the lowering blood pressure effect of safranal was more potent than crocin (Imenshahidi et al., 2010).

Ethanolic and aqueous extracts of *C. sativus* petals dose-dependently reduced blood pressure in the rats. In this work, aqueous extract (50 mg/g) lowered blood pressure by 17 mmHg compared with the control group. It has been suggested that *C. sativus* petal extracts induced this effect by relaxation of vascular smooth muscle or affecting the heart or both. Nonetheless, the more probable mechanism of the hypotensive effect of extract may contribute to a reduction in peripheral vascular resistance due to the relaxation of vascular smooth muscle (Fatehi et al., 2003).

Reduction of deoxycorticosterone acetate–induced hypertension or increase in MSBP due to treatment with aqueous extract of saffron (10, 20, and 40 mg/kg/day) was also shown in a dose-dependent manner, but this effect on blood pressure was absent in normotensive rats. Furthermore, evidence showed that saffron generates the antihypertensive effects for relatively a short period of time. Therefore, it was suggested that saffron induced short-term blood pressure regulation (Imenshahidi et al., 2013). Likewise, admiration of saffron aqueous ethanolic extract (0.1, 0.5, 1.0, and 5.0 mg%) produced a negative inotropic and chronotropic effect, which was comparable to the effects of diltiazem. It was suggested that the muscle relaxant properties of the extract could be responsible for the heart contractility of the extract (Boskabady et al., 2008).

In the isoproterenol-induced myocardial injury model in rats, the effects of *C. sativus* were evaluated. In this model, isoproterenol enhanced the level of troponin in the serum and reduced the activity of glutathione peroxidase in the heart muscle compared with control group. In addition, isoproterenol-induced heart muscle damages by more than 70%. Saffron remarkably

decreased excessive destruction of the tissue and significantly decreased serum levels of heart troponin I (Joukar et al., 2010). On heart ischemia-reperfusion–induced lethal cardiac arrhythmias model, *C. sativus* (100 mg/kg/day) administration significantly decreased ventricular tachycardia (VT)/ventricular fibrillation (VF) numbers, durations, and also the severity of arrhythmia compared with the untreated group. The PR and QTcn intervals of electrocardiogram were significantly longer in the *C. sativus* (200 mg/kg/day) group (Joukar et al., 2013).

Responses to the endothelium-dependent relaxant effect of acetylcholine (ACh) were improved by treatment with crocetin (15, 30 mg/kg) in a dose-dependent manner in aorta isolated from high cholesterol diet–fed rabbits. Oral admiration of crocetin restored the maximal relaxation compared with the control group dose-dependently. Crocetin increased the of serum nitric oxide (NO) concentration, increased vessel cyclic GMP (cGMP) content as well as unregulated mRNA expression of endothelial NO synthase (eNOS) compared with control group (Tang et al., 2006).

Increased systolic blood pressure (SBP) and heart rate after administration of diazinon (DZN) normalized by the crocin (50 mg/kg) which might be due to relation response of vascular smooth muscle (Razavi et al., 2013).

The effects of kaempferol on endothelium-dependent (bradykinin) and independent (sodium nitroprusside) relaxation of porcine coronary arteries were indicated. Kaempferol (3×10^{-6} M) increased relaxations created by bradykinin and sodium nitroprusside (Xu et al., 2015). It was demonstrated that crocin (1 μM) has preventive effects on the cell apoptosis induced by H_2O_2, in cardiovascular diseases (Xu et al., 2006).

Quercetin, 10^{-6} and 10^{-4} M, inhibited the contractions induced by noradrenaline, high KCl, and Ca^{2+} in a concentration-dependent manner. This effect was observed when the quercetin was added before or after the induction of contractions. Quercetin also increased the aortic cyclic AMP content (Duarte et al., 1993a).

The contractions elicited by KCl, noradrenaline, or phorbol 12-myristate,13-acetate were also relaxed by kaempferol and quercetin (Duarte et al., 1993a,b).

Saffron and Its Components Relaxant Effect on GI and Urogenital Smooth Muscle

Saffron petals extract relaxant effect was examined on isolated ileum of guinea pig. Findings revealed that administration of *C. sativus* aqueous and ethanol extracts decreased contractile responses to electrical field stimulation (EFS) of guinea pig ileum. Furthermore, the aqueous extract (560 mg/mL) reduced the contractile responses to epinephrine (1 μM) in guinea pig ileum (Fatehi et al., 2003).

Kaempferol (3–60 μM) and quercetin (1–100 μM) reversed contractions induced by KCl (60 μM) in rat uterus in a concentration-dependent way. However, the inhibitory effect of cAMP-dependent protein kinases (TPCK, 3 μM) antagonized the effect of quercetin and kaempferol. To sum up, cAMP contributes to the relaxant effects of quercetin and kaempferol on KCl (60 mM)-induced tonic contraction (Revuelta et al., 1997). Table 17.1 presents the saffron and its components' relaxant effect on different types of smooth muscles.

MECHANISMS RESPONSIBLE FOR THE RELAXANT EFFECT OF SAFFRON AND ITS COMPONENTS ON SMOOTH MUSCLES

The saffron and its components' relaxant effect could be due to different mechanisms such as stimulatory effect on β2-adrenoreceptors, inhibitory effect on muscarinic receptor, inhibitory effect on histaminic (H_1) receptors, inhibitory effect on calcium channel, endothelium-dependent relaxation (EDR) effect, and the effect of saffron and its constituent on intracellular cAMP as well as their effect on intracellular cAMP.

β2-Adrenoreceptors Stimulatory Effect of Saffron and Its Components

The β-adrenoceptors have been divided into at least three groups, β1, β2, and β3, which are commonly expressed in cardiac muscle, airway smooth muscle, and adipose tissue, respectively (Somlyo and Somlyo, 1994). Inhalation of selective β2-agonists is commonly used as the most effective bronchodilatory drug and therefore, for the management of obstructive airway diseases. Selective β2-agonists drugs bind to the β2-adrenoceptor and trigger the activation of certain G-proteins followed by the production of cyclic adenosine monophosphate (cAMP) in airway smooth muscle leading to bronchodilation (Cazzola et al., 2013). Cyclic AMP induces its effect through activating several effector molecules such as cAMP-dependent protein kinase (PKA). Probably, PKA decreases the cytosolic Ca^{2+} level via preventing the influx of Ca^{2+} by the cell membrane K^+ channels activation and via stimulation of the sarcoplasmic reticulum (SR) Ca^{2+} uptake, which results in decreased Ca^{2+}-dependent myosin light-chain kinase (MLCK) activity. In addition, stimulation of PKA directly increased the activity of myosin light-chain phosphatase (MLCP). Subsequently, diminished MLCK activity and increased MLCP activity results in reduced phosphorylation of MLC and vasodilatation (Morgado et al., 2012).

TABLE 17.1
Saffron and Its Components' Relaxant Effect on Smooth Muscles.

Smooth Muscle (SM) Type	Solution	Effect	Reference
Trachea smooth muscle	AE and EE of stigma	Relaxant effects on TSM precontracted by methacholine	Boskabady and Aslani (2006)
	Safranal	TSM relaxant effect	Boskabady and Aslani (2006)
	AE of stigma	Reduced tracheal responsiveness to methacholine	Byrami et al. (2013)
	Safranal	Reduced tracheal responsiveness to methacholine in sensitized animals by OVA	Byrami et al. (2013)
	AE of stigma and safranal	Antitussive effect	Hosseinzadeh and Ghenaati (2006)
	Quercetin	In vivo and in vitro bronchodilatory effect	Joskova et al. (2011)
Vascular smooth muscle	AE of stigma, safranal and crocin	Lowered MAP and heart rate	Imenshahidi et al. (2010)
	AE and EE of petals	Lowers BP dose-dependently in isolated rat vas deferens	Fatehi et al. (2003)
	AE of stigma	Decreased MSBP in hypertensive rats	Imenshahidi et al. (2013)
	AE and EE of stigma	Reduced cardiac contractile and heart rate	Boskabady et al. (2008)
	AE of stigma	Decreased intensity of tissue destruction	Joukar et al. (2010)
	AE of stigma	Decreased durations and also arrhythmia severity	Joukar et al. (2013)
	Crocetin	Improved EDR in response to acetylcholine	Tang et al. (2006)
	Crocin	Restored elevation of HR and reduction of SBP induced by diazinon	Razavi et al. (2013)
	Kaempferol	Enhanced relaxations produced by bradykinin and sodium nitroprusside	Xu et al. (2015)
	Crocin	Preventive effects on the cell apoptosis, in cardiovascular diseases	Razavi et al. (2013)
	Quercetin	Inhibitory effects on contractions produced by NA, high KCl and Ca^{2+} in a concentration-dependent manner	Duarte et al. (1993a)
	Quercetin and kaempferol	Relaxant effects on rat aortic strips contraction	Duarte et al. (1993a,b)
Gastrointestinal smooth muscle	AE and EE of petals	Decreased EFS of guinea-pig ileum	Fatehi et al. (2003)
	Kaempferol and quercetin	Relaxed the tonic contraction induced by KCl	Revuelta et al. (1997)

AE, aqueous extract; EE, ethanolic extract; TSM, tracheal smooth muscle; OVA, ovalbumin; SBP, systolic blood pressure; HR, heart rate; EFS, electrical field stimulation; MAP, mean arterial blood pressure; BP, blood pressure; MSBP, mean systolic blood pressure; EDR, endothelium-dependent relaxation; NA, noradrenaline.

The stimulatory effect on the β2-adrenergic receptor is one of the possible mechanisms for natural products such as saffron and its constituents inducing smooth muscles relaxation (Chaudhary et al., 2012). In fact, saffron and safranal β2-adrenergic stimulatory effect were demonstrated in isolated guinea pig TSM (Nemati et al., 2008). The safranal β2-adrenergic stimulatory effect was evaluated by conducting isoprenaline cumulative concentration-response curves (nonspecific β-adrenergic agonist) induced relaxation of precontracted TSM. The study was performed in the presence of two components of aqueous ethanolic extracts of saffron (0.1 and 0.2 g %), safranal (1.25 and 2.5 μg), propranolol (10 nM), and saline. Although the extract and safranal induced leftward shifts in isoprenaline curves when compared to saline, propranolol showed a rightward shift in the curve. Therefore, *C. sativus* extract and its components safranal showed a stimulatory effect of the on β2-adrenoreceptors (Nemati et al., 2008). Consequently, these findings suggested β2-agonistic activities of the *C. sativus* and its constituent, as a potential medicinal plant in the treatment of obstructive airways disorders.

Although stimulation of β2-adrenoreceptors might account for *C. sativus*—induced relaxation of TSM, the adrenergic receptors blocking the effect of the plant petal on vas deferens smooth muscle was also suggested (Fatehi et al., 2003). It was shown that the *C. sativus* petals' extract decreased contractile responses to EFS in isolated rat vas deferens (Fatehi et al., 2003). This study suggested that adrenergic receptors involved in the relaxation of vas deferens by *C. sativus* petals' extract.

Muscarinic Receptor Blocking Effects of Saffron and Its Components

Five subtypes including M 1 to 5 are located in various organs, such as vascular and airway smooth muscles (Harvey, 2012). It has been well known that muscarinic receptor agonists such as acetylcholine, methacholine, and carbachol cause contraction of airway smooth muscle in vivo and in vitro. In addition, anticholinergic drugs, for instance, the atropine, have been used to treat asthma and obstructive airway diseases for several years (Gosens et al., 2006). It was shown that the relaxant effect of *C. sativus* and safranal was mediated by the blocking of muscarinic receptors of TSM. Methacholine concentration-response curves were nonparallelly shifted to right due to saffron extract and safranal caused significant, but the maximum response to methacholine was not achieved in the presence of the extract and safranal. These results indicated a functional antagonism effect of the plant and its component on TSM (Neamati and Boskabady, 2010).

Histaminic Antagonistic Activity of Saffron and Its Components

Evidence revealed that histamine affects the smooth muscle of numerous tissues, including bronchioles, intestine, uterus, arterioles, and spleen. Administration of exogenous histamine to guinea pigs generates symptoms and signs of respiratory changes similar to those generated after challenge with the certain antigen (Togias, 2003). Three types of histamine receptors have currently been identified. H_1-receptors mediate bronchoconstriction and H_1-receptor activation, which seems to stimulate inositol trisphosphate (IP3) generation and transient Ca^{2+} release from intracellular stores, followed by continued generation of IP, IP_2, and IP_3, which is linked to prolonged Ca^{2+} influx in cultured human bronchial smooth muscle cells (Goldie et al., 1986).

In a number of studies, the histaminic (H_1) antagonistic effect of plants in smooth muscle cells have been reported (Boskabady et al., 2011a, 2006). Saffron inhibitory effect on histamine (H_1) receptors was evaluated by the effect of aqueous-ethanolic extracts of the plant on the histamine-induced contraction of guinea pig TSM. Three concentrations of the extracts (0.025 g%, 0.05 g %, and 0.1 g%) lead to a parallel rightward shift in histamine concentration-response curve, and the maximum response to histamine was obtained in the presence of the extract. Chlorpheniramine also showed a similar effect to that of saffron (Boskabady et al., 2010).

Therefore, saffron showed a competitive antagonistic (inhibitory) effect on histamine H_1 receptors that could be contributed to the plant relaxant effect on TSM. Similarly, the effect of safranal (0.63, 1.25, and 2.5 μg/mL) on histamine (H_1) receptors in guinea pig TSM were evaluated. The findings showed that safranal shifted histamine-response curves to the right, causing achievement of maximum response to histamine and increased EC50 (effective concentration of histamine causing 50% of maximum response). These results indicated competitive antagonistic effect of safranal at histamine H_1 receptors which may be considered as one possible mechanism for the relaxant action of safranal on TSM (Boskabady et al., 2011a).

Calcium Channel Antagonist Activity of Saffron and Its Components

Intracellular calcium and associated calcium channels play a significant role in the regulation of smooth muscle activity. Therefore, any change in intracellular/extracellular calcium concentration results in various disturbance such as intestinal spasm and bronchoconstriction. In this regard, calcium channel antagonists commonly used in different abnormalities such as

cardiovascular disorders and hypertension. These drugs induce a common inhibition on the L-type calcium channels in smooth muscle cells and result in relaxation.

Several studies have demonstrated that medicinal plants induce a relaxant effect on smooth muscles via blocking of the calcium channels (Boskabady et al., 2005a, 2005b; Rakhshandah et al., 2010). For instance, crocin could inhibit the influx of extracellular Ca^{2+} and also release intracellular Ca^{2+} stores in the endoplasmic reticulum in cultured bovine aortic smooth muscle cells (He et al., 2004). Furthermore, the effect of saffron on Ca^{2+} influx in the aorta smooth muscle of rat was evaluated by radioactive tracer ^{45}Ca. Data showed that the Ca^{2+} influxes induced by norepinephrine (1.2 μmol/L and KCl of 100 mmol/L) were remarkably inhibited by saffron concentration-dependently. The finding indicated that the plant can block the influx of extracellular Ca^{2+} via potential-dependent Ca^{2+} channels and receptor-operated Ca^{2+} channels (Liu et al., 2005). Similarly, in another study, the chronic treatment with saffron had a hypotensive effect that was attributed to the smooth muscles inhibitory effect through calcium channel blocking or inhibition of sarcoplasmic reticulum Ca^{2+} release into the cytosol (Boskabady and Aslani, 2006).

In addition, crocin could inhibit the elevation of intracellular Ca^{2+} in the smooth muscle cells in an atherosclerosis model. When the cells were treated with Ox-LDL in the presence of extracellular Ca^{2+}, maximum intracellular Ca^{2+} have generated. However, preincubation of cells with crocin for 4 h, concentration-dependently, inhibited the Ox-LDL-induced intracellular Ca^{2+} elevation. In the lack of extracellular Ca^{2+}, Ox LDL-treated smooth muscle cell revealed maximum intracellular Ca^{2+}, and preincubation with crocin for 4 h significantly blocked the maximum response (He et al., 2005).

Flavonoid compounds, which are widely found as secondary metabolites in various plants including saffron, have an important function such as vasodilation. It has been suggested that the vasodilatory effect of these plants attributed to inhibition of PKC (protein kinase C) or reduction of Ca^{2+} uptake (Duarte et al., 1993a,b). It was proposed that crocetin attenuated the PKC activity in the fraction of membrane, which leads to a reduction of blood pressure by an inhibitory effect on proliferation in vascular smooth muscle cells (Zhou et al., 2010).

EDR Effect of Saffron and Its Components

Nitric oxide (NO) predominantly regulates the vascular tone. NO exerted vasodilatation largely through the activation of soluble guanylyl cyclase (sGC), resulting in cGMP production (Gao, 2010). The endothelium-dependent vasodilatation of crocetin has also been documented by upregulation of the mRNA expressions of eNOS in both in vitro and in vivo studies by its action on eNOS activity and NO production. These results suggest increased NO synthesized in response to crocetin by endothelial cells, which is the major endothelium-derived relaxing factor in the rat aortas (Tang et al., 2006).

Kaempferol may also have relaxation effects by endothelium-derived and exogenous NO as well as endothelium-dependent hyperpolarization through large-conductance calcium-activated potassium channel ($K_{Ca}1.1$ channels) (Xu et al., 2015).

Intracellular cAMP Effects of Saffron and Its Components

Substantial evidence indicates that kaempferol exerts significant relaxation in KCl-induced tonic contraction in isolated rat uterus, which largely mediated through the cAMP and antagonized by Rp-cAMPS, an antagonist of cAMP. In fact, the cAMP, transcription, and protein synthesis are involved in the kaempferol-induced relaxation in rat uterus (Revuelta et al., 2000). However, this mechanism also should be evaluated for saffron and its components in further studies. Table 17.2 presents the possible mechanisms of the saffron and its components relaxant effect.

CLINICAL APPLICATIONS OF SAFFRON AND ITS COMPONENTS RELAXANT EFFECT ON SMOOTH MUSCLES

There are evidence regarding the clinical applications of saffron and its active ingredients in various disorders (Pourmasoumi et al., 2018; Razak et al., 2017). For instance, the effect of saffron and its constituents was studied in patients with depression (Tabeshpour et al., 2017), premenstrual syndrome (Agha-Hosseini et al., 2008), erectile dysfunction (Mohammadzadeh-Moghadam et al., 2015), Alzheimer's disease (Farokhnia et al., 2014), and Metabolic syndrome (Kermani et al., 2017a,b). With regard to the plant relaxant effect on various types of smooth muscle, saffron may also have therapeutic values in the disorders including cardiovascular, respiratory, GI, and urogenital tracts disorders (summarized in Table 17.3).

Cardiovascular System

In most cardiovascular disorders such as heart failure, coronary artery disease, stroke, and peripheral vascular disease, hypertension is the major risk factor worldwide (Ogihara et al., 2005). Several adverse effects have been reported for the available antihypertension drugs for

TABLE 17.2
The Possible Mechanisms of Saffron and Its Components' Relaxation Effect on Smooth Muscles.

Smooth Muscle (SM) Type	Possible Mechanisms	Extract/Constituent and Studied Dose/Concentration	Reference
Trachea	β2-adrenoceptor stimulatory effects	Saffron AE extract (0.1 and 0.2 g%) and safranal (1.25 and 2.5 μg)	Nemati et al. (2008)
Trachea	Muscarinic and cholinergic receptor blocking effects	Saffron AE extract and safranal	Neamati and Boskabady (2010)
Trachea	Histaminic antagonistic activity	Saffron AE extract (0.02, 0.05 and 0.1 g%) Safranal (0.63, 1.25, and 2.5 μg/mL)	Boskabady et al. (2010) Boskabady et al. (2011a,b)
Vascular	Calcium channel antagonist activity	Crocin (1×10^{-8}, 1×10^{-7}, 1×10^{-6} mol/L)	He et al. (2004)
Vascular	Inhibitory effect on PKC or Ca^{2+} uptake	Kaempferol	(Duarte et al. 1993b)
Vascular	Inhibitory effect on PKC activity	Crocetin	Zhou et al. (2010)
Vascular	Increased endothelial NOS activity and production of NO	Crocetin	Tang et al. (2006)
Vascular	Activation of K_{Ca} 1.1 channels	Kaempferol (3×10^{-6} M)	Xu et al. (2015)
Uterus	Increased intracellular cAMP and PKA	Kaempferol (3–60 mM)	Revuelta et al. (2000)

PKC, protein kinase C; PKA, protein kinase A.

these drugs. It was suggested that saffron and its components produced hypotensive and cardiovascular protective effects (Mancini et al., 2014; Razavi et al., 2018). Intravenous administration of saffron aqueous extract (2.5, 5, and 10 mg/kg), safranal (0.25, 0.5, and 1 mg/kg), and crocin (50, 100, and 200 mg/kg) lowered the mean arterial blood pressure in normotensive and hypertensive rats induced by desoxycorticosterone acetate dose-dependently (Imenshahidi et al., 2010). Furthermore, administration of crocin (50, 100, and 200 mg/dL) for 5 weeks in hypertensive animals also showed that crocin reduced mean arterial blood pressure dose-dependently (Imenshahidi et al., 2014).

In the study conducted in healthy volunteers by Modaghegh and colleagues, saffron with a higher dose (400 mg) significantly decreased systolic blood pressure and mean arterial pressures (Modaghegh et al., 2008). However, in a double-blind, randomized clinical trial which was conducted on metabolic syndrome patients, no significant change in BP after the administration of 100 mg of crocin for 6 weeks was observed (Kermani et al., 2017a). In addition, a meta-analysis of 11 published studies did not exhibit any significant change in systolic blood pressure after saffron consumption (Pourmasoumi et al., 2018). Based on contradictory results, further experiments are required to fully evaluate effects of saffron and its constituents on cardiovascular disorders.

Respiratory System

In vitro or in vivo animal studies indicated the bronchodilator effect of saffron in asthma and COPD through several mechanisms including antihistamine, anticholinergic, and β2-adrenoreceptors stimulation (Mokhtari-Zaer et al., 2015). Unfortunately, there have not yet been any controlled clinical studies investigating the bronchodilatory effect of the plant and its components on respiratory disorders such as asthma, COPD, or other obstructive pulmonary diseases. To confirm the bronchodilatory effects of saffron and its active compound, clinical studies should be performed. However, saffron and its components showed a potent relaxant effect on TSM as well as their stimulatory effect on β2-adrenoreceptors, inhibitory effect on muscarinic and histamine (H_1) receptors, as well as the effect on calcium channels, strongly suggest the bronchodilatory effect of saffron and its component.

TABLE 17.3
Possible Clinical Applications of Saffron and Its Components' Relaxant Effect on Smooth Muscles.

System	Disease	Effects	Possible Mechanism	Extract/ Constituents	Reference
Respiratory	Asthma COPD Other obstructive diseases	Relaxant effects on guinea pig tracheal chains	–	Safranal, AE, and EE of stigma	Boskabady and Aslani (2006)
		Reduction of TR		AE of stigma	Byrami et al. (2013)
		Antitussive effect		AE of stigma and safranal	Hosseinzadeh and Ghenaati (2006)
		In vivo and in vitro bronchodilation		Quercetin	Joskova et al. (2011)
		Bronchodilatory	β2– adrenoreceptors stimulatory	Aq, Eth Ext of stigma, safranal	Nemati et al. (2008)
		Bronchodilatory	Anticholinergic and antimuscarinic	Aq, Eth Ext of stigma, safranal	Neamati and Boskabady (2010)
		Bronchodilatory	Histaminic (H_1) receptor antagonism		Boskabady et al. (2011a)
Cardiovascular	Hypertension	Reduction of blood pressure	_	Aq. Ext., safranal, crocin	Imenshahidi et al. (2010)
		Reduction of blood pressure	_	Aq. and Eth. Ext of petals	Fatehi et al. (2003)
		Reduction of blood pressure	_	Aq. Ext of stigma	Imenshahidi et al. (2013)
		Decreased SBP and heart rate induced	Vascular SM relaxation	Crocetin	Razavi et al. (2013)
		Endothelium-dependent relaxation of aorta	Increased expression of eNOS, and cGMP	Crocetin	Tang et al. (2006)
		Relaxation of porcine coronary arteries	Preventive effects on the cell apoptosis	Kaempferol	Xu et al. (2006)
		Relaxation of rat aortic strips	Increased the aortic cAMP	Quercetin and kaempferol	Duarte et al. (1993a,b)
		Reduced heart rate and contractility	Vascular SM relaxation	Aq-Eth. Ext of stigma	Boskabady et al. (2008)
Gastrointestinal	constipation	Decreased EFS of guinea-pig ileum	–	AE and EE of petals	Fatehi et al. (2003)
Urogenital	Sexual dysfunctions	Improved erectile dysfunction	–	saffron gel	Mohammadzadeh-Moghadam et al. (2015)
	Premenstrual syndrome	Relieving premenstrual syndrome symptoms	–	capsule saffron 30 mg	Agha-Hosseini et al. (2008)
		Relaxed tonic contraction	Muscarinic stimulation	Kaempferol and quercetin	Revuelta et al. (1997)

Ext, extract; Aq., aqueous; Eth, ethanolic; TR, tracheal responsiveness; SM, smooth muscle; SBP, systolic blood pressure.

GI Tract

Rezaee Khorasany and his colleagues have published a review detailing the therapeutic effects of saffron in GI disorder including hepatotoxicity, liver cancer, fatty liver, colon cancer, stomach cancer, pancreas cancer, hyperlipidemia, peptic ulcer, ulcerative colitis, and ileum contractions (Khorasany and Hosseinzadeh, 2016). However, most of the studies have been carried our on isolated tissues or murine models of GI disorders. For instance, It has been reported that the petal extracts of *C. sativus* diminished the evoked contractions in isolated guinea-pig ileum (Fatehi et al., 2003). In fact, the saffron extract exhibited a spasmolytic effect in tissues precontracted by EFS. In fact, it was shown that electrical stimulation resulted in ACh released from activated synapses (Bornstein et al., 2004). It is suggested that ACh binds to M3 postsynaptic smooth muscle receptors and cause contraction of ileum smooth muscles. Nonetheless, the efficacy of saffron in GI tract disorders remains an open empirical question. Controlled clinical studies of saffron have yet to be conducted to determine the efficacy of this plant for people who have suffered from GI disorders.

Urogenital Tracts

Several experimental and clinical studies have shown a therapeutic effect of saffron and its component on sexual disfunction (Hosseinzadeh et al., 2008; Shamsa et al., 2009). In a study on male rats, crocin (100, 200, and 400 mg/kg) and the extract of the plant (160 and 320 mg/kg) enhanced mounting frequency, erection frequency, and intromission frequency. However, they reduced mounting latency, ejaculation latency, and intromission latency, while safranal did not have any effect on the mentioned parameters (Hosseinzadeh et al., 2008).

Although the previous studies conducted to determine the relaxant effect of saffron on smooth muscle, some studies have revealed the spasmodic action on smooth muscle-like uterine (Sadraei et al., 2003). In fact, *C. sativus* is regarded as an abortifacient agent, as it increases uterine muscle contraction (Hosseinzadeh

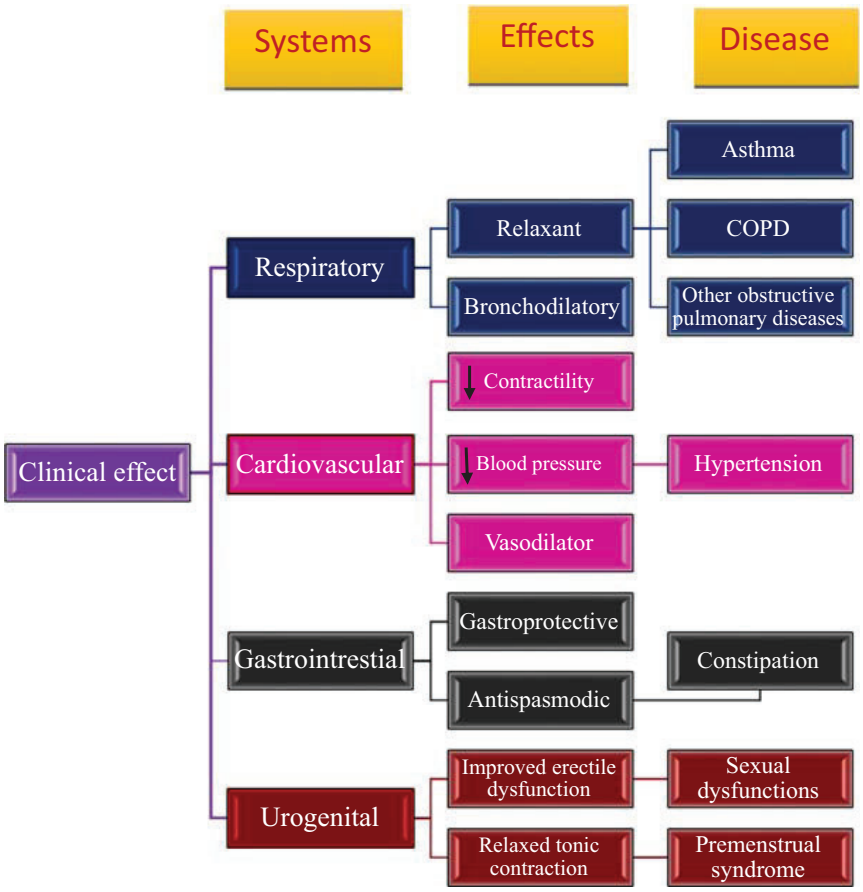

FIG. 17.1 Possible clinical applications of the relaxant effect of saffron and its constituents.

and Nassiri-Asl, 2013). In an *in vitro* experiment, saffron extract (200−1600 lg/mL) increased the spontaneous rhythmic contraction in isolated rat uterine (Sadraei et al., 2003). Furthermore, it has been reported that saffron consumption, particularly in the last trimester of gestation, can generate preterm delivery due to increased uterine contractions in female mice (Zeinali et al., 2009). It should be noted that *C. sativus* contained various components so each component could have different actions on muscular tissue. Interestingly, kaempferol, as a flavonol from saffron petal, showed a potent relaxant effect on KCl-precontracted rat uterus (Revuelta et al., 2000). In addition, in Chinese medicine, saffron usage is recommended for difficult labor, menorrhagia, and postpartum hemorrhage (Gruenwald, 1998).

However, little research has been performed to investigate the potential therapeutic effects of saffron in the urogenital tract disorders. Thus, based on such preclinical evidence, saffron could be extensively evaluated in clinical trials for urogenital tract diseases, and future studies should fill this knowledge gap. Possible clinical effect of saffron and its constituents are presented in Table 17.2 and Fig. 17.1.

CONCLUSION

The results of those evaluated in vivo and in vitro animal studies demonstrated relaxant effects of saffron extract, safranal, and quercetin, which approved the bronchodilatory and antitussive properties of the plant. In different hypertensive animal models and in vitro isolated vascular and heart preparations, the relaxant and hypotensive effects of *C. sativus* extract and its constituents (safranal, crocetin, crocin, quercetin, and kaempferol) have been shown. Several pathways, including protecting effect against heart toxic agent, antiarrhythmia property, improving endothelium-dependent relaxation response to acetylcholine, increasing serum level of nitric oxide (NO), upregulating vessel activity and mRNA expression of endothelial NO synthase (eNOS), as well as vessel cyclic GMP (cGMP) content, have been suggested for cardioprotective and vasodilatory effects of plant and its constituents. In addition, saffron petal extracts and its flavonoids kaempferol and quercetin showed relaxant effects on GI (ileum) and urogenital (uterus) smooth muscle preparations.

The saffron and its components' relaxant effect on smooth muscles of various organs, including blood vessels, trachea, GI, and urogenital, have been shown to be mediated via different mechanisms such as stimulatory effect on β2-adrenoreceptors, inhibitory effect on muscarinic receptor, inhibitory effect on histaminic (H_1 receptor) receptors, inhibitory effect on calcium channel, EDR effect, and effect of saffron and its constituent on intracellular cAMP and effect of saffron and its constituent on intracellular cAMP.

The result of the reviewed studies suggested the therapeutic effect of saffron and its constituents on cardiovascular, GI, respiratory, and urogenital disorders. However, few clinical studies investigated those beneficial effects on related systems. Therefore, more clinical trials should be designated for demonstration of preventive and therapeutic effects of saffron and its constituents due to their smooth muscle relaxant effect.

REFERENCES

Abdullaev, F.I., 2002. Cancer chemopreventive and tumoricidal properties of saffron (*Crocus sativus* L.). Exp. Biol. Med. 227, 20−25.

Abe, K., Saito, H., 2000. Effects of saffron extract and its constituent crocin on learning behaviour and long-term potentiation. Phytother. Res. 14, 149−152.

Agha-Hosseini, M., Kashani, L., Aleyaseen, a, Ghoreishi, a, Rahmanpour, H., Zarrinara, a R., Akhondzadeh, S., 2008. *Crocus sativus* L. (saffron) in the treatment of premenstrual syndrome: a double-blind, randomised and placebo-controlled trial. BJOG 115, 515−519.

Aung, H.H., Wang, C.Z., Ni, M., Fishbein, A., Mehendale, S.R., Xie, J.T., Shoyama, A.Y., Yuan, C.S., 2007. Crocin from *Crocus sativus* possesses significant anti-proliferation effects on human colorectal cancer cells. Exp. Oncol. 29, 175.

Bayrami, G., Boskabady, M.H., 2012. The potential effect of the extract of *Crocus sativus* and safranal on the total and differential white blood cells of ovalbumin-sensitized Guinea pigs. Res. Pharm. Sci. 7, 249−255.

Bornstein, J.C., Costa, M., Grider, J.R., 2004. Enteric motor and interneuronal circuits controlling motility. Neurogastroenterol. Motil. 16 (Suppl. 1), 34−38.

Boskabady, M.H., Aslani, M.R., 2006. Relaxant effect of *Crocus sativus* (saffron) on Guinea-pig tracheal chains and its possible mechanisms. J. Pharm. Pharmacol. 58, 1385−1390.

Boskabady, M.H., Farkhondeh, T., 2016. Antiinflammatory, antioxidant, and immunomodulatory effects of *Crocus sativus* L. And its main constituents. Phytother. Res. 30, 1072−1094.

Boskabady, M.H., Kiani, S., Azizi, H., 2005a. Relaxant effect of *Cuminum cyminum* on Guinea pig tracheal chains and its possible mechanism (s). Indian J. Pharmacol. 37, 111.

Boskabady, M.H., Kiani, S., Haghiri, B., 2005b. Relaxant effects of *Ocimum basilicum* on Guinea pig tracheal chains and its possible mechanism (s). DARU J. Pharm. Sci. 13, 28−33.

Boskabady, M.H., Kiani, S., Rakhshandah, H., 2006. Relaxant effects of *Rosa damascena* on Guinea pig tracheal chains and its possible mechanism (s). J. Ethnopharmacol. 106, 377−382.

Boskabady, M.H., Rahbardar, M.G., Jafari, Z., 2011a. The effect of safranal on histamine (H1) receptors of Guinea pig tracheal chains. Fitoterapia 82, 162–167.

Boskabady, M.H., Rahbardar, M.G., Nemati, H., Esmaeilzadeh, M., 2010. Inhibitory effect of *Crocus sativus* (saffron) on histamine (H1) receptors of Guinea pig tracheal chains. Pharmazie 65, 300–305.

Boskabady, M.H., Seyedhosseini Tamijani, S.M., Rafatpanah, H., Rezaei, A., Alavinejad, A., 2011b. The effect of *Crocus sativus* extract on human lymphocytes' cytokines and T helper 2/T helper 1 balance. J. Med. Food 14, 1538–1545.

Boskabady, M.H., Shafei, M.N., Shakiba, A., Sefidi, H.S., 2008. Effect of aqueous-ethanol extract from *Crocus sativus* (saffron) on Guinea-pig isolated heart. Phytother. Res. 22, 330–334.

Byrami, G., Boskabady, M.H., Jalali, S., Farkhondeh, T., 2013. The effect of the extract of *Crocus sativus* on tracheal responsiveness and plasma levels of IL-4, IFN-gamma, total NO and nitrite in ovalbumin sensitized Guinea-pigs. J. Ethnopharmacol. 147, 530–535.

Cazzola, M., Page, C.P., Rogliani, P., Matera, M.G., 2013. β2-agonist therapy in lung disease. Am. J. Respir. Crit. Care Med. 187, 690–696.

Chaudhary, M.A., Imran, I., Bashir, S., Mehmood, M.H., Rehman, N., Gilani, A.-H., 2012. Evaluation of gut modulatory and bronchodilator activities of Amaranthus spinosus Linn. BMC Complement Altern. Med. 12, 166.

Duarte, J., Perez-Vizcaino, F., Zarzuelo, A., Jimenez, J., Tamargo, J., 1993a. Vasodilator effects of quercetin in isolated rat vascular smooth muscle. Eur. J. Pharmacol. 239, 1–7.

Duarte, J., Pérez, F.V., Utrilla, P., Jiménez, J., Tamargo, J., Zarzuelo, A., 1993b. Vasodilatory effects of flavonoids in rat aortic smooth muscle. Structure-activity relationships. Gen. Pharmacol. 24, 857–862.

Farokhnia, M., Shafiee Sabet, M., Iranpour, N., Gougol, A., Yekehtaz, H., Alimardani, R., Farsad, F., Kamalipour, M., Akhondzadeh, S., 2014. Comparing the efficacy and safety of *Crocus sativus* L. with memantine in patients with moderate to severe Alzheimer's disease: a double-blind randomized clinical trial. Hum. Psychopharmacol. 29, 351–359.

Fatehi, M., Rashidabady, T., Fatehi-hassanabad, Z., 2003. Effects of *Crocus sativus* petals' extract on rat blood pressure and on responses induced by electrical field stimulation in the rat isolated vas deferens and Guinea-pig ileum. J. Ethnopharmacol. 84, 199–203.

Fernández, J.-A., 2004. Biology, biotechnology and biomedicine of saffron. Recent Res. Dev. Plant Sci. 2, 127–159.

Feyzi, R., Boskabady, M.H., Seyedhosseini Tamijani, S.M., Rafatpanah, H., Rezaei, S.A., 2016. The effect of safranal on Th1/Th2 cytokine balance. Iran. J. Immunol. 13, 263–273.

Gao, Y., 2010. The multiple actions of NO. Pflügers Arch. J. Physiol. 459, 829–839.

Ghadrdoost, B., Vafaei, A.A., Rashidy-Pour, A., Hajisoltani, R., Bandegi, A.R., Motamedi, F., Haghighi, S., Sameni, H.R., Pahlvan, S., 2011. Protective effects of saffron extract and its active constituent crocin against oxidative stress and spatial learning and memory deficits induced by chronic stress in rats. Eur. J. Pharmacol. 667, 222–229.

Gholamnezhad, Z., Koushyar, H., Byrami, G., Boskabady, M.H., 2013. The extract of Crocus sativus and its constituent safranal, affect serum levels of endothelin and total protein in sensitized Guinea pigs. Iran. J. Basic Med. Sci. 16, 1022–1026.

Goldie, R.G., Spina, D., Henry, P.J., Lulich, K.M., Paterson, J.W., 1986. In vitro responsiveness of human asthmatic bronchus to carbachol, histamine, beta-adrenoceptor agonists and theophylline. Br. J. Clin. Pharmacol. 22, 669–676.

Gosens, R., Zaagsma, J., Meurs, H., Halayko, A.J., 2006. Muscarinic receptor signaling in the pathophysiology of asthma and COPD. Respir. Res. 7, 73.

Gruenwald, J., 1998. PDR for Herbal Medicines. Medical Economics Company.

Harvey, R.D., 2012. Muscarinic receptor agonists and antagonists: effects on cardiovascular function. In: Muscarinic Receptors. Springer, pp. 299–316.

He, S.-Y., Qian, Z.-Y., Tang, F.-T., 2004. Effect of crocin on intracellular calcium concentration in cultured bovine aortic smooth muscle cells. Acta Pharm. Sin. 39, 778–781.

He, S.-Y., Qian, Z.-Y., Tang, F.-T., Wen, N., Xu, G.-L., Sheng, L., 2005. Effect of crocin on experimental atherosclerosis in quails and its mechanisms. Life Sci. 77, 907–921.

Hosseinzadeh, H., Ghenaati, J., 2006. Evaluation of the antitussive effect of stigma and petals of saffron (*Crocus sativus*) and its components, safranal and crocin in Guinea pigs. Fitoterapia 77, 446–448.

Hosseinzadeh, H., Nassiri-Asl, M., 2013. Avicenna's (Ibn sina) the canon of medicine and saffron (*Crocus sativus*): a review. Phytother. Res. 27, 475–483.

Hosseinzadeh, H., Noraei, N.B., 2009. Anxiolytic and hypnotic effect of *Crocus sativus* aqueous extract and its constituents, crocin and safranal, in mice. Phytother. Res. 23, 768–774.

Hosseinzadeh, H., Sadeghnia, H.R., 2007. Effect of safranal, a constituent of *Crocus sativus* (saffron), on methyl methanesulfonate (MMS)-induced DNA damage in mouse organs: an alkaline single-cell gel electrophoresis (comet) assay. DNA Cell Biol. 26, 841–846.

Hosseinzadeh, H., Shamsaie, F., Mehri, S., others, 2009. Antioxidant activity of aqueous and ethanolic extracts of *Crocus sativus* L. stigma and its bioactive constituents, crocin and safranal. Pharmacogn. Mag. 5, 419.

Hosseinzadeh, H., Younesi, H.M., 2002. Antinociceptive and anti-inflammatory effects of *Crocus sativus* L. stigma and petal extracts in mice. BMC Pharmacol. 2, 7.

Hosseinzadeh, H., Ziaee, T., Sadeghi, a, 2008. The effect of saffron, *Crocus sativus* stigma, extract and its constituents, safranal and crocin on sexual behaviors in normal male rats. Phytomedicine 15, 491–495.

Imenshahidi, M., Hosseinzadeh, H., Javadpour, Y., 2010. Hypotensive effect of aqueous saffron extract (*Crocus sativus* L.) and its constituents, safranal and crocin, in normotensive and hypertensive rats. Phytother. Res. 24, 990–994.

Imenshahidi, M., Razavi, B.M., Faal, A., Gholampoor, A., Mousavi, S.M., Hosseinzadeh, H., 2013. The effect of

chronic administration of saffron (*Crocus sativus*) stigma aqueous extract on systolic blood pressure in rats. Jundishapur J. Nat. Pharm. Prod. 8, 175.

Imenshahidi, M., Razavi, B.M., Faal, A., Gholampoor, A., Mousavi, S.M., Hosseinzadeh, H., 2014. Effects of chronic crocin treatment on desoxycorticosterone acetate (doca)-salt hypertensive rats. Iran. J. Basic Med. Sci. 17, 9.

Jahanbakhsh, Z., Rasoulian, B., Jafari, M., Shekarforoush, S., Esmailidehaj, M., Mohammadi, M.T., Aghai, H., Salehi, M., Khoshbaten, A., 2012. Protective effect of crocin against reperfusion-induced cardiac arrhythmias in anaesthetized rats. EXCLI J 11, 20–29.

Joskova, M., Franova, S., Sadlonova, V., 2011. Acute bronchodilator effect of quercetin in experimental allergic asthma. Bratisl. Lek. Listy 112, 9–12.

Joukar, S., Ghasemipour-Afshar, E., Sheibani, M., Naghsh, N., Bashiri, A., 2013. Protective effects of saffron (Crocus sativus) against lethal ventricular arrhythmias induced by heart reperfusion in rat: a potential anti-arrhythmic agent. Pharm. Biol. 51, 836–843.

Joukar, S., Najafipour, H., Khaksari, M., Sepehri, G., Shahrokhi, N., Dabiri, S., Gholamhoseinian, A., Hasanzadeh, S., 2010. The effect of saffron consumption on biochemical and histopathological heart indices of rats with myocardial infarction. Cardiovasc. Toxicol. 10, 66–71.

Kermani, T., Kazemi, T., Molki, S., Ilkhani, K., Sharifzadeh, G., Rajabi, O., 2017a. The efficacy of crocin of saffron (*Crocus sativus* L.) on the components of metabolic syndrome: a randomized controlled clinical trial. J. Res. Pharm. Pract. 6, 228.

Kermani, T., Zebarjadi, M., Mehrad-Majd, H., Mirhafez, S.-R., Shemshian, M., Ghasemi, F., Mohammadzadeh, E., Mousavi, S.H., Norouzy, A., Moghiman, T., Sadeghi, A., Ferns, G., Avan, A., Mahdipour, E., Ghayour-Mobarhan, M., 2017b. Anti-inflammatory effect of Crocus sativus on serum cytokine levels in subjects with metabolic syndrome: a randomized, double-blind, placebo-controlled trial. Curr. Clin. Pharmacol. 12, 122–126.

Khorasany, A.R., Hosseinzadeh, H., 2016. Therapeutic effects of saffron (*Crocus sativus* L.) in digestive disorders: a review. Iran. J. Basic Med. Sci. 19, 455.

Liu, N., Yang, Y., Mo, S., Liao, J., Jin, J., 2005. Calcium antagonistic effects of Chinese crude drugs: preliminary investigation and evaluation by 45Ca. Appl. Radiat. Isot. 63, 151–155.

Mancini, A., Serrano-Diaz, J., Nava, E., D'Alessandro, A.M., Alonso, G.L., Carmona, M., Llorens, S., 2014. Crocetin, a carotenoid derived from saffron (Crocus sativus L.), improves acetylcholine-induced vascular relaxation in hypertension. J. Vasc. Res. 51, 393–404.

Modaghegh, M.-H., Shahabian, M., Esmaeili, H.-A., Rajbai, O., Hosseinzadeh, H., 2008. Safety evaluation of saffron (*Crocus sativus*) tablets in healthy volunteers. Phytomedicine 15, 1032–1037.

Mohammadzadeh-Moghadam, H., Nazari, S.M., Shamsa, A., Kamalinejad, M., Esmaeeli, H., Asadpour, A.A., Khajavi, A., 2015. 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 Complement. Altern. Med. 20, 283–286.

Mokhtari-Zaer, A., Khazdair, M.R., Boskabady, M.H., 2015. Smooth muscle relaxant activity of Crocus sativus (saffron) and its constituents: possible mechanisms. Avicenna J. Phytomed. 5, 365.

Morgado, M., Cairrao, E., Santos-Silva, A.J., Verde, I., 2012. Cyclic nucleotide-dependent relaxation pathways in vascular smooth muscle. Cell. Mol. Life Sci. 69, 247–266.

Mousavi, S.H., Moallem, S.A., Mehri, S., Shahsavand, S., Nassirli, H., Malaekeh-Nikouei, B., 2011. Improvement of cytotoxic and apoptogenic properties of crocin in cancer cell lines by its nanoliposomal form. Pharm. Biol. 49, 1039–1045.

Neamati, N., Boskabady, M.H., 2010. Effect of Crocus sativus (saffron) on muscarinic receptors of Guinea pig tracheal chains. Funct. Plant Sci. Biotechnol. 4, 128–131.

Nemati, H., Boskabady, M.H., Ahmadzadef Vostakolaei, H., 2008. Stimulatory effect of *Crocus sativus* (saffron) on β 2-adrenoceptors of Guinea pig tracheal chains. Phytomedicine 15, 1038–1045.

Nilakshi, N., Gadiya, R.V., Abhyankar, M., Champalal, K.D., 2011. Detailed profile of Crocus sativus. Int. J. Pharma Bio Sci. 2, 530–540.

Ogihara, T., Matsuzaki, M., Matsuoka, H., Shimamoto, K., Shimada, K., Rakugi, H., Umemoto, S., Kamiya, A., Suzuki, N., Kumagai, H., 2005. The combination therapy of hypertension to prevent cardiovascular events (COPE) trial: rationale and design. Hypertens. Res. 28, 331.

Pourmasoumi, M., Hadi, A., Najafgholizadeh, A., Kafeshani, M., Sahebkar, A., 2018. Clinical evidence on the effects of saffron (Crocus sativus L.) on cardiovascular risk factors: a systematic review meta-analysis. Pharmacol. Res. 139, 348–359.

Premkumar, K., Abraham, S.K., Santhiya, S.T., Gopinath, P.M., Ramesh, A., 2001. Inhibition of genotoxicity by saffron (Crocus sativus L.) in mice. Drug Chem. Toxicol. 24, 421–428.

Rakhshandah, H., Boskabady, M.H., Mossavi, Z., Gholami, M., Saberi, Z., 2010. The Differences in the relaxant effects of different fractions of Rosa damascena on Guinea pig tracheal smooth muscle. Iran. J. Basic Med. Sci. 13, 126–132.

Razak, S.I.A., Anwar Hamzah, M.S., Yee, F.C., Kadir, M.R.A., Nayan, N.H.M., 2017. A review on medicinal properties of saffron toward major diseases. J. Herbs, Spices, Med. Plants 23, 98–116.

Razavi, B.M., Alyasin, A., Hosseinzadeh, H., Imenshahidi, M., 2018. Saffron induced relaxation in isolated rat aorta via endothelium dependent and independent mechanisms. Iran. J. Pharm. Res. 17, 1018–1025.

Razavi, M., Hosseinzadeh, H., Abnous, K., Motamedshariaty, V.S., Imenshahidi, M., 2013. Crocin restores hypotensive effect of subchronic administration of diazinon in rats. Iran. J. Basic Med. Sci. 16, 64.

Revuelta, M.P., Cantabrana, B., Hidalgo, A., 1997. Depolarization-dependent effect of flavonoids in rat uterine smooth muscle contraction elicited by $CaCl_2$. Gen. Pharmacol. 29, 847–857.

Revuelta, M.P., Cantabrana, B., Hidalgo, A., 2000. Mechanisms involved in kaempferol-induced relaxation in rat uterine smooth muscle. Life Sci. 67, 251–259.

Rios, J.L., Recio, M.C., Giner, R.M., Manez, S., 1996. An update review of saffron and its active constituents. Phytother. Res. 10, 189–193.

Sadraei, H., Ghannadi, A., Takei-bavani, M., 2003. Effects of Zataria multiflora and Carum carvi essential oils and hydro-alcoholic extracts of Passiflora incarnata, Berberis integerrima and Crocus sativus on rat isolated uterus contractions. Int. J. Aromather. 13, 121–127.

Shamsa, A., Hosseinzadeh, H., Molaei, M., 2009. Evaluation of *Crocus sativus* L . (saffron) on male erectile dysfunction : a pilot study. Phytomedicine 16, 690–693.

Somlyo, A.P., Somlyo, A.V., 1994. Signal transduction and regulation in smooth muscle. Nature 372, 231.

Srivastava, R., Ahmed, H., Dixit, R.K., others, 2010. Crocus sativus L.: a comprehensive review. Pharmacogn. Rev. 4, 200.

Tabeshpour, J., Sobhani, F., Sadjadi, S.A., Hosseinzadeh, H., Mohajeri, S.A., Rajabi, O., Taherzadeh, Z., Eslami, S., 2017. A double-blind, randomized, placebo-controlled trial of saffron stigma (*Crocus sativus* L.) in mothers suffering from mild-to-moderate postpartum depression. Phytomedicine 36, 145–152.

Tang, F.T., Qian, Z.Y., Liu, P.Q., Zheng, S.G., He, S.Y., Bao, L.P., Huang, H.Q., 2006. Crocetin improves endothelium-dependent relaxation of thoracic aorta in hypercholesterolemic rabbit by increasing eNOS activity. Biochem. Pharmacol. 72, 558–565.

Togias, A., 2003. H1-receptors: localization and role in airway physiology and in immune functions. J. Allergy Clin. Immunol. 112, S60–S68.

Xu, G.-L., Qian, Z.-Y., Yu, S.-Q., Gong, Z.-N., Shen, X.-C., 2006. Evidence of crocin against endothelial injury induced by hydrogen peroxide in vitro. J. Asian Nat. Prod. Res. 8, 79–85.

Xu, G., Yu, S., Gong, Z., Zhang, S., 2005. [Study of the effect of crocin on rat experimental hyperlipemia and the underlying mechanisms]. Zhongguo Zhongyao Zazhi 30, 369–372.

Xu, Y.C., Leung, S.W.S., Leung, G.P.H., Man, R.Y.K., 2015. Kaempferol enhances endothelium-dependent relaxation in the porcine coronary artery through activation of large-conductance Ca(2+) -activated K(+) channels. Br. J. Pharmacol. 172, 3003–3014.

Zeinali, F., Anvari, M., Dashti, R.M.H., Hosseini, S.M., 2009. The effects of different concentrations of saffron (Crocus sativus) decoction on preterm delivery in mice. Planta Med. 75, PI29.

Zhou, C., Xiang, M., He, S., Qian, Z., 2010. Protein kinase C pathway is involved in the inhibition by crocetin of vascular smooth muscle cells proliferation. Phytother. Res. 24, 1680–1686.