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**Running Head**: Potential clinical applications of saffron

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## Abstract

*Crocus sativus* L. (Saffron) has long been known for multiple target therapeutic uses. The plant metabolism is well investigated and the main metabolites related to saffron organoleptic qualities are crocin, crocetin, picrocrocin and safranal. Particularly, the most abundant of them, such as crocin and safranal, are investigated for their multiple biological activities and known as potential drugs. We aimed to review the constituent features of the plant, along with its potential therapeutic effects in depression, neurodegenerative diseases, diabetes mellitus, atherosclerosis, cancer and sexual dysfunction. A systematic literature search was conducted in PubMed, Medline, Scopus, EMBASE, with particular attention to preclinical and clinical studies. Although saffron and its components showed potential clinical applications, further investigations are necessary to confirm the effective use of "Red Gold" and its real applications in clinical practice.

## 1. Introduction

Saffron has long been known for its medical, aromatic and coloring qualities (Betti & Schmdit 2006; Moghaddasi, 2010; Bhargava, 2011). Due to its taste and yellow-orange colour, it was used as spice in Persian, Arab, European and Indian cuisine and it is a precious ingredient for liquors, candies and food supplements (Deng *et al.,* 2002; Bathaie & Mousavi, 2010). It is considered the most expensive spice in the world, therefore nicknamed "Red Gold". The flowers are traditionally hand-picked around mid-October, at the crack of dawn. The stigmas are taken from flowers, dried and finally powdered (Bathaie & Mousavi, 2010). It is estimated that more than 90% worldwide production of saffron come from Iran and about 5-6 tons of saffron is being produced annually in the Herat region of Afghanistan (Moradi &Turhan, 2017). The production of saffron is often related to very small extension and widely distributed, with a strong geographic influence on the final quality (Anastasaki *et al.,* 2009).

Many scientific papers deal with saffron and its active ingredients (Abdullaev, 1993; Hosseinzadeh & Nassiri, 2013). Additionally, saffron potential therapeutic role in cancer, diabetes, atherosclerosis and depression is well documented (Abdullaev & Espinosa-Aguerre, 2004; Abdullaev, 2002; Ghasemi *et al.*, 2015; Shirali *et al.*, 2013; He *et al.*, 2005).

Most of the studies underline saffron antioxidant role and anti-inflammatory effects, *in vitro*, mainly due to carotenoid constituents, forming complex ligand-polynucleotides which could protect DNA and RNA from disruptive chemical reactions (Kanakis *et al.*, 2009; Boskabady & Farkhondeh, 2016; Chiavaroli *et al.*, 2017).

In order to investigate the applicative potential of saffron as therapeutic agent, a brief overview was performed focusing on depression, Alzheimer and Parkinson diseases, diabetes and sexual dysfunctions, deeply related to increased burden of inflammation and oxidative stress. For each condition, preclinical and clinical studies were analyzed and summarized, focusing on the efficacy of dried plant, extract or single active principle.

Despite being multiple mechanistic studies, only a few clinical trials proved that saffron could improve depression sympthoms and stimulate cognitive functions in patients suffering from Alzheimer's disease (Tsolaki *et al.*, 2016; Broadhead *et al.*, 2016; Farokhnia *et al.*, 2014). Due to its supposed aphrodisiac effects, saffron has also been tested in preclinical and clinical studies regarding male and female sexual disorders, mainly caused by major depression (Hosseinzadeh *et al.*, 2008). In figure 1 and table 1 are summarized the potential therapeutic applications and preclinical and clinical studies performed on saffron and its constituents.

## 2. Principal constituents of saffron

Crocus sativus L. (Saffron) is a monocotyledon specie of the Iridaceae family. Originating from south-Europe and south-West Asian region, it is widely distributed due to the high adaptability that

permitted its cultivation since a long time (Bhat & Broker, 1953).

Saffron is a perennial herb cultivated in countries with mild and dry climate, such as Iran, India, Greece, Morocco, Spain, Italy, Turkey, Pakistan, Azerbaijan, China and Egypt.

For most of its life, the plant appears only as hypogea bulb characterized by short life cycle. In less than three months the bulb opens new leaves, develops flowers and reproduces by bulb divisions.

The flowers that are marginally covered by a tunica, consist in 6 tepals perigone, whose color is varying according to the genetic variability (Lunau et al., 2016), 3 yellow stamens and a tripartite style with three stigmas of 2-3 cm connected at the base. Stigmas, being the most valuable spice appear as filaments of intense red color with fimbriate apical margins. The styles are less consistent, cylindrical, yellowish brown to pale yellowish orange color and sometimes are present in the commercial spice still connected with stigmas.

The flowering season is late summer and finishes in few weeks. During this time, every morning at the sunshine flowers are manually collected, before opening. Then, each flower is opened and the stylo and stigmas are collected pulling them out or the stigmas alone are separated cutting the stylo with fingernails. Tepals and stamens, usually known as byproducts and discarded, could have promising applicative potentialities (Menghini *et al.,* 2018). The subsequent step of drying plays a key role in the quality of final product resulting from a typical strong aromatic odor, bitter/aromatic taste and red-yellow color.

Saffron stigmas contain 14-16% water, 11-13% nitrogenous matters, 12-15% sugars, 41-44% soluble extract, 0.6-0.9% essential oil, 4-5% fibres and 4-6% ashes. Compared to other foods, saffron is particularly riched in vitamins like riboflavin and thiamine (56-138 and 0.7-4 µg/g, respectively) (Bhat & Broker, 1953; Christodoulou *et al.,* 2015).

The quality of the spice used as medicinal herb or food ingredient is mainly depending on the

presence of four metabolites, such as crocin, crocetin, picrocrocin, and safranal, that are responsible for color, bitter taste and aroma as well as for the salutistic effects of saffron (Srivastava *et al.*, 2010).

The color of saffron is mainly related to qualitative and quantitative composition of crocins, a mix of glycoside derivatives of crocetin, which represents approximately 6–16% of dry stigmas (Gregory *et al.*, 2005). The main active constituents are crocins and other carotenoid glycosides derived from crocetin, the yellow pigment of saffron (Fernández & Pandalai, 2004). Crocin and crocetin are atypical carotenoids, characterized by a 20 carbon atom chain (instead of the typical 35-40 carbon atom chain of other pigments) and two carboxylic groups, at the extremities (Fig. 2). The presence of the sugar moiety makes crocins an unusually water soluble carotenoid in nature. Crocin aglycone derives from spontaneous hydrolysis of the water insoluble crocetin further converted in different glycolsilated derivatives. The presence of other carotenoids, such as β-crocetin (monometilcrocetin), γ-crocetin (dimetilcrocetin), α- and β-carotene, lycopene, and zeaxanthin, influences the spice color (Bhat & Broker, 1953). Crocetin is considered the main antioxidant constituent, and as such it has been studied in more detail as a valid therapeutic candidate (Frank, 1961). Chemical studies on saffron described several derivatives of crocins that occur as pair of cis-trans isomers, including different saccharidic moieties, such as gentiobiosyl and glucosyl (Tarantilis *et al.*, 1994; Alavizadeh & Hosseinzadeh, 2014).

Masi and collaborators (2016) identified 25 different derivatives of crocins for a total quantity of 302-548 mg/g dry stigmas, depending of geographic origin. Picrocrocin (4-β-hydroxy-cyclocitral glucoside) is responsible for the saffron bitter taste (Buchecker & Eugster, 1973) and ranging between 1 and 13% of dry matter, represents the second most abundant metabolite (Masi *et al.*, 2016; Alonso *et al.*, 2001). The stigma contains also an essential oil resulting in a complex mixture of more than 160 volatile components (Carmona *et al.*, 2007; Kanakis *et al.*, 2004), mainly

characterized by safranal (up to 70% of essential oil and up to 2% of dry stigmas) (Masi *et al*, 2016; Maggi *et al.,* 2009), which is responsible for the aromatic note of saffron (Buchecker & Eugster, 1973). Safranal is a cyclic terpenic aldehyde (2,6,6-trimetil-1,3-cyclohexadiene-1-carboxaldehyde) usually not present in fresh plant. During the drying phase it derives from oxidation of the picrocrocin aglycone 4-β-hydroxy-cyclocitral. Fig. 2 shows the structures of crocin, safranal and crocetin.

From zeaxanthin, via enzymatic activity derive both the water insoluble crocetin-dialdehyde and picrocrocin (Bouvier *et al.,* 2003). From crocetin derives the complex mixture of water soluble cis- and trans-crocin glycoside esters, responsible for dyeing property of stigmas. On the other hand, from picrocrocin, derives safranal, the most relevant metabolite present in the volatile fraction. The conversion of picrocrocin in safranal is mainly related to the drying process and influenced by temperature or alkaline-acid hydrolysis (Carmona *et al.,* 2006; Himeno & Sano, 1987; Iborra *et al.,* 1992).

It has been demonstrated that optimal drying temperature for safranal generation is above 80°C (Gregory *et al.,* 2005). According to this pathway the amount of the precursor zeaxanthin is critical for quality of spice, at least from phytochemical point of view. The bitterness of spice is directly dependent on the amount of zeaxanthin metabolized, as well as the formation of crocetin, and inversely related to the aromatic note of spice. Moreover, the ratio between aroma and bitter is strictly influenced by drying process (Carmona *et al.,* 2005).

The presence of multiple metabolites derived from the same metabolic pathway related to the quality of final product imposes to reconsider the classical phytochemical rule which identifies in high amount of active principles the high quality product. In the network of saffron metabolites the highest amount of ones could not be the optimal condition, because it is surely balanced by the less presence of another. In this context, we could imagine the biosynthetic pathway as a complex of two

balances. The biggest balance, having fulcrum in the metabolism of zeaxanthin, modulates equilibrium between the bitterness and dyeing. At the bitterness arm a second balance is fixed compensating, in function of drying process, the bitterness (picrocrocin) for aromatic properties (safranal). At the dyeing arm is fixed a third balance modulating the equilibrium between the antioxidant/crocetin and mixture of water soluble pigments (crocins).

The best quality saffron, particularly if related to the organoleptic properties, could be identified not to the highest amount but to the optimal equilibrium between all metabolites. In this context, some traditional procedures applied to the production of saffron spice could result more efficient than modern and standardized process. For example, Italian saffron PDO “Zafferano dell’Aquila” is produced following a codified drying process with sieve disk directly on wood embers. This process that could be considered toasting rather than drying is completely handmade and coupling the experience of operator to the short time thermal shock allows to obtain a high quality balance between color, bitter and aroma, as confirmed by ISO/Technical Specification (TS) 3632-1/2 standards (2003).

Chemical composition of crocins, picrocrocin, and safranal providing the unique color, taste and flavor in Saffron is the most important indicator of its quality and commercial value in agreement with ISO/TS 3632-1/2 (2011; 2010). The reference standard extraction process is with water, at room temperature, at plant/solvent ratio 0.5 mg/ml (ISO/TS 3632-1/2, 2003).

The poorness of saffron agronomic yield (to obtain a kilo of dried stigma are necessary over 150.000 flowers) along with the very laborious and handmade production determine the high cost for final buyer and the fame of the most expensive spice. Since the long time use adulteration and sophistication are well known and methods for plant identity determination are well defined, of increasing interest is the chemical approach as guarantee of geographical origin. In particular, a type of adulteration, including total substitution of saffron constituents by well known adulterants such

as tartrazine and sunset yellow along with other components including propan-1,2-diol, propan-2-ol, triacetin and free saturated fatty acids, was recently found (Ordoudi et al., 2017).

The volatile fractions of saffron from different origin were analyzed via an electronic nose and GC–MS (Carmona et al., 2006) as well as chemometric approach was applied to quantitative determination of crocins, picrocrocin and safranal by RP-HPLC-DAD and NIR (Nescatelli et al., 2017) and mineral elements were analyzed by inductively coupled plasma-mass spectrometry (D'Archivio *et al.,* 2014) as qualitative and quantitative markers for saffron traceability. On the basis of the correlation between saffron color and place of production an alternative method based on image processing technology was proposed for a qualitative standard definition and spice authentication (Kiani & Minaei, 2016).

Other compounds are identified and could be at least in part, related to the pharmacological activities of plant and extracts, such as flavonoids, mainly kaempferol-glycosides representing 10-16% of dry weight (Masi *et al.,* 2016) and other phenols in lower amount less than 0.4% (Menghini *et al.,* 2018).

## 3. Potential therapeutic applications of saffron

### 3.1. Depression

Clinical studies have demonstrated that saffron hydro-alcoholic extract at the dose of 20-30 mg/day twice daily, compared to fluoxetine and imipramine, has improved mild and moderate depression (Akhondzadeh *et al.,* 2004, 2005, 2007; Noorbala *et al.,* 2005). More recently, Talaei and collaborators (2015) reported that 30 mg/day for 4 weeks of crocin significantly improved mood in patients with major depression, but the sample size and short term of the clinical trial limit the scientific value of these results. In a double-blind, in parallel, randomized, placebo-controlled clinical trial, the efficacy of a novel saffron stigma standardized extract has been evaluated in mood

disorders. The treatment with 28 mg/day of the extract improved, as reported by self evaluation, mood, stress, anxiety and sleep quality in healthy adults (Kell *et al.*, 2017). The administration of crocin (30 mg per day for 8 weeks) reduced the symptoms of depression in subjects with metabolic syndrome (Jam *et al.*, 2017), while treatment with saffron (15 mg/day or 30 mg/day) improved the depressive symptoms in post-menopausal healthy women (Kashani *et al.*, 2018). A limited number of clinical evidences indicate that saffron supplementation could improve symptoms of depression; however the small size studies, protocol heterogeneity and small number of patients limit reproducibility and comparability between the studies.

The antidepressant effects of saffron are attributed to safranal and crocins, because they inhibit neuronal reuptake of dopamine (DA), serotonin (5-HT) and norepinephrine (NE) (Hosseinzadeh *et al.*, 2004; Khazdair *et al.*, 2015). In addition, monoamine oxidase (MAO) inhibitory properties of crocin and safranal were also evaluated in order to assess their influence on catecholamine and 5-HT levels in the brain. In particular, crocin was demonstrated to be a non-competitive inhibitor of the human MAO-A and MAO-B in the micromolar range, by means of binding to allosteric sites on the enzyme, whereas safranal was inactive toward both isoforms (De Monte et al., 2014). Moreover, *in vivo* studies suggested that crocin has antidepressant-like effect by increasing cAMP response element binding (CREB), brain-derived neurotrophic factor (BDNF) and nerve growth factor inducible (VGF) levels in rat hippocampus (Vahdati Hassani *et al.*, 2014; Ghasemi *et al.*, 2015; Razavi *et al.*, 2017).

### 3.2. Alzheimer's and Parkinson's diseases

Extracts of saffron and its components have been tested as adjuvant treatment in Alzheimer's and Parkinson's diseases (Ahmad *et al.*, 2005; Akhondzadeh *et al.*, 2010a, 2010b; Pitsikas *et al.*, 2015). Alzheimer's disease is characterized by a pathological deposition of β-amyloid peptide (Aβ) plaques in the brain and neurofibrillary clusters. Different authors reported the antioxidant and anti-

apoptotic properties of saffron and its constituents (Hatziagapiou *et al.*, 2018; Inoue *et al.*, 2018). Other mechanisms have been reported in the neurodegenerative disease (Nam *et al.*, 2010). In this context, the inhibitory effect of crocins on acetylcholinesterase activity (Geromichalos *et al.*, 2012) and the reduction of various proinflammatory and neurotoxic factor levels (Finley *et al.*, 2017) have been described. The antioxidant properties of stigma extracts of saffron and its effects on the Aβ1-40 fibrillogenesis have been evaluated in a double-blind, randomized study performed on 46 patients with Alzheimer's disease (Akhonzadeh *et al.*, 2010a). Group treated with capsules containing 15 mg of saffron, twice a day for 16 weeks, showed improvement of cognitive functions compared to placebo. In another study (Akhondzadeh *et al.*, 2010b), including 54 patients with mild-to-moderate Alzheimer's disease, the same dosage of saffron was compared to donepezil, 10 mg/day. After 22 weeks, both treatments showed a comparable decrease of the psychometric measures, including the Alzheimer's Disease Assessment Scale (ADAS) and Clinical Dementia Rating Scale (CDR). Incidence of side effects was comparable in the two treatment groups, while vomiting was associated with the donepezil treatment. Furthermore, a randomized single-blind 12 month study showed that saffron improved the cognitive decline in 17 participants with amnesia (Tsolaki *et al.*, 2016). Moreover, saffron proved comparable to memantine in reducing cognitive decline in patients with moderate to severe Alzheimer's disease (Farokhnia *et al.*, 2014). However, confirmatory studies with larger sample sizes and longer follow-up are necessary.

Pan and collaborators (2016), in an *in vitro* model of Parkinson's disease, demonstrated that safranal could act as preventive agent against rotenone-induced oxidative stress and apoptosis by regulating Keap1/Nrf2 signaling pathway. Ahmad and collaborators (2005) have studied the therapeutic potential of crocetin in a rat model of Parkinson's disease induced by 6-hydroxydopamine. Histopathological analysis in the substantia nigra suggested a neuroprotective effect of crocetin.

Finally, crocin was found to improve the Parkinson-like behavioral deficit in rat (Mohammadzadeh *et al.*, 2018).

On the other hand, different reports described neuroprotective effect of saffron, also confirmed in age related macular degeneration, a retinal neurodegenerative disease. Oxidative stress and chronic inflammation play a significant role in the pathogenesis of age related macular degeneration (Bisti *et al.*, 2014). Actually, although the action ways are still under investigation, a direct antioxidant activity, with a reduction of the oxidative stress related to the initiation and progression of the disease, exerted by various saffron components has been hypothesized (Bisti *et al.*, 2014).

The saffron neuroprotective effect could be, at least in part, justified from the pharmacokinetics of its components.

### 3.3. Diabetes mellitus

Diabetes mellitus is a metabolic disorder characterized by hyperglycemia, and increased production of reactive oxygen species (ROS). Oxidative stress, due to an imbalance between the production of ROS and antioxidant mechanisms, plays a key role in the pathogenesis of diabetes (Bonnefort-Rousselot, 2002; Rajaei *et al.*, 2013). Diabetes has long been associated with other comorbidities, including psychiatric and degenerative diseases (Herder *et al.*, 2018; Fei *et al.*, 2013; Wang *et al.*, 2017). Recently, we observed that saffron high quality by-product extracts were able to exert significant protective effects *in vitro*, including inhibitory enzyme activity effects on α-glucosidase, α-amylase and cholinesterases (Menghini *et al.*, 2018).

Accordingly, *in vivo* studies have demonstrated that crocins induce anti-oxidant effects in diabetic rats (Kianbakht & Hajiaghaee, 2011; Altinoz *et al.*, 2015; Naghizadeh *et al.*, 2014). In addition, crocetin seems to increase insulin secretion from pancreatic β-cells, therefore lowering blood glucose levels (Shirali *et al.*, 2013; Assimopoulou *et al.*, 2005). Kang and colleagues (2012)

reported that saffron strongly increased glucose uptake and insulin sensitivity in muscle cells and enhanced the phosphorylation of AMP-activated protein kinase (AMPK)/acetyl-CoA carboxylase (ACC) and mitogen-activated protein kinases (MAPKs ), but not phosphatidylinositol 3-kinase/Akt (PI 3-kinase/Akt), *in vitro.*

In a randomized, single-blind, placebo-controlled clinical trial, including 208 patients with type 2 diabetes mellitus, saffron has been associated with cinnamon, cardamom and ginger. Supplementary herbals showed significant beneficial effects on cholesterol, but not glycemic control, oxidative stress and inflammation (Azimi *et al.*, 2014). On the other hand, saffron hydroalcholic extract was found to improve blood glucose control by decreasing fasting blood sugar in 54 type 2 diabetes mellitus patients (Milajerdi *et al.*, 2018). Based on contradictory results reported in *in vitro* and *in vivo* studies, the effects of saffron in diabetic patients should undergo further investigations.

### 3.4. Atherosclerosis

*In vivo* studies have shown that saffron lowers blood pressure and serum cholesterol levels, therefore improving the clinical course of atherosclerosis and cardiac ischemia (Imenshahidi *et al.*, 2010; Gainer, 1993; Huang *et al.*, 2016). One of the key points in atherosclerosis pathogenesis is the adhesion and migration of leukocytes beyond endothelial cells; advanced glycation end-products (AGEs) promote the expression of vascular cell adhesion molecule 1 (VCAM-1), intercellular adhesion molecule-1 (ICAM-1) and atheroma formation. In a study on bovine endothelial cells, Xiang and colleagues (2006) found that crocetin suppresses AGEs and consequently leukocyte migration and ICAM-1 expression (Xiang *et al.*, 2006), while other authors point to protective effects of crocin in the endothelium (Xu *et al.*, 2007; Alavizadeh &Hosseinzadeh, 2014).

Furthermore, crocin decreases the external cholesterol in macrophages and inhibits the uptake of oxidized low density lipoproteins (OX-LDL), therefore reducing the formation of foam cells which

are involved in the pathogenesis of atherosclerosis (He *et al.*, 2005).

In addition, *in vivo* studies (Christodoulou *et al.*, 2014) have demonstrated that the administration of watery extracts of saffron, in transgenic mice for ApoE, reduces atherosclerotic plaques by modifying their composition. Saffron improves the stability of the atherosclerotic plaque (Christodoulou *et al.*, 2018). Safranal has also shown important cardiovascular effects (Kamalipour *et al.*, 2011). *In vivo* studies have demostrated that saffron is able to decrease myocardial damage in a model of ischemia-reperfusion injury, increasing the phosphorylation of Akt/GSK-3b/eNOS and reducing the expression of IKK-b/NF-KB (Bharti *et al.*, 2012; Fatehi Hassanabad *et al.*, 2004; Hosseinzadeh & Sadeghnia, 2005; Rezaee & Hosseinzadeh, 2013).

### 3.5. Cancer

Clinical studies aimed to evaluate the potential effects of saffron on human cancer, either alone or in association with conventional anticancer therapy, have not been performed, yet. Recent preclinical data, both *in vivo* and *in vitro*, have shown that saffron extracts and its main active components could have anticancer effects. Saffron has been reported to have inhibitory effects on tumor cells (Garcia-Olmo *et al.*, 1999; Nair *et al.*, 1991; Escribano *et al.*, 2000; Geromichalos *et al.*, 2014; Samarghandian *et al.*, 2014).

Saffron extracts, either topically or orally/intravenously administered, showed excellent results against skin tumors. In particular, an *in vivo* study by Das and collaborators (2010) showed an improvement in skin structure in saffron-treated mice, in skin carcinoma. Additionally, both saffron watery extract and crocetin inhibit the progression of gastric tumors in a dose-dependent manner, *in vivo* (Bathaie *et al.*, 2013). Furthermore, in preclinical studies saffron was found to induce cellular apoptosis in colorectal, pancreas and bladder tumors (Sun *et al.*, 2013; Bathaie *et al.*, 2013; Bakshi *et al.*, 2010).

The antitumor effects of saffron could involve several mechanisms. Saffron and its components might have inhibitory effects on the DNA and RNA synthesis (Nair *et al.*, 1991, 1995; Smith, 1998). Another possible anticancer mechanism, due mainly to carotenoids, is related to inhibition of free radical chain reactions (antioxidant effect) (Abduallev & Frenkel; 1999; Molnár *et al.,* 2000), and it has been reported the role of crocetin in decreasing lipid peroxidation in lung cancer (Inoue *et al.,* 2005). The cytotoxic effect of carotenoid could be linked to their interactions with topoisomerase II, a regulatory enzyme involved in DNA supercoiling in the replication cycle (Molar *et al.,* 2000).

### 3.6. Sexual dysfunction

Erectile dysfunction affects 10-52% of men between 40 and 70 years old (Porst, 2004) and is associated with a wide range of organic and psychogenic conditions, including hypertension, hypercholesterolemia, cardiovascular disease, and depression (Burnett, 2006).

Vasodilatation mediators, such as nitric oxide (NO), released by nervous system and endothelial cells in the corpora cavernosa of the penis are key components of erection mechanisms. NO activates soluble guanylyl cyclase, which increases 3',5'-cyclic guanosine monophosphate (cGMP) levels. Acting as a second messenger molecule, cGMP regulates the activity of calcium channels as well as intracellular contractile proteins affecting the relaxation of vascular smooth muscle (Yafi *et al.,* 2017). Phosphodiesterase-5 inhibitors (sildenafil, vardenafil and tadalafil) inhibit cGMP hydrolysis, inducing vasodilation (Montorsi *et al.,* 2003; Montague *et al.,* 2004). Phosphodieterase-5 inhibitors are associated with unpleasant side-effects, such as headaches, flushing, dyspepsia, nasal congestion, visual and cardiac disturbances (Porst, 2004). Minor therapeutic approaches in sexual dysfunction involve central nervous system stimulation through apomorphine (Heaton, 2000; Montorsi *et al.,* 2003) or medical herbs (Dhawan *et al.,* 2003; Tharakan *et al*, 2005).

Saffron has long time considered an aphrodisiac drug (Madan, 1966). Preclinical and clinical studies support saffron as a treatment for male and female sexual disorders, particularly if related to major depression (Hosseinzadeh *et al.,* 2008). On the other hand, administration of antidepressants such as serotonin reuptake inhibitors (SSRIs) is associated with sexual disorders and it has been observed that the use of saffron improves sexual disorders induced by fluoxetine and other SSRIs (Modabbernia *et al.,* 2012). Accordingly, saffron (30 mg/day) for 4 weeks was found to improve some of sexual problems induced by fluoxetine, including arousal, lubrification and pain in 38 women with major depression (Kashani *et al.,* 2013). Hosseinzadeh and collaborators (2008) reported that crocins improve sexual behavior in rodents; crocetin has been shown to induce endothelium-dependent relaxation by increasing eNOS activity (Tang *et al.,* 2006). Other studies show that crocins could inhibit extracellular $Ca^{2+}$ influx, and intracellular $Ca^{2+}$ release from deposits of endoplasmic reticulum (He *et al.,* 2004), which contributes to relaxation of the corpus cavernosum smooth muscle with consequent erection (Williams *et al.,* 2005).

Although preclinical data support this hypothesis, inconsistent results emerged from two clinical trials performed to evaluate the efficacy of saffron on erectile dysfunction (Shamsa *et al.,* 2009; Safarinjad *et al.,* 2010). In a pilot study conducted by Shamsa and collaborators (2009), 20 patients were treated with 200 mg saffron tablets/day. After 10 days, overall positive effects were observed with a significant increase in erectile and orgasmic functions such as sexual desire and general satisfaction. On the other hand, Safarinejad and collaborators (2010) emphasize the lack of beneficial effects on sexual dysfunction.

Cai and collaborators (2013), compared the effects of Serenoa repens and a commercial formula containing Serenoa, Pinus massoniana and Saffron on 129 patients with lower urinary tract symptoms related to benign prostatic hyperplasia in a prospective, multicentre, phase 3 study. Clinical, instrumental analyses and questionnaires were used to measure the improvement of quality

of life at the end of the study. The commercial formula was found to significantly improve the quality of life of patients, specifically in terms of sexual function.

Additionally, the same extract mixture also exerted protective effects in an *in vitro* pharmacological model consisting in isolated rat prostate specimens challenged with an inflammatory stimulus (E. Coli lipopolysaccharide) (Chiavaroli *et al.*, 2017).

### 3.7. Other potential therapeutic uses

Other various possible therapeutic uses of saffron and its constituents have been reported. In particular, anti-anxiety, anticonvulsant and hypnotic effects of C. sativus L. (Hosseinzadech & Noraei, 2009; Pitsikas *et al.*, 2008; Hosseinzadeh & Talebzadeh, 2005) have been described. Pitsikas and collaborators (2008) have shown that crocin, at the dose of 50 mg/kg, induces anti-anxiety effects in rats. Furthermore, Hosseinzadeh and Noraei (2009) observed that safranal can bind to benzodiazepine (BZ) receptor subtypes BZ1, BZ2 and BZ3, and therefore improve insomnia in mice. A wide body of evidence suggests that saffron could play a pivotal role in the treatment of different digestive system disorders, including gastric ulcer, hepatitis without jaundice, inflammatory necrotic hepatitis, liver cirrhosis, ulcerative colitis (Khorasany & Hosseinzadeh, 2016).

## Conclusion

In summary, saffron and its components show significant pharmacological properties and could exert potential therapeutic effects in a wide spectrum of diseases, such as depression, atherosclerosis, diabetes mellitus, neurodegenerative diseases, cancer and sexual dysfunction. Despite there being promising preclinical results, further investigations are necessary to confirm the effective use of “Red Gold” and its possible applications in clinics.

**Legends**

Figure 1. Potential therapeutic applications of Saffron.

Table 1. Studies performed on *Crocus sativus L.* (Saffron) and its constituents.

Figure 2. Chemical structures of crocin (**1**), safranal (**2**) and crocetin (**3**).

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