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Potential Healthy Effects of Saffron Spice (*Crocus sativus* L. Stigmas) Consumption

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ABSTRACT

Saffron (*Crocus sativus* L.), has been used since ancient ages in food for its flavouring, aromatic and colouring properties but also for its biomedical activity. In the past years many efforts have been made in order to demonstrate scientifically the healthy effects attributed to saffron consumption since Dioscorides' time. More than 400 papers have been published in the last decade related to antioxidant properties, cancer, neuronal injury and sedative effect, among others. It has been found that its antioxidant activity is the major responsible for many of the properties that helps to prevent or diminish some diseases. But the majority of these research use animals, making difficult to understand the human application. In this review, a first attempt to translate animal doses to human intake when saffron is included on the diet is carried out, in order to make an estimation of the potential healthy effects in humans.

Keywords: antioxidant properties, *Crocus sativus* L., healthy effects, human equivalent doses, saffron intake
Abbreviations: b.w., body weight; **BSA**, body surface area; **HED**, human equivalent dose; **MI**, myocardial infarction; **PMS**, premenstrual syndrome

CONTENTS

INTRODUCTION

Since ancient ages, spices have placed a major role in cooking, cosmetics, perfumery, global exploration, economics and medicine (Dog 2006). Saffron (*Crocus sativus* L.), is an example of a multi-purpose spice widely used for many centuries. Starting in Mesopotamia, where saffron was used in religious celebrations and for curative purposes; continuing with Phoenicians, where used it to dye cloths, and in ancient Rome, used as a treatment and dye, as well as in perfumes and ointments (Giaccio 2004; Carmona *et al*. 2006). Also used by Cleopatra (69-30 B.C.), it was a cosmetic, phitotherapy and a nail, hair and lips dye. Healing properties of saffron are well known since ancient times, as said by Dioscorides Pedacio, a Greek medical practitioner of the first century, who considered it as sexual stimulant, anti-inflammatory and as a drunkenness impediment. Since then, saffron has been considered as anodyne, antidepressant, a respiratory decongestant, antispasmodic, aphrodisiac, diaphoretic, emmenagogue, expectorant and sedative, among others (Abdullaev and Espinosa-Aguirre 2004).

Recently, research on saffron properties has covered a great interest, demonstrated by the increase of the number of publications in scopus and science direct databases, as shown in **Fig. 1**, where it can be observed the exponential augment, especially from 1996. Approximately, every 2 years, publications duplicate its number, being in 2009 about 5 times more than in 2000. Many reviews have been published in the past recent years (Deng *et al*. 2002; Abdullaev and Espinosa-Aguirre 2004; Schmidt *et al*. 2007; Soeda *et al*. 2007; Kianbakht 2008), but some properties of saffron have been particularly investigated, as seen in **Fig. 2**, where antioxidant, nervous system damage and cancer properties cover a great number of publications, almost 3 to 5 times more than the rest, follow by cardiovascular injury and antinociceptive effects.

The current paper provides an overview of saffron investigations on its biological activity and diseases preven-

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Invited Review

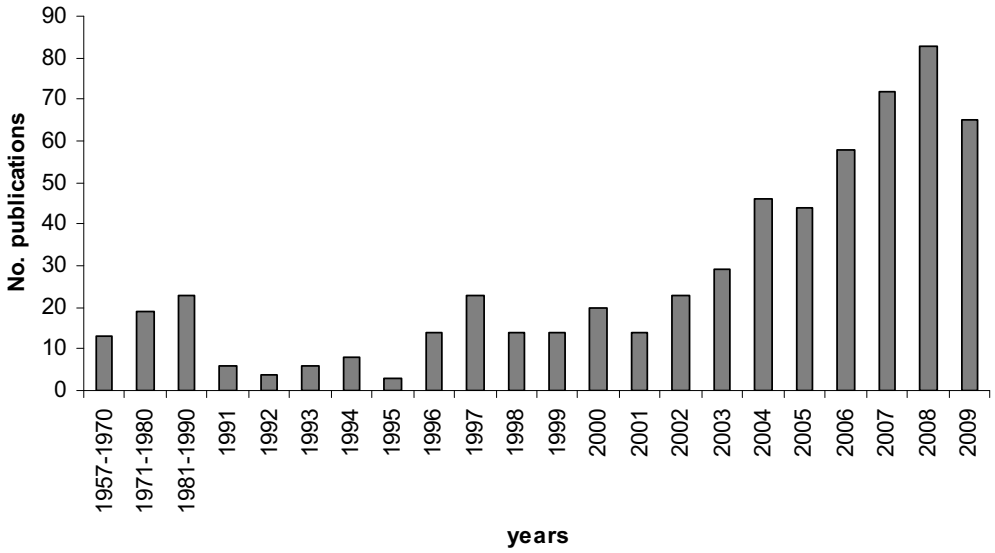


Fig. 1 Biomedical properties of saffron publications during the past years (based on scopus.com and sciencedirect.com).

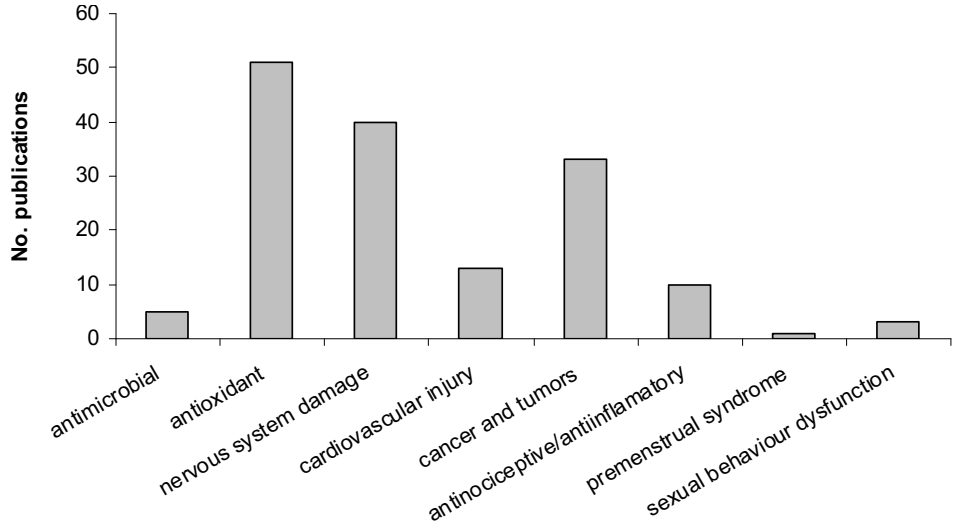


Fig. 2 Principal saffron properties investigated during the past decade (based on scopus.com and sciencedirect.com).

tion during the past decade. In addition, an attempt to relate saffron consumption with its potential healthy benefits when added to different food, so that, the effects found to be effective on animals, will be estimated in humans.

BIOLOGICAL ACTIVITIES OF SAFFRON

The main biological activity of saffron is based on its great antioxidant ability; in fact the antioxidant properties of saffron are well known and have been widely studied since this property is responsible for many of its biomedical attributes. A radical scavenging activity is involved in aging processes, anti-inflammatory, anticancer and wound healing activities, among others (Assimopoulou *et al.* 2005), so many efforts have been made in order to find natural products that posses this property. Assimopoulou *et al.* (2005) suggests that saffron could be used in functional foods, drinks with antioxidant activity and in pharmaceutical and cosmetic preparations, as well as, food supplement with antioxidant properties. Saffron extracts exhibited a remarkable intracellular antioxidant activity. Moreover, the antioxidant efficiency observed in ethanol saffron extracts was equivalent to 116 mg α-tocopherol/g (Chen *et al*. 2008). So that, it can be assumed that this property is responsible for preventing many diseases which mechanisms involve oxidation, such as neurodegenerative injury (Urrutia *et al*. 2007) and cardiovascular diseases, which are described below and injury in kidney or brain tissues caused by ischemia-reperfusion (I/R) (Hosseinzadeh *et al*. 2007b). In addition, treating thermal induced burn wounds with saffron extract cream (20%) result in a significantly increased re-epithelialization that could be explained for the antioxidant effects of this spice (Khorasani *et al.* 2008).

Other important property which converts saffron in a beneficial spice for health is their antimicrobial activity. This one has been studied under different saffron parts; it is well known that many spices such as garlic and basil are antibacterial agents (Low Dog 2006). Ethyl acetate extracts of stigma, stamen and leaves were tested on *Sthaphylococcus aureus*, *Staphylococcus epidermis*, *Escherichia coli*, *Micrococcus luteus*, *Candida albicans*, *Cladosporium* sp. and *Aspergillus niger*, finding that the leave extract did not show antimicrobial activity at a concentration of 100 mg/ml. The antifungal activity of stigma was higher than stamen; in contrast, the antibacterial activity of stamen was higher than the rest of the parts studied (Vahidi *et al*. 2002).

On the other hand, the anti-*Helicobacter pylori* activity of saffron extracts, safranal and crocin was investigated using aqueous and methanol extracts and four antibiotics as control. All isolates were susceptible to methanol and aqueous saffron extracts, being the minimum inhibitory concentrations of methanol saffron extract, crocin and safranal 677, 26.5 and 16.6 μg/ml, respectively (Nakhaei *et al*. 2008). In other series of studies were determined other antiulcer properties of saffron, suggesting that saffron inhibits gastric acid secretion and stimulates mucus secretion which is a

barrier to prevent damage (Al-Mofleh *et al*. 2006). In this work saffron extracts at 250 mg/kg b.w. produce a significant decrease in the volume of gastric secretion and ulcer index in the animals tested. In addition, Kianbakht and Mozaffari (2009) studied the effects of pretreated rats with saffron extract (25, 100 and 250 mg/kg b.w.), crocin (2.5, 5 and 10 mg/kg b.w.) and safranal (0.25, 2 and 5 ml/kg b.w.), finding that these extracts, prevent gastric lesions, increase lipid peroxidation and decrease glutathione levels induced by indomethacin, effects that are comparable to omeprazole, an inhibitor protons pump, which is used as an antiulcerogenic agent.

These properties of saffron could be applied as possible therapeutic agent for a several diseases as demonstrated by several biomedical studies.

BIOMEDICAL STUDIES WITH SAFFRON

Nervous system damage

1. Neuronal injury

Saffron and its constituents: crocetin glycosides and picrocrocin were demonstrated to cause protective effects on neuronal injury acting as an antioxidant. Crocin, among the rest of the components, results the most potent antioxidant, capable of combating ischemic stress-induced neuron death (Saleem *et al*. 2006; Ochiai *et al*. 2007). In addition, 727.5 mg/kg b.w. of safranal in rats showed protective effects on hippocampal tissue from rats under ischemic conditions, elevating antioxidant capacity of the hippocampus (Hosseinzadeh and Sadeghnia 2005). Ochia *et al.* (2004) suggest that crocin inhibits apoptosis in a model cellular for neuronal differentiation, PC-12 cell line, and combats the serum/glucose deprivation-induced ceramide formation in PC-12 cells by increasing glutathione (GSH) levels and preventing the activation of a pathway for neural cell death.

2. Diabetic neuropathy

Diabetic neuropathy is one of the most frequent complications of diabetes. Vascular and neural diseases are closely related; in fact microvascular dysfunction occurs together with the progression of neural dysfunction. Neuronal ischemia is a well-established characteristic of diabetic neuropathy. The mechanisms of neurotoxicity from high glucose levels are poorly understood, but an increase on reactive oxygen species has been proposed as possible mechanism. Saffron, as antioxidant, can have neuroprotective effects. Saffron extracts and crocin were studied in glucose-induced neurotoxicity, using PC12 cells as a suitable *in vitro* model of diabetic neuropathy, showing that saffron extract (5 and 25 mg/ml) and crocin (10 and 50 μM) could decrease the toxicity caused by glucose, suggesting that saffron and crocin could be potentially useful in diabetic neuropathy treatment (Mousavi *et al.* 2009).

3. Retinal function

Recently, it was published by Maccarone *et al*. (2008) that saffron and carotene extracts (1 mg/kg b.w. /d) as feed supplementation in rats, mitigates retinal damage induced by exposure to continuous bright light (1000 lux) during 24 hrs. They mentioned that the antiapoptotic characteristic of saffron makes it interesting in the treatment of retinal neurodegenerative disease; moreover, it reduces photoreceptor death induced by environmental stresses. In another study using retinal cell cultures from bovine and primate eyes, crocin protected the photoreceptors against blue light or white light-mediated damage in a concentration dependent manner (10–160 μM) (Laabich *et al.* 2006). Finally, saffron can significantly inhibit the elevation of glutamic acid concentration, fact that contributes to neurodegeneration of retina, thus, prevents retina damage (Yang X-G *et al.* 2006). For a different type of retinal malfunction, such as ischemic retinopathy and age-related macular degeneration, which are the leading ocular diseases that cause blindness, it has been studied that crocin analogs increase the blood flow in the retina and choroid and facilitate retinal function recovery, leading to the conclusion that crocin analogs could be used to treat this problem (Xuan *et al.* 1999).

4. Alzheimer's disease

Alzheimer's disease is the most common form of dementia among people over 65 years old which is characterized by cognitive impairment and memory deterioration, promoted by deposition of amyloid *β*-peptide (A*β*) fibrils that is caused by oxidation. Thus, to identify agents inhibiting the pathogenesis of Alzheimer's disease, the antioxidant properties of *C. sativus* were examined on A*β* fibrils and compared with that of tomato and carrot by Papandreou *et al*. (2006). The results showed that saffron extracts at concentrations of 300 and 600 μg/ml had twice the antioxidant activity than tomato and carrot extracts. In addition, *C. sativus* stigmas extract significantly inhibited the formation of amyloid fibrils in a concentration and time-dependent manner. In conclusion, the study resulted to demonstrate that saffron extract has antioxidant and antiamyloidogenic activity; as a result, it has a positive effect on cognitive function, indicating that saffron may be valuable for prevention or delay of Alzheimer's disease. Recently, a clinical trial with 54 patients of 55 years old or older, with mild-to-moderate Alzheimer's disease, using a saffron capsule of 30 mg/day, provides preliminary evidence of saffron possible therapeutic effect (Akhondzadeh *et al.* 2010).

5. Parkinson's disease

Parkinson's disease is a terminal, progressive neurodegenerative disorder. A cure has not been developed yet, so many efforts for relief the symptoms have been done. The causes of the disease are marked by generation of excessive free radicals but the exact mechanism is still unclear (Ahmad AS *et al.* 2005; Ahmad M *et al.* 2005; von Bohlen und Halbach *et al.* 2005). The neuromodulatory effects of crocetin (75 μg/kg b.w.) were studied resulting in a neuronal protection from a catecholaminergic neurotoxin that causes loss of cells in the substantia nigra (Ahmad AS *et al.* 2005), mechanism that could be helpful for reducing Parkinson.

6. Seizures

Since traditional medicine, saffron has been used as anticonvulsant agent, but its mechanisms of action deserve further study. Seizures are produced when neurons are activated in an unusually synchronous manner, disturbing the balance between excitation and inhibition and altering several classic neurotransmitters systems such as the glycine, glutamatergic and GABAergic (Engelborghs *et al.* 2000). Depressant effects on the central nervous system are at least partly responsible for inhibiting the alterations mentioned above. Given that the current therapeutic treatment using antiepileptic drugs is associated with side-effects, plants, such as saffron, would be helpful in this treatment, as shown by Hosseinzadeh and Khosravan (2002), who found that ethanolic (0.2-2.0 g/kg b.w.) and aqueous (0.08-0.80 g/kg b.w.) saffron extracts increased the latency of convulsions induced by pentylenetetrazol (PTZ), a popular chemoconvulsant, in a dose-dependent manner and decreased the duration of tonic seizures caused by electroshock. Safranal (0.15-0.35 mg/kg b.w.) showed anticonvulsant behaviour as well, in PTZ-induced seizures (Hosseinzadeh and Talebzadeh 2005). Besides, Hosseinzadeh and Sadeghnia (2007) studied deeply these properties of safranal showing that peripheral administration of safranal (72.75, 145.5 and 291 mg/kg b.w.) exerts a dose dependent decrease in minimal clonic seizure (MCS) induced by PTZ and first generalized tonic-clonic seizures (GTCS). The exact mechanisms of saffron action are unclear yet.

7. Learning behaviour

Several studies have reported that saffron extracts and two of its main ingredients crocin and crocetin, improved memory and learning skills in ethanol-induced learning behavior impairments in mice and rats (Sugiura *et al.* 1994; Abe *et al.* 1999; Abe and Saito 2000), suggesting that oral administration of saffron may be useful as treatment for neurodegenerative disorders and related memory impairment. Recently, rats treated with 30 and 60 mg/kg b.w. of saffron extracts were capable of discriminate between familiar and novel objects (Pitsikas and Sakellaridis 2006), finding the enhancing effects of crocetin esters on memory and its implication in the mechanisms underlying recognition and spatial memory (Pitsikas *et al.* 2007).

8. Anxiety

The traditional therapeutic potential of crocetin esters in anxiety was investigated using a light/dark chamber test in rats. The results showed that crocin at 50 mg/kg b.w. reduced the anxiety of animals but the mechanism that might account for this effect was not determined (Pitsikas *et al.* 2008). In addition, the anxiolytic and hypnotic effects of saffron (56, 80, 320 and 560 mg/kg b.w.) and safranal (0.15 and 0.35 ml/kg b.w.) were similar to diazepam, which is used in pharmacology as a sedative. Safranal was confirmed as anxiolytic in a dose-dependent manner (Hosseinzadeh and Noraei 2009).

9. Sedative/relaxant

The sedative effects of saffron are well known since traditional medicine, and it was confirm by Boskabady and Aslani (2006). Aqueous-ethanolic saffron extract (0.15-0.6%g) and safranal (0.15-0.60 ml containing 0.2 mg/ml solution) showed a potent relaxant effect that is comparable or even higher than theophylline, a relaxing drug. To corroborate the mechanism of action, another study was published, suggesting that relaxation is due to saffron stimulatory effects on β-adrenergic receptors being superior to its agonist's available (Nemati *et al.* 2008). β-adrenoceptors agonists, such as saffron, stimulate the liver, kidneys, increase heart rate and heart contractility rate (Boskabady *et al.* 2008), vasodilatation due to petals (Fatehi *et al.* 2003) and bronchodilation, to which can be attributed the proven antitussive effect of safranal and ethanolic extracts of saffron stigma (Hosseinzadeh and Ghenaati 2006). Relaxant properties of saffron could be useful for treating different conditions described below.

10. Depression

Herbal treatments, including saffron, as antidepressant agents have been widely studied. There is strong evidence that, stigmas, petals, safranal and crocetin esters of saffron exert an antidepressant activity. Since a few years ago, efforts has been made by some research groups, especially in Iran, in order to know the doses of the different extracts that can be useful to treat this disorder. It was observed during 6 weeks 30 patients, that if saffron (30 mg/day) is compared with imipramine (100 mg/day), a antidepressant drug, saffron could be of therapeutic benefit in the treatment to mild to moderate depression (Akhondzadeh *et al.* 2004). As well as safranal (0.15-0.5 mg/kg b.w.) and crocin (50-600 mg/kg b.w.), that were proved to be effective on mice (Karimi *et al.* 2001). Fluoxetine activity, which is a common drug used for treating this disorder, can be compared with aqueous and ethanolic saffron extracts and with kaempferol obtained from saffron petals (Hosseinzadeh *et al.* 2004, 2007a). In the same way, the effect of kaempferol has been studied in 40 depressed patients (between 18 and 55 years) concluding that a treatment of 30 mg/day of a petal extract during 8 weeks and 30 mg/day of a stigma extract during 6 weeks can be helpful for treating this condition (Noorbala *et al.* 2004, 2005; Moshiri *et al.* 2006; Akhondzadeh *et al.* 2007). Finally, Akhondzadeh *et al.* (2008) concluded that, being petals less expensive than stigmas and exerting the same activity could represent a new alternative treatment.

Cardiovascular injury

1. Atherosclerosis

Hyperlipidemia is characterized for abnormal levels of lipids or lipoproteins in the blood stream causing thickness of the arteries' wall leading to a cardiovascular disease named atherosclerosis. Since several efforts have been made in order to know more about this mechanism and its prevention, the possibility of using antioxidants, such as crocin, as an inhibitor of this disease has been evaluated. There is evidence that crocin (25, 50 and 100 mg/kg b.w.) decrease greatly the content of cholesterol, triglyceride and density lipoprotein in blood and increase the content of high density lipoprotein (He *et al.* 2005; Xu *et al.* 2005). Moreover, thiobarbituric acid reactive substances decrease and plasma lipid levels remain unchanged in high lipid diet rabbits (Zheng *et al.* 2006). Sheng *et al.* (2006) confirmed that crocin (25, 50 and 100 mg/kg b.w.) significantly reduced serum triglyceride, total cholesterol, LDL cholesterol and VLDL cholesterol. In the same way, crocin suppressed the absorption of fat and cholesterol. In addition, crocetin can prevent the adhesion of leukocyte to bovine endothelial cells (BEC), which is important because adhesion and migration of leukocyte to endothelial cells is one of the early key steps in the atherosclerosis. This activity may be related to the antioxidant properties of saffron and protection for mitochondrion (Xiang *et al.* 2006). Furthermore, Sheng *et al.* (2006) found that the hypolipidemic effect of crocin was due to its inhibition of pancreatic lipase activity, being this enzyme the key to digestion and absorption of fat, so much effort has been directed to search an inhibitor. Crocin doses from 0.1 to 10,000 μg/ml, result in a dose-dependent, reversible inhibition of lipase that was more potent than the inhibition of gastric lipase (Sheng *et al.* 2006). Recently, another study revealed that saffron had superior hypolipidemic effect than crocin (Asdaq *et al.* 2009).

2. Myocardial infarction

Myocardial infarction (MI) is an acute condition of necrosis of the myocardium that occurs as a result of imbalance between myocardial demand and coronary blood supply. It is well established that reactive oxygen species have been implicated in the pathophysiology of MI and antioxidants suppress its formation. Therefore, the effects of crocin in cardiotoxicity isoproterenol induced were studied. Crocin at 20 mg/kg b.w./day, administered during 21 days, significantly modulated hemodynamic and antioxidant derangements, suggesting a cardioprotective effect through modulation of oxidative stress in such a way that maintains the redox status of the cell (Goyal *et al.* 2010; Joukar *et al.* 2010). In adittion, crocetin has beneficial effects on blocking inflammatory cascades caused by hemorrhage/resuscitation on cardiac injury at doses of 50 mg/kg b.w. (Yan *et al.* 2010).

3. Peripheral vascular diseases

It has been reported that the platelet-rich thrombi are the indispensable sources of thromboembolic complications, such as atherosclerosis, heart attacks, strokes, and peripheral vascular diseases. Therefore, inhibition of platelet functions represents a promising approach for the prevention and treatment of cardiovascular diseases, such as thrombosis. Crocetin effects on platelet activity and thrombosis formation were demonstrated showing a dose-dependent inhibition of platelet aggregation and significantly attenuation of dense granule release, as well as, prolonged the occlusive time in electrical stimulation-induced carotid arterial throm-

bosis. These findings suggest that the favourable impacts of crocetin on platelet activity and thrombosis formation may be related to the inhibition of Ca_2 elevation in stimulated platelets (Yang *et al.* 2008). In accordance with these results, other study using blood from healthy volunteers evaluated the inhibitory activity of saffron extract on human platelets, confirming a dose-dependent inhibition (Jessie and Krishnakantha 2005).

4. Insulin resistance

Insulin resistance is a condition in which normal levels of insulin are inadequate to produce a normal insulin response, situation that is linked to genetic and environmental factors, causing hyperinsulinemia, hypertension, dyslipidemia and being one of the principal factors for developing Diabetes mellitus type 2, which may lead in a cardiovascular disease. Crocetin at doses of 20 mg/kg b.w. and specially 40 mg/kg b.w. is capable of attenuate the development of insulin resistance and the abnormalities mentioned above, as well as, restoring free fatty acid metabolism disorders, which may explain the biochemical and nutritional basis of its inhibitory action (Xi *et al.* 2007).

Cancer and tumours

Chemoprevention is defined as the use of natural or synthetic agents to prevent of block the development of cancer. The chemopreventive and antitumoral potential properties of saffron and several other spices against cancer have been extensively studied during the last decade, proposing different hypotheses for the mode of action of its constituents. The cytotoxic effect of saffron extract (200-2000 µg/ml) was evaluated by Tavakkol-Afshari *et al*. (2008) in HepG2 and HeLa malignant cell lines, resulting in a decrease of viability of malignant cells in a concentration and time-dependent manner, fact confirmed by Feizzadeh *et al.* (2008). Saffron doses inducing 50% cell growth inhibition (IC50) values against HeLa and HepG2 were determined as 800 and 950 µg/ml after 48 hrs, respectively. It was concluded that saffron can cause cell death in which apoptosis or programmed cell death plays an important role (Tavakkol-Afshari *et al.* 2008). The cytotoxic and antitumor properties of saffron petals have been also studied, being the IC50 values of stigma and petal extract against tumour, 5.3 and 10.8 mg/ml (Hosseinzadeh *et al.* 2005), respectively. On the other hand, the genotoxic potential of anti-tumour drugs limits their efficacy in the treatment of cancers, so a study was designed to ascertain the chemoprotective potential of saffron against the genotoxicity of cisplatin, cyclophosphamide and mitomycin, three well known antitumor drugs. Saffron doses of 20, 40 and 80 mg/kg b.w. significantly inhibited the cellular DNA damage induced by the antitumor drugs, suggesting that saffron could be an adjuvant in chemotherapeutic applications (Premkumar *et al.* 2006).

1. Skin cancer

Skin carcinogenesis is a malignant growth of the epidermis that could be caused by UV-A and UV-B- radiation that generate free radicals in the cells. It was found that a saffron infusion (200 mg/kg b.w./d) has a beneficial action when given before and after the induction of skin carcinogenesis. Saffron ingestion inhibited the formation of skin papillomas and simultaneously reduced their size, fact that at least in part, may be due to the induction of cellular defense systems (Das *et al.* 2010).

2. Pancreatic cancer

Pancreatic cancer accounts for a high mortality rate because it has a very poor prognosis, so new therapeutic alternatives are really needed. Given saffron antitumour activity *in vitro* and *in vivo*, the proliferation of pancreatic adenocarcinoma cells is significantly inhibited due to a crocetin treatment (4 mg/kg b.w. /d) in mice. Also, pancreatic cancer growth was also significantly inhibited because of crocetin oral treatment (Dhar *et al.* 2009).

3. Breast cancer

Crocus sativus and different types of *Crocus taxa,* endemic in Greece, containing hydrophilic carotenoids show a dose-dependent inhibitory effect on breast cancer cells proliferation, attributing this effect to crocin contents in saffron (Chryssanthi *et al.* 2007). Some authors (Bathaie *et al.* 2007; Kanakis *et al.* 2007a, 2007b) mentioned that saffron carotenoids interact with DNA and induce some conformational changes on it, having crocetin the most potential.

4. Lung cancer

This type of cancer is the leading cause of cancer related mortality worldwide; so many efforts have been done in order to reduce it. A treatment of 20 mg/kg b.w. of crocetin dissolved in dimethyl sulphoxide was administered in mice, resulting in a reversion of the pathological changes observed in cancerous animals proving the antitumor ability of this compound (Magesh *et al.* 2006).

5. Colorectal cancer

Saffron inhibition on three colorectal cancer cell lines (HCT-116, SW-480 and HT-29) was studied, finding a dose-dependent inhibition of malignant cells growth, being crocin the major responsible of this activity. Moreover, crocin did not affect normal cells growth (Aung *et al.* 2007).

Antinociceptive effects

The antinociceptive, a well known property of saffron, due to their content of flavonoids, tannins, anthocyanins, alkaloids and saponins which was confirmed using safranal at doses between 0.1 and 0.5 ml/kg b.w. (Hosseinzadeh and Shariaty 2007). However, the mechanism responsible remains to be investigated (Hosseinzadeh and Younesi 2002).

Premenstrual syndrome

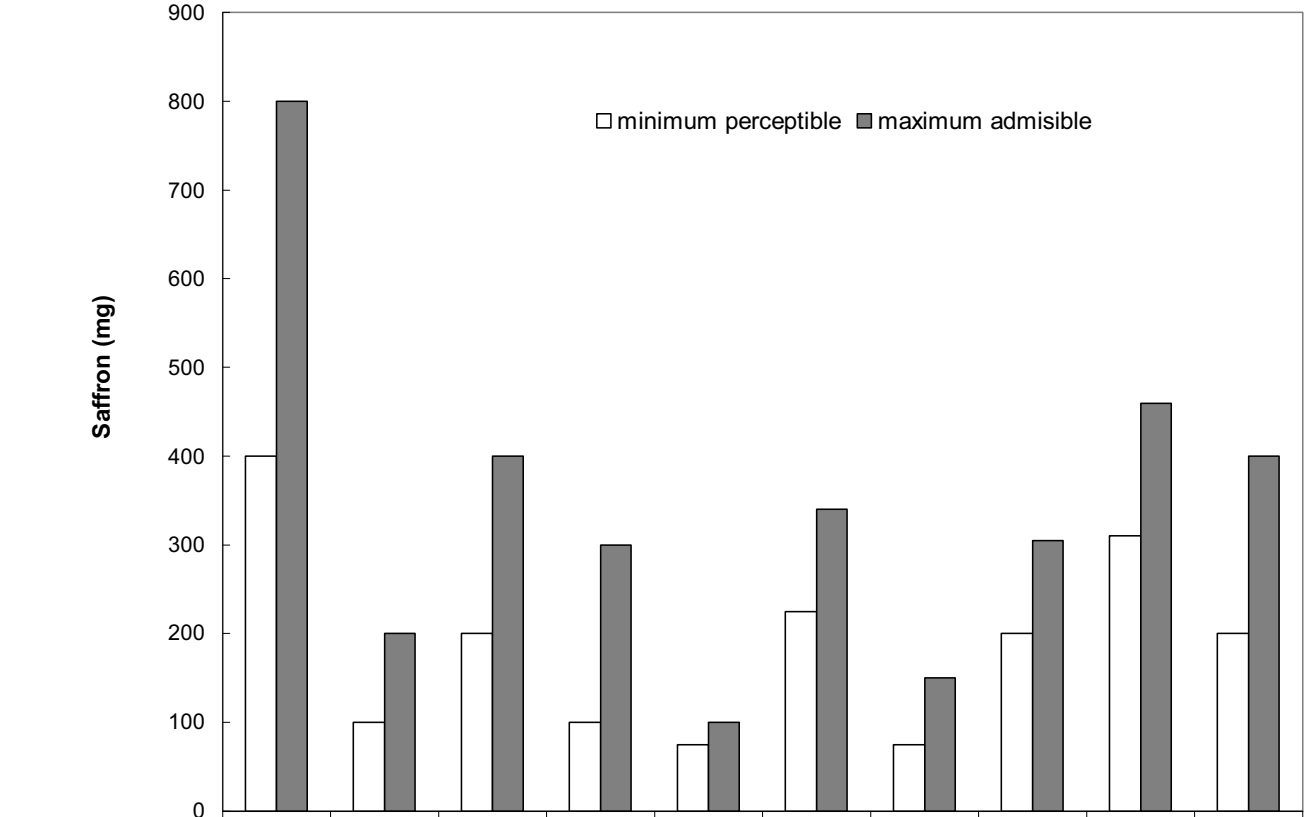
Premenstrual syndromes (PMS) are among the most common health problems reported by women of reproductive age characterised by emotional, behavioural and physical symptoms. There is an overlap between symptoms of depression and those associated with PMS, so saffron also resulted to be effective on treating this syndrome. Women between 20 and 45 years received 15 mg of saffron capsule twice a day, resulting in a relief of several symptoms. Even if the study is in line with previous reports, further research in this area is needed, because it is the first clinical trial done (Agha-Hosseini *et al.* 2008).

Sexual behaviour dysfunction and infertility

Sexual dysfunction is a serious medical and social symptom that occurs in 10-52% of men and 25-63% of women (Porst 2004). Since the available drugs and treatments for these problems have limited efficacy or side-effects, a series of plants, such as saffron, have been proved to have aphrodisiac effects. This fact is confirmed in a study using saffron extracts (80, 160 and 320 mg/kg b.w.) and crocin (100, 200 and 400 mg/kg b.w.), resulting an activity compared to sildenafil, an phosphodiesterase inhibitor, commonly used for treating erectile dysfunction, unlike safranal that showed a vasodilator effect (Hosseinzadeh *et al.* 2008). Recently, a pilot study was published, conducted with twenty male patients with erectile dysfunction, in which 200 mg of dried saffron stigma were taken orally during ten days every morning. After this period of time there was a statistically significant improvement on sexual function with increased number and duration of erectile events (Shamsa *et al.* 2009).

Table 1 Biomedical properties of saffron studied using patients.

Properties	References	Doses (saffron mg)	Frequency
Sexual behaviour dysfunction and infertility	Shamsa *et al.* 2008	200	Daily, 10 days
	Heidary *et al.* 2008	50	3 times a week, 12 weeks
Alzheimer	Akhondzadeh *et al.* 2010	30	Daily, 22 weeks
Depression	Akhondzadeh *et al.* 2004; Noorbala *et al.* 2004, 2005; Moshiri *et al.* 2006; Akhondzadeh Basti *et al.* 2007	30	Daily, 6-weeks
	Akhondzadeh Basti *et al.* 2008	30	Daily, 8-weeks
PMS	Agha-Hosseini *et al.* 2008	30	Daily, 8-weeks



	Oils	Fish soup	Meat soup	Mashed vegetables	Stew	Bread	Rice	Croissant	Pasta	Infussion
minimum perceptible	400	100	200	100	75	225	75	200	310	200
maximum admisible	800	200	400	300	100	340	150	305	460	400

Fig. 3 Minimum and maximum admissible saffron doses in different dishes (mg/L). Based on but modified from Verdú Cantó 2009.

Aside, the effects of saffron on men with idiopathic infertility was proved to be effective, based on an intake of 50 mg of saffron 3 times a week during 3 months, but further research are needed (Heidary *et al.* 2008).

Other studies

More biological applications of saffron and its constituents have been studied such as encephalomyelitis (Ghazavi *et al.* 2009), the hormone changes in pituitary-testis axis of mice (Nazem *et al.* 2009), possible fertility improvement (Ai *et al.* 2009), as a treatment for hemorrhagic shock (Yang R *et al.* 2006), the effects on the fetal development of mice (Golalipour *et al.* 2008), the efficacy against pneumonia (Mannan *et al.* 2006), pancreas-protective effects of saffron ethanolic extracts (Mohajeri *et al.* 2009), protective effects against nephrotoxicity (Boroushaki and Sadeghnia 2009), tyrosinase inhibitory activity (Li and Wu 2002), morphine dependence inhibition (Sahraei *et al.* 2008), among others, but further study needs to be done, in order to know more about the mechanisms of action of saffron and its constituents.

SAFFRON INTAKE AND HUMAN EQUIVALENT DOSES TRANSLATION

Many reviews have been published in the past recent years (Deng *et al.* 2002; Abdullaev and Espinosa-Aguirre 2004; Schmidt *et al.* 2007; Soeda *et al.* 2007; Kianbakht 2008) in order to synthesize saffron properties and its related research, but it is difficult to understand the human repercussion of these studies because most of them use animal doses that are not directly related neither to human doses nor saffron consumption. Studies using patients, principally Iranian, are very few, as presented in **Table 1**, treating problems related to sexual behaviour and depression, principally. For treating sexual behaviour, the studies used 50 and 200 mg during several weeks, in order to increase the number and duration of erectile events. In depression studies, they use unique doses, independently of the body weight of the patient, being 30 mg daily of saffron during 6 or 8 weeks the most common dose used for depression that cause an improvement.

The 30 mg dose per day can easily be attained eating saffron in different food dishes, as shown in **Fig. 3**, which presents minimum perceptible and maximum admissible doses of saffron (mg) per litre of different dishes such as soups, rice, pasta and pastry products, including a saffron

Table 2 K_m factors of different species for conversion of animal doses to human equivalent doses based on BSA.

Species	Weight (kg)	BSA (m^2)	K_m factor
Adult Human	60	1.6	37
Guinea pig	0.4	0.05	8
Rat	0.15	0.025	6
Mouse	0.02	0.007	3

FDA Draft Guidelines 2002

infusion. Most of them can be prepared using between 300 and 500 mg maximum of saffron per litre of food, being oil preparation the product that use more saffron, but its concentration is diluted taking into account that oil is used to prepare a wide variety of dishes and not eat alone. Saffron infusion represents a good way to increase saffron consumption because it is drink directly, without quantity restrictions. A cup of 100-150 ml can contain the active compounds corresponding to the mentioned dose of 30 mg of saffron per day.

In order to compare animal doses used in the majority of the studies with possible human doses, it is necessary to transform these quantities using the body surface area (BSA) normalization, because of converting the safe starting dose based on body weight alone, can result an inappropriate comparison between studies because of the lack of correlations for oxygen utilization, caloric expenditure, blood volume, circulating plasma proteins and renal functions between various mammalian species and differently sized members of the same species, including humans (Reagan-Shaw *et al.* 2007). In this study the recommendation of the U.S. Food and Drug Administration of using BSA normalization has been employed for the purpose to calculate the hypothetical human equivalent doses (HED), parameter used on initial clinical trials in healthy adult volunteers.

The customary approach for calculation of BSA uses the Du Bois height-weight formula: BSA (m2) equals body weight (kg b.w.)$^{0.425}$ multiplied by height (cm)$^{0.725}$ multiplied by 0.007184, has been re-evaluated in similar forms with updated constants, however scientific evidence does not favour one alternative formula over another (Sawyer

Table 3 Human equivalent doses calculated for the different saffron animal studies.

Effects	Reference	Animal	Saffron product	Frequency	Saffron equivalent doses (mg/kg b.w.)[a]	HED (mg/person)[b]
Biological activities						
Antioxidant	Hosseinzadeh *et al.* 2007b	Rats	Ethanolic extract	Mono dose	5 - 80	57 - 908
			Crocin		172 - 1379	1 957 - 15 657
			Safranal		14 505 - 72 523	164 646 - 823 229
Ulcers	Al-Mofleh *et al.* 2006	Rats	Extract	Mono dose	250	2 838
	Kianbakht and Mozaffari 2009	Rats	Extract		25 - 250	284 - 2 838
			Crocin		8 - 35	88 - 391
			Safranal		362 613 - 725	4 116 144 - 8 232
					225	286
Nervous system damage						
Neuronal injury	Hosseinzadeh and Sadeghnia 2005	Mice	Safranal	Mono dose	109 234	1 239 956
Retinal function	Maccarone *et al.* 2008	Rats	Extract	Mono dose	1	11
Parkinson	Ahmad M *et al.* 2005	Rats	Crocin	Daily, 7 days	0.3	3
Seizures	Hosseinzadeh and Khosravan 2002	Mice	Ethanolic extract	Mono dose	0.2 - 2	1 - 11
			Aqueous extract		0.1 - 0.8	0.45–5
	Hosseinzadeh and Talebzadeh 2005	Mice	Safranal		21 757 - 50 766	123 484 - 288 130
	Hosseinzadeh and Sadeghnia 2007	Rats	Safranal		10 923 - 43 694	123 996 - 495 982
Learning behaviour	Pitsikas and Sakellaridis 2006	Rats	Extract	Mono dose	30 - 60	341 - 681
	Pitsikas *et al.* 2007	Rats	Crocin	Daily	52 - 103	587 - 1 174
Anxiety	Pitsikas *et al.* 2008	Rats	Crocin	Mono dose	172	1 957
	Hosseinzadeh and Noraei 2009	Mice	Extract		56 - 560	318 - 3 178
			Safranal		21757 - 50 766	123 484 - 288 130
Sedative/relaxant	Hosseinzadeh and Ghenaati 2006	Guinea pigs	Ethanolic extract	Mono dose	100 - 800	1 514 - 12 108
			Safranal		36 261 - 108 784	548 819 - 1 646 457
Depression	Karimi *et al.* 2001	Mice	Safranal	Mono dose	23 - 75	128 - 426
			Crocin		172 - 2 079	979 - 11 743
	Hosseinzadeh *et al.* 2004	Mice	Aqueous extract		160 - 320	908 - 1 816
			Ethanolic extract		200 - 800	1 135 - 4 541
Cardiovascular injury						
Atherosclerosis	Sheng *et al.* 2006	Rats	Crocin	Daily, 10 days	86 - 1 250	979 - 14 189
	Asqad *et al.* 2009	Rats	Extract	Daily, 5 days	25 - 100	284 - 1 135
Myocardial infarction	Goyal *et al.* 2009	Rats	Crocin	Daily, 21 days	69	783
	Yan *et al.* 2010	Rats	Crocin		172	1957
Pheripheral vascular diseases	Yang *et al.* 2008	Rats	Crocin	Daily, 2 days	86 - 625	979 - 7 095
Insulin resistance	Xi *et al.* 2007	Rats	Crocin	Daily, 8 weeks	69 - 138	783 - 1 566
Cancer and tumours						
Cancer and tumours	Premkumar *et al.* 2006	Mice	Extract	Daily, 5 days	20 - 80	114 - 454
Skin cancer	Das *et al.* 2009	Mice	Extract	Daily, 7 days	200	1 135
Pancreatic cancer	Dhar *et al.* 2009	Mice	Crocin	Daily, 30 days	14	78
Lung cancer	Magesh *et al.* 2006	Mice	Crocin	Daily, 4 weeks	69	391
Antinociceptive effects						
Antinociceptive	Hosseinzadeh and Shariaty 2007	Mice	Safranal	Mono dose	14 505 - 72 522	82 323 - 411 614
Sexual behaviour dysfunction						
Sexual behaviour dysfunction	Hosseinzadeh *et al.* 2008	Rats	Extract	Mono dose	80 - 320	908 - 3 632

[a] Doses were converted to mg/kg b.w. of saffron equivalent, taking into account a saffron humidity of 9%, 0.66% safranal content and 32% on dry basis of crocetin content
[b] HED were calculated using K_m factors based on BSA. The final HED was multiplied by a body weight of 70 kg

and Ratain 2001; Wang and Hihara 2004; Verbraeclen *et al.* 2006; Reagan-Shaw *et al*. 2007). BSA is often represented in mg/m2 and can be translated to human equivalent doses (HED) in mg/kg b.w. according to this formula: HED (mg/kg) equals to animal dose (mg/kg) multiplied by animal Km/ human Km (Reagan-Shaw *et al*. 2007) using factors named K_m factors, for the different species summarized on **Table 2**.

This study pretends to calculate a tentative HED from the animal doses of the different saffron studies and link it to saffron consumption in different dishes. This work it is a first attempt to suggest a tentative reference for human doses that can not be taken lightly, because of the lack of pharmacokinetics studies in the bibliography and other very important data such as LD_{10} values, bioavailability, absorption and elimination kinetics of the saffron compounds in humans. Human equivalent doses for the different saffron properties are shown in **Table 3**, calculations are based on the range of doses proved on each animal study that did not cause toxicity and exerted a noticeable effect. Doses were converted to mg/kg b.w. of saffron equivalent, taking into account an average saffron moisture of 9% (Carmona *et al.* 2006), up to 0.66% safranal content (Maggi *et al.* 2009) and up to 32% crocetin ester content (Sánchez *et al.* 2009). Differences between aqueous and ethanolic extracts were not considered. The final human dose was multiply by a body weight of 70 kg.

From **Table 3**, it can be observed that some of these doses are really approachable for adults, such as antioxidant activity (57-908 mg), depression (128-426 mg) and learning behaviour (341-681), being seizures (1-11 mg) and Parkinson the diseases that needs less saffron doses for its prevention or amelioration. In the other hand, studies which used safranal, show doses that can not be achieved by just eating dishes with saffron, taking into account that safranal content in saffron represents a 0.66% of its composition and in the studies the majority of safranal source was a standard of high purity.

Saffron intake in food, as shown by data presented in this work, could represent a good way to achieve different biological effects and taken daily as a habitude, could represent a preventing method for many diseases, specially taken as an infusion.

CONCLUSION

Saffron has been investigated during years in a wide variety of different biological effects. A big part of these effects are achieved by its antioxidant properties, since it is responsible for many chemical reactions that have effects on preventing many diseases, such cardiovascular and neuronal injury, among others. It is recommended further investigation and clinical trials because of deficiencies on some studies conducted and the lack of pharmacokinetics studies in order to have a better correlation between animal and human doses. Saffron consumption in food could represent a good source for preventing many diseases. Doses of clinical trials made in human patients can be achieved by consuming saffron in food, especially as an infusion.

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