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

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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, antidepres- sant, 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-
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 *Staphylococcus 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
that crocin inhibits apoptosis in a model cellular for neuronal tissue from rats under ischemic conditions, a therapeutic agent for several diseases as demonstrated by genic agent.

an inhibitor proton pump, which is used as an antiulcerative drug. It was found that these extracts, prevent gastric lesions, increase lipid peroxidation and decrease glutathione levels induced by indomethacin, effects that are comparable to omeprazole, an inhibitor proton pump, which is used as an antulcerogenic 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 microcrocine 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 protecting 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). Ochiai 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 supplement 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 (Aβ) 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 Aβ fibrils and compared with that of tomato and carrot by Papandreu 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 stigmata 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.

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 synchronized 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 treatments for 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 pentylentetrazol (PTZ), a popular chemoconvulsant, in a dose-dependent manner and decreased the duration of tonic seizures caused by electroshock. Saffranol (0.15-0.35 mg/kg b.w.) showed anticonvulsant behaviour as well, in PTZ-induced seizures (Hossein- zadeh 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 impair-ment. Recently, rats treated with 30 and 60 mg/kg b.w. of saffron extracts were capable of discriminate between famili-ar and novel objects (Pitsikas and Sakellaridis 2006), find-ing 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. re-duced 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 safranal (56, 80, 320 and 560 μg/kg b.w.) and safranal (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 tradi-tional medicine, and it was confirm by Boskabady and Aslanli (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 cor-roborate the mechanism of action, another study was pub-lished, suggesting that relaxation is due to saffron stimu-latory 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), vasodilation due to petsals (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 Ghenaatii 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 safranal (30 mg/day) is treated with imipramine (100 mg/day), a antidepressant drug, saffron could be of therapeutic benefit in the treatment to mild to moderate depression (Ahkondzadeh 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 (Karimirad et al. 2001). Flouoxetine activity, which is a com-mon 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; Ahkondzadeh et al. 2007). Finally, Ahkondzadeh et al. (2008) concluded that, being petsals 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 re-duced serum triglyceride, total cholesterol, LDL cholesterol and VLDL cholesterol. In the same way, crocin suppressed the absorption of fat and cholesterol. In addition, crocin can prevent the adhesion of leukocyte to bovine endothelial cells (BEC), which is important because adhesion and mig-ration 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, revers-ible inhibition of lipase that was more potent than the in-hibition of gastric lipase (Sheng et al. 2006). Recently, an-other study revealed that saffron had superior hypolipide-mic 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 isoprotorenol induced were studied. Crocin at 20 mg/kg b.w./day, administered during 21 days, signifi-cantly modulated hemodynamic and antioxidant derange-ments, suggesting a cardioprotective effect through modula-tion of oxidative stress in such a way that maintains the redox status of the cell (Goyal et al. 2010; Joukar et al. 2010). In addition, crocin has beneficial effects on block-ing inflammatory cascades caused by hemorrhage/resus-ci-tation 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 periphe-ral vascular diseases. Therefore, inhibition of platelet func-tions represents a promising approach for the prevention and treatment of cardiovascular diseases, such as thrombo-sis. Crocetin effects on platelet activity and thrombosis for-mation were demonstrated showing a dose-dependent in-hibition 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 Ca2+ 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 using saffron extracts (80, 160 and 320 mg/kg b.w.) and crocin (100, 200 and 400 mg/kg b.w.) resulted in 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. (Hosseinizadeh and Shariati 2007). However, the mechanism responsible remains to be investigated (Hosseinizadeh and Younesi 2002).

Prenmenstrual syndrome

Prenmenstrual syndromes (PMS) are among the most common health problems reported by women of reproductive age characterized 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, a phosphodiesterase inhibitor, commonly used for treating erectile dysfunction, unlike safranal that showed a vaso dilator effect (Hosseinizadeh 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).
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>
<thead>
<tr>
<th>Properties</th>
<th>References</th>
<th>Doses (saffron mg)</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sexual behaviour dysfunction and infertility</td>
<td>Shamsa et al. 2008</td>
<td>200</td>
<td>Daily, 10 days</td>
</tr>
<tr>
<td></td>
<td>Heidary et al. 2008</td>
<td>50</td>
<td>3 times a week, 12 weeks</td>
</tr>
<tr>
<td>Alzheimer</td>
<td>Akhondzadeh et al. 2010</td>
<td>30</td>
<td>Daily, 22 weeks</td>
</tr>
<tr>
<td></td>
<td>Agha-Hosseini et al. 2008</td>
<td>30</td>
<td>Daily, 8-weeks</td>
</tr>
</tbody>
</table>

Fig. 3 Minimum and maximum admissible saffron doses in different dishes (mg/L). Based on but modified from Verdú Cantó 2009.
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 concentration is diluted taking into account that oil is used to prepare 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 et al. 2002).

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

<table>
<thead>
<tr>
<th>Species</th>
<th>Weight (kg)</th>
<th>BSA (m²)</th>
<th>$K_b$ factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adult Human</td>
<td>60</td>
<td>1.6</td>
<td>37</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>0.4</td>
<td>0.05</td>
<td>8</td>
</tr>
<tr>
<td>Rat</td>
<td>0.15</td>
<td>0.025</td>
<td>6</td>
</tr>
<tr>
<td>Mouse</td>
<td>0.02</td>
<td>0.007</td>
<td>3</td>
</tr>
</tbody>
</table>

*FDA Draft Guidelines 2002

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

<table>
<thead>
<tr>
<th>Effects</th>
<th>Reference</th>
<th>Animal</th>
<th>Saffron product</th>
<th>Frequency</th>
<th>Saffron equivalent doses (mg/kg b.w.)</th>
<th>HED (mg/person)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Antioxidant</strong></td>
<td>Hosseinzadeh et al. 2007b</td>
<td>Rats</td>
<td>Ethanolic extract</td>
<td>Mono dose</td>
<td>5 - 80</td>
<td>57 - 908</td>
</tr>
<tr>
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<td></td>
<td>Crocin</td>
<td></td>
<td>172 - 1379</td>
<td>1 957 - 15 657</td>
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<td></td>
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<td></td>
<td>Safranal</td>
<td></td>
<td>14 505 - 72 523</td>
<td>164 646 - 823 229</td>
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<tr>
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<td>Rats</td>
<td>Extract</td>
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<td>250</td>
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<td>Rats</td>
<td>Extract</td>
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<td>4 116 144 - 8 232</td>
<td>286</td>
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<tr>
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<td>Hosseinzadeh and Sadeghnia 2005</td>
<td>Mice</td>
<td>Safranal</td>
<td>Mono dose</td>
<td>109 234</td>
<td>1 239 956</td>
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<td>Maccarone et al. 2008</td>
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<td>Extract</td>
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<td>Parkinson</td>
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<td>Crocin</td>
<td>Daily, 7 days</td>
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<td>Seizures</td>
<td>Hosseinzadeh and Khosravan 2002</td>
<td>Mice</td>
<td>Ethanolic extract</td>
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<td>0.2 - 2</td>
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<td>Mono dose</td>
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<td>0.45 - 5</td>
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<td>10 923 - 43 694</td>
<td>123 996 - 495 982</td>
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<td>Learning behaviour</td>
<td>Pitsikas and Sakellaridis 2006</td>
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<td>Mono dose</td>
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<td>Anxiety</td>
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<td>Daily</td>
<td>52 - 103</td>
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<td>Guinea pigs</td>
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<td>Sheng et al. 2006</td>
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<td>Crocin</td>
<td>Daily, 10 days</td>
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<td>Asqad et al. 2009</td>
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<td>Extract</td>
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<td>Yan et al. 2010</td>
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<td>Cancer and tumours</td>
<td>Xi et al. 2007</td>
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<td>Daily, 8 weeks</td>
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<td>Antinociceptive effects</td>
<td>Hosseinzadeh and Shariaty 2007</td>
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<td>82 323 - 411 614</td>
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<td>Hosseinzadeh et al. 2008</td>
<td>Rats</td>
<td>Extract</td>
<td>Mono dose</td>
<td>80 - 320</td>
<td>908 - 3 632</td>
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</tbody>
</table>

$^a$ 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 $K_b$ factors based on BSA. The final HED was multiplied by a body weight of 70 kg

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and Ratain 2001; Wang and Hihara 2004; Verbraeck 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 Km 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 is it 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 LD50 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 (Sanchez et al. 2009). Different 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 Parkinsonian symptoms 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 preventing method for many diseases, specially as an infusion. This work it is a first attempt to suggest a tentative reference for human doses that can not be achieved by just eating saffron. The final human dose was multiply by a body weight of 70 kg.

CONCLUSION

Saffron has been investigated during years in a wide variety of different biological effects. A big part of the 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, on 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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