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Immunomodulatory, Anti-oxidant and Cardio-protective Activities of Style & Stigma of *Z'afrān (Crocus sativus L.)*: A Unani Medicine used for promotion of healthy ageing

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Abstract

Z'afrān (Crocus sativus L.), commonly known as saffron, is a perennial stemless herb that is widely cultivated in Iran (commonly) and other countries such as India, Spain and Greece. In India it is cultivated in the Kashmir valley, especially in the Pampore and Budgam at about 1600 m. The degeneration continues in the human body with increasing age. The resultant effects, though, cannot be overcome but by the help of certain measures, the ageing process can be slowed down and the diseases of old age can either be prevented or treated. The Unani single drug *Z'afrān* is one of the most important in Unani Medicine used for longevity and geriatric care since long time and has *Mu'mmir* (Longevity promoting agent), *Muqawwī-i-Ḥarārat Gharīziyya* (Tonic for Innate heat), *Muqawwī-i-Qalb* (Cardiotonic), *Muqawwī-i-Dimāgh* (Brain tonic), *Muqawwī-i-Baṣar* (Eye tonic), *Muqawwī-i-Kabid* (Hepatotonic), *Muqawwī-i Bāh (*Aphrodisiac*), Muḥallil-i-Waram* (Anti-inflammatory), *Mufarriḥ* (Exhilarant), etc. properties. It is used in *Ḍu'f-i-Ḥarārat Gharīziyya* (Innate heat insufficiency), *Amrāḍ-i-Qalb* (Cardiac diseases), *Ḍu'f-i-Qalb* (Cardiac insufficiency), *Ḍu'f-i-Kabid* (Hepatic insufficiency), *Ḍu'f-i-Dimāgh* (Cerebrasthenia), *Ḍu'f-i-Baṣar (*Poor eyesight), *Nisyān* (Forgetfulness) etc. It showed radical immunomodulatory, anti-ageing, anti-oxidant, cardio-protective, memory enhancer anti-depressant, antianxiety activities. In this paper, an attempt has been made to review the Unani pharmacological actions & therapeutic uses for a comprehensive understanding of the importance of *Z'afrān (Crocus sativus* L.) for promotion of longevity and healthy ageing.

Keywords

Mu'mmir, Muqawwī-i-Qalb, Ḍu'f-i-Dimāgh, Mufarriḥ, Exhilarant etc.

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Introduction	The drug of *Z'afrān* consists of dried style and stigma of *Crocus sativus* L., Syn. *Crocus officinalis* (L.) Honck. (Family-Iridaceae). A small, bulbous, perennial, 15 to 25 cm high. It is cultivated in the Kashmir valley, especially in the Pampor plateau, at about 1600 m. (Anonymous, 2009).	 **Fig. *Zafran***
Vernacular Names	English: Saffron; Hindi: *Kaysar*; Urdu: *Z'afrān*; Arabic: *Kurkum, Z'afrān, Ḥalūq, Jād* ; Persian: *Kīmās. (Khān,* 2013*; Ibn Sīnā,*1987*; Ibn Baytār,* 1986*; Kabīruddin,* 2000*; Ghani,*YNM*)*	
Temperament	*Ḥār* (Hot)[2] *Yābis* (Dry)[1] *(Khān,* 2013*; Ibn Sīnā,*1987*; Ibn Baytār,* 1986*; Kabīruddin,* 2000*)*	
Chemical Constituents	• The characteristic components of saffron are crocin-(responsible for the color), picrocrocin- (responsible for the bitter taste), and safranal-(responsible for odor and aroma) (Evans, 2009). Saffron contains more than 150 volatile and aroma-yielding compounds. It also has many non-volatile active components, many of which are carotenoids including zeaxanthin, lycopene, and various α- and β-carotenes (Liakopoulou-Kyriakides and Kyriakidis, 2002). The volatiles with a very strong odor are consistent of more than 34 components that are mainly terpenes, terpene alcohols, and their esters. Non-volatiles include crocins 14 that are responsible for the red or reddish-brown color of stigmas together with carotenes, crocetin, picrocrocin (a glycosidic precursor of safranal), the bitter substance and safranal the major organoleptic principle of stigmas (Wallis, 1946). However, saffron's golden yellow-orange color is primarily due to α-crocin. This crocin is *trans*-crocetin di-(β-D-gentiobiosyl) ester. This means that the crocin underlying saffron's aroma is a digentiobiose ester of the carotenoid crocetin. Crocins themselves are a series of hydrophilic carotenoids that are either monoglycosyl or di-glycosyl polyene esters of crocetin (Liakopoulou-Kyriakides and Kyriakidis, 2002). Meanwhile crocetin is a conjugated polyene dicarboxylic acid that is hydrophobic and thus oil soluble. When crocetin is esterified with two water-soluble gentiobioses (which are sugars), a product results that is itself water soluble. The resultant α-crocin is a carotenoid pigment that may comprise more than 10% of dry saffron's mass. The two esterified gentiobioses make α-crocin ideal for coloring waterbased (nonfatty) foods such as rice dishes (Wallis, 1946). • A hypothetical protocrocin of the fresh plant is decomposed on drying into one molecule of crocin and two molecules of picrocrocin. Crocin on hydrolysis yields gentiobiose and crocetin, while picrocrocin yields glucose and safranal (Evans, 2009). The bitter glucoside picrocrocin is responsible for saffron's flavor. Picrocrocin is a union of an aldehyde subelement known as safranal and a carbohydrate. It has insecticidal and pesticidal properties and may comprise up to 4% of dry saffron. Safranal is less bitter than picrocrocin and may comprise up to 70% of dry saffron's volatile fraction in some samples. A second element underlying saffron's aroma is 2-hydroxy-4, 4, 6-trimethyl-2, 5-cyclohexa-dien-1-one, the scent which has been described as "saffron, dried hay-like (Hosseinzadeh *et al.,* 2003). • Crocetin, riboflavin, picrotoxin, thiamine, picrocrocin, crocin, carotenoids, essential oil, bitter glycoside are the main constituents present in *Crocus sativus L.* (Anonymous, 2009).	

Pharmacological Actions	• *Mu'mmir* (Longevity promoting agent) • *Muqawwī-i-Ḥarārat Gharīziyya* (Tonic for Innate heat) • *Muqawwī-i-Qalb* (Cardiotonic) • *Muqawwī-i-Dimāgh* (Brain tonic) • *Muqawwī-i-Baṣar* (Eye tonic) • *Muqawwī-i-Kabid* (Hepatotonic) • *Muqawwī-i Bāh (*Aphrodisiac*)* • *Muḥallil-i-Waram* (Anti-inflammatory) • *Mufarriḥ* (Exhilarant) • *Muḥarrik-i-Bāh* (Libido stimulant) • *Mudirr-i-Bawl* (Diuretic) • *Mudirr-i-Ḥayḍ* (Emmenagogue) • *Qābiḍ* (Astringent) • *Dāfi'-i-Ta'ffun* (Antiseptic) • *Jāli* (Detergent) *(Khān,* 2013*; Ibn Sīnā,*1987*; Ibn Baytār,* 1986*;* Al-Harawi,2002; *Kabīruddin,* 2000*; Ghani,*YNM; Anonymous, 2009*)*
Therapeutic Uses	• *Ḍu'f-i-Ḥarārat Gharīziyya* (Innate heat insufficiency) • *Amrāḍ-i-Qalb* (Cardiac diseases) • *Ḍu'f-i-Qalb* (Cardiac insufficiency) • *Ḍu'f-i-Kabid* (Hepatic insufficiency) • *Ḍu'f-i-Dimāgh* (Cerebrasthenia) • *Ḍu'f-i-Baṣar (*Poor eyesight) • *Ḍu'f-i-Bāh (*Sexual debility*)* • *Nisyān* (Forgetfulness) (*Khān,* 2013*; Ibn Sīnā,*1987*; Ibn Baytār,* 1986*;* Al-Harawi,2002; *Kabīruddin,* 2000*; Ghani,*YNM; Anonymous, 2009*)*
Important Formulations	*Dawa ul Kurkum, Dawa ul Misk Mo'tadil sada, Dawa ul Misk Mo'tadil Jawāhar wālā, M'ajūn Dabīd ul Ward.* (Anonymous,2009)
Pharmacological / Clinical studies (evidence based)	**Anti-oxidant activity** • The imbalance between reactive oxygen species (ROS) production and Anti-oxidant level is directly linked to the pathogenesis of diseases. The enhancement of Anti-oxidant level or reduction of reactive species level is maintained through Anti-oxidant properties of plants or their derivatives. Numerous studies based on *in-vivo* and *in-vitro* have confirmed that *Crocus sativus* has a significant Anti-oxidant activity. Anti-oxidant activity of saffron has been observed in extract of stigma and such extract shows role in the reduction of chlorophyll damage, lipid peroxidation, and protein oxidation. Similarly, other finding has confirmed that saffron stigma contains superior Anti-oxidant activity. Earlier findings have demonstrated that active and inactive constituents of saffron extract have high Anti-oxidant activity and saffron petal extract showed Anti-oxidant activity. (Rahmani et al.,2017; Assimopoulou et al.,2005; Goli et al.,2012) • More studies demonstrated that constituent of saffron such as crocin has a potent Anti-oxidant activity. Lebanon based finding demonstrated that saffron notably decreased lipid peroxidation as well increased superoxide dismutase activity when compared to control group. Crocin showed role in the inhibition of lipid peroxidation and restored SOD activity. Stigma of *Crocus sativus* contains more Anti-oxidant activity as compared to tomatoes and carrots. (Rahmani et al.,2017; Asdaq et al.,2010; Makhlouf et al.,2011; Ochiai et al.,2004) • Baba et al (2015) study was conducted to evaluate and compare the chemical composition and Anti-oxidant activity of three different tissue types of *Crocus* viz: stigma, corm, and leaf. The phytochemical analysis

carried out using LC-MS showed that the major constituents identified were flavonoids like kaempferol, taxifolin, naringenin, etc. and apocarotenoids including crocin, crocetin, and their derivatives. Also, the total phenolic, flavonoid, and carotenoid contents were determined. The Anti-oxidant property of these tissue types was also investigated and compared by biochemical assays like, DPPH, NBT, and FRAP. The ethanolic fraction of stigma demonstrated the strongest Anti-oxidant activity which could be attributed to its highest content of phenolics and flavonoids. Stigma extract was further evaluated for its role in alleviating oxidative stress in plants, yeast, and bacteria. The results revealed that stigma extract reduced methylviologen induced chlorophyll damage, lipid peroxidation, and protein oxidation in plants thereby rendering them more tolerant to stress. It also showed to alleviate H_2O_2 mediated oxidative stress tolerance in bacteria and yeast.

Immunomodulatory activity

- Samarghandian et al. (2017) investigated the immunomodulatory effects of the aqueous saffron extract on streptozotocin (STZ)-induced diabetic rats. During the study, rats were divided into the following groups of 9 animals each: control, untreated diabetic, three saffron extract-treated diabetic groups. Diabetes was induced by STZ in rats. Saffron was administered 3 days after STZ administration; these injections were continued to the end of the study (4 weeks). At the end of 4-week period, blood was drawn for biochemical assays and the abdominal aorta was removed for detecting the inflammatory cytokines expression. As result, the saffron decreased blood glucose, malondialdehyde, nitric oxide, total lipids, triglycerides, cholesterol levels significantly ($p < 0.01$) and increased glutathione level, catalase, and superoxide dismutase activities in the saffron-treated diabetic groups compared with the untreated groups, in a dose dependent manner ($p < 0.05$, $p < 0.01$, $p < 0.001$). On the other hand, saffron-treated diabetic rats inhibited the expression of inflammatory cytokines in the abdominal aorta versus the untreated diabetic rats.
- Kianbakht and Ghazavi (2011) reported immunomodulatory activities of *Crocus sativus* without any adverse effects.
- Zeinali et al. (2019) summarized the protective roles of *C. sativus* and its constituents against the pathogenesis of immune diseases and understanding a better management of the same. The study indicated that the compounds with immunoregulatory properties may be effective for prevention and treatment of such diseases.

Activity in Memory Impairment

- Saffron extract improved ethanol-induced impairments of learning behaviours in mice, and prevented ethanol-induced inhibition of hippocampal long-term potentiation, a form of activity-dependent synaptic plasticity that may underlie learning and memory. Accordingly, saffron extract or its active constituents, crocetin and crocin, could be useful as a treatment for neurodegenerative disorders accompanying memory impairment (Esmail Al-Snaf ,2016; Abe and Saito ,2000)
- Alzheimer's disease was characterized pathologically by deposition of amyloid beta-peptide (Abeta) fibrils. Oxidation was thought to promote Abeta fibril formation and deposition. To identify agents inhibiting the pathogenesis of Alzheimer's disease, the Anti-oxidant properties of extract of Crocus sativus stigmas and its effect on Abeta (1-40) fibrillogenesis was investigated in-vitro. The Anti-oxidant properties were determined by measuring the ferric-reducing Anti-oxidant power and Trolox-equivalent Anti-oxidant capacity, while its effects on Abeta-aggregation and

	fibrillogenesis were studied by thioflavine T-based fluorescence assay and by DNA binding shift assay. The water: methanol (50:50, v/v) extract of Crocus sativus stigmas possessed good Anti-oxidant properties, higher than those of tomatoes and carrots, and inhibited Abeta fibrillogenesis in a concentration and time-dependent manner. The main carotenoid constituent (trans-crocin-4) the digentibiosyl ester of crocetin, inhibited Abeta fibrillogenesis at lower concentrations than dimethylcrocetin, revealing that the action of the carotenoid was enhanced by the presence of the sugars. The result suggested the possible use of Crocus sativus stigma constituents for inhibition of aggregation and deposition of Abeta in the human brain (Papandreou etal.,2006) • Saffron extract was investigated in preventing D-galactose and NaNO2 induced memory impairment and improving learning and memory deficits in amnestic mice. The learning and memory functions in ovariectomized mice were examined by the one-way passive and active avoidance tests. In active avoidance test, training in amnestic treated (AT) and amnestic prophylaxis (AP) groups, was improved, there was a significant difference between them and the amnestic control (AC) group. In passive avoidance test, animal's step through latency, as an index for learning, in all test groups was significantly greater than control group. Total time spent in dark room (DS), which opposed the memory retention ability, in AC was significantly greater than AT group at 1 and 2 hours after full training, while there was no significant difference in this parameter between AP and AT (Esmail Al-Snaf ,2016;;Dashti et al.,2012) • The acute effects of an alcohol extract of Crocus sativus (CS-extract) were studied on learning and memory in step through (ST) and step down (SD) tests in normal, trained and memory-impaired mice. A single oral administration of CS-extract had no effects on memory registration consolidation or retrieval in normal mice. CS-extract reduced the ethanol-induced impairment of memory registration both in ST and SD tests and the ethanol-induced impairment of memory retrieval in SD test. CS extract decreased the motor activity (MA) and prolonged the sleeping time induced by hexobarbital (Esmail Al-Snaf ,2016; Zhang et al.,1994) • Long-term potentiation (LTP) was thought as a generative mechanism underlying learning and memory via storing information in central nervous system. Electro-neurophysiological assay for LTP was generally used in screening the drugs that can facilitate learning and memory. Methanol extract of saffron (MES) being able to facilitate LTP-induction, and can antagonize the inhibiting effect of 30% ethanol on LTP induction (30 pulses/60 Hz) (Esmail Al-Snaf ,2016; He et al.,2009) • The effects of Crocus sativus, and its active constituent crocin was evaluated on learning and memory loss and the induction of oxidative stress in the hippocampus by chronic stress. Rats were injected with saffron extract, crocin or vehicle over a period of 21 days while being exposed to chronic restraint stress (6 h/day). Then, animals were trained and tested on a water-maze spatial memory task. They performed four trials per day for 5 consecutive days, and this was followed by a probe trial two days later. At the end of the behavioral testing, several parameters of oxidative stress in the hippocampus were measured. Treatment with saffron extractor crocin blocked the ability of chronic stress to impair spatial learning and memory retention. Crocin significantly decreased plasma levels of corticosterone, as measured after the end of stress. The results indicated that saffron and its active constituent crocin can prevent the impairment of learning and memory as well as the oxidative stress damage to the

hippocampus induced by chronic stress (Esmail Al-Snaf ,2016; Ghadrdoost et al., 2011)

- The effect of aqueous extracts of saffron was investigated in morphine-induced memory impairment. On the training trial, the mice were subjected to an electric shock on entering into the dark compartment. Twenty-four and forty-eight hours later, the time latency for entering the dark compartment was recorded and defined as the retention trial. The mice were divided into (1) control, (2) morphine which received morphine before the training in the passive avoidance test, (3-5) three groups treated by 50, 150 and 450 mg/kg of saffron extract before the training trial, and (6 and 7) the two other groups received 150 and 450 mg/kg of saffron extract before the retention trial. The time latency in morphine-treated group was lower than control ($p<0.01$). Treatment of the animals by 150 and 450 mg/kg of saffron extract before the training trial increased the time latency at 24 and 48 hours after the training trial ($p<0.05$ and $p<0.01$). Administration of both 150 and 450 mg/kg of the extract before retention trials also increased the time latency ($p<0.01$). The results revealed that the saffron extract attenuated morphine-induced memory impairment (Esmail Al-Snaf ,2016; Naghibi et al.,2012)
- Inhibitors of acetylcholine breakdown by acetylcholinesterase (AChE) constituted the main therapeutic modality for Alzheimer's disease. The inhibition of AChE activity of saffron extract and its constituents was studied by in-vitro enzymatic and molecular docking studies. Saffron extract showed moderate AChE inhibitory activity (up to 30%), but IC50 values of crocetin, dimethylcrocetin, and safranal were 96.33, 107.1, and 21.09 µM, respectively. Kinetic analysis showed mixed-type inhibition, which was verified by in silico docking studies. Safranal interacted only with the binding site of the AChE, but crocetin and dimethylcrocetin bind simultaneously to the catalytic and peripheral anionic sites (Esmail Al-Snaf ,2016; Geromichaloset al.,2012)
- The efficacy of Crocus sativus was studied in the treatment of patients with mild-to-moderate Alzheimer's disease. Fifty-four Persian adults, 55 years of age or older were participated in a 22-week, double-blind study of parallel groups of patients with AD. The main efficacy measures were the change in the Alzheimer's disease Assessment Scale-cognitive subscale and Clinical wztia Rating Scale-Sums of Boxes scores compared with baseline. Adverse events (AEs). Participants were randomly assigned to receive a capsule saffron 30 mg/day (15 mg twice per day) or donepezil 10 mg/day (5 mg twice per day). Saffron at this dose was found to be effective similar to donepezil in the treatment of mild-to-moderate AD after 22 weeks. The frequency of AEs was similar between saffron extract and donepezil groups with the exception of vomiting, which occurred significantly more frequently in the donepezil group (Esmail Al-Snaf ,2016; Akhondzadeh et al.,2010)

Cardio-protective activity

- Saffron and its constituents have also supported the evidences of cardio-protective effects. Rat model-based study has confirmed that whole saffron pretreatment or its individual constituents such as safranal pretreatment considerably decrease the serum LDH and CK-MB level, as well as myocardial lipid peroxidation as compared to isoproterenol – induced animals. Crocin, an ingredient of saffron revealed its protective effects of cardio-toxicity through reducing lipid peroxidation as well as alleviating apoptosis. In a similar study based on rat models, it was concluded that crocin improves toxic effects of diazinon through decreasing lipid peroxidation and restoring altered contractile and relaxant responses in

aorta. The cardio-protective effect of saffron active constituents including crocin has been confirmed through regulation of oxidative stress. The finding concluded that *Crocus sativus* perfused during electrolysis might trap radical oxygen species and significantly improve myocardial function. (Rahmani et al., 2017).

Anti-obesity activity

- Saffron showed anti-obesity and anorectic effects in the obese rat models. It's property of reducing the leptin level in obese cases indicates that saffron reduces fat mass and increases insulin sensitivity. In an experimental protocol, it was performed to assess the anti-obesity effects of ethanolic extracts of saffron and crocin. Results of this study demonstrated that saffron extract notably decrease the food consumption by obese rats as compared to control groups. Furthermore, crocin showed a noteworthy decrease on rate of body weight gain, total fat deposition and regulates the weight ratio of epididymal fat to body. (Rahmani et al., 2017).

Anti-convulsant activity

- The results of the experiments performed on mice to evaluate the Anti-convulsant activities of safranal and crocin, indicated that safranal reduced the seizure duration, delayed the onset of convulsions as well as protected mice from death. This study further investigated that crocin did not show this Anti-convulsant activity at all. (Rahmani et al.,2017; Hosseinzadeh & Talebzadeh, 2005)

Anti-cancer activity

- Abdullae (2002) demonstrated that saffron extract itself and its main constituents, the carotenoids, possess chemo-preventive properties against cancer.
- Chemoprevention using readily available natural substances from vegetables, fruits, herbs and spices is one of the significantly important approaches for cancer prevention in the present era. Among the spices, *Crocus sativus* L. has generated interest because pharmacological experiments have established numerous beneficial properties including radical scavenging, anti-mutagenic and immuno-modulating effects. Studies in animal models and with cultured human malignant cell lines have demonstrated antitumor and cancer preventive activities of saffron and its main ingredients. This review provides a brief insight into the Anti-cancer properties of saffron and its components. (Bhandari, 2015).
- The topical application of a saffron extract has been shown to inhibit both the initiation and the promotion of cancer by a common carcinogen, DMBA, which is used to induce skin cancer for experimental purposes. The saffron extracts have been shown to significantly prolong-almost by three-fold-the life spans of mice undergoing experimental chemotherapy with the toxic Anti-cancer drug, cisplatin. Saffron also partially prevented the decrease in body weight, hemoglobin levels and leukocyte counts associated with that form of chemotherapy (Nair, 1991).
- Oral administration of saffron extract inhibited the growth of mouse tumors that were derived from three different kinds of cancer cells and significantly increased the life spans of treated tumor-bearing mice (Chermahini *et al.,* 2010).

Additional activities:

- The style & stigma of *Crocus sativus* L. has been reported to possess anti-depressant, antianxiety, anti-diabetic, anti-inflammatory, analgesic activity, anti-microbial, anti-viral, anti-hypertensive, anti-hyperglycemic, anti-nociceptive, gastro-protective, nephro-protective, hepato-protective, CNS protective, anxiolytic, aphrodisiac, neuro-protective etc. activities. (Anonymous,2021; Esmail Al-Snaf ,2016; Rahmani et al.,2017)

CONCLUSION:

Style & Stigma of *Crocus sativus* L. contains more than 150 volatile and aroma-yielding compounds mainly terpenes, terpene alcohol, and their esters. The active constituents present in *Z'afrān* has *Mu'mmir* (Longevity promoting agent), *Muqawwī-i-Ḥarārat Gharīziyya* (Tonic for Innate heat), *Muqawwī-i-Qalb* (Cardiotonic), *Muqawwī-i-Dimāgh* (Brain tonic) etc. properties. It The role of saffron including crocin and crocetin in the management of numerous diseases and used in Ḍu'f-*i-Ḥarārat Gharīziyya* (Innate heat insufficiency), *Amrāḍ-i-Qalb* (Cardiac diseases), *Ḍu'f-i-Qalb* (Cardiac insufficiency), *Ḍu'f-i-Kabid* (Hepatic insufficiency), *Ḍu'f-i-Dimāgh* (Cerebrasthenia) *etc.* The style & stigma of *Crocus sativus* L. has been reported to possess anti-depressant, antianxiety, anti-inflammatory, gastro-protective, nephro-protective, hepato-protective, CNS protective, aphrodisiac, neuro-protective etc. activities. Regular use of Style & Stigma of *Z'afrān* in diet in prescribed doses may help delay ageing process, strengthen body faculties, enhance memory, prevent and control chronic diseases like obesity, hypertension and thus ensure longevity and healthy ageing.

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