MINIREVIEW

Cancer Chemopreventive and Tumoricidal Properties of Saffron (*Crocus sativus L.*)

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Since cancer is the most common cause of death in the world population, the possibility that readily available natural substances from plants, vegetables, herbs, and spices may be beneficial in the prevention of cancer warrants closer examination. Saffron in filaments is the dried, dark red stigmata of *Crocus sativus* L. flowers and it is used as a spice, food colorant, and a drug in medicine. A growing body of research has demonstrated that saffron extract itself and its main constituents, the carotenoids, possess chemopreventive properties against cancer. This review discusses recent literature data and our results on the cancer chemopreventive activities of saffron and its main ingredients. [Exp Biol Med Vol. 227(1):20–25, 2002]

Key words: saffron *Crocus sativus L.*; antitumor; anticarcinogenic; antimutagenic activities; cytotoxicity; chemoprevention

Cancer continues to represent the largest cause of mortality in the world and claims over 6 million lives each year (1). An extremely promising strategy for cancer prevention today is chemoprevention, which is defined as the use of synthetic or natural agents (alone or in combination) to block the development of cancer in human beings. Plants, vegetables, herbs, and spices used in folk and traditional medicine have been accepted currently as one of the main sources of cancer chemopreventive drug discovery and development (2). A large and increasing number of patients in the world use medicinal plants and herbs for health purposes. Therefore, scientific scrutiny of their therapeutic potential, biological properties, and safety will be useful in making wise decisions about their use. For example, one in three people in the United States has used at least one form of alternative medicine (3). From ancient times to the present, saffron has been used as a spice for flavoring and coloring food preparations, as a perfume, and also as a dye or ink. In folklore medicine, as well as in modern pharmacy, saffron has been reputed to be useful (Fig. 1) in the treatment of numerous human diseases (4–13).

Commercial saffron is produced from dried stigmas of *Crocus sativus* L., a member of the large family *Iridaceae*, and is cultivated in Azerbaijan, France, Greece, India, Iran, Italy, Spain, China, Israel, Morocco, Turkey, Egypt, and Mexico (14–16). Saffron is produced worldwide at an annual rate of 50 tons with a commercial cost of about 50 million dollars (16). The main reason for its great cost is that saffron is still cultivated and harvested as it has been for millennia—by hand.

The chemical composition of saffron has attracted the interest of several research groups during the last decades, and among the estimated more than 150 volatile and several nonvolatile compounds of saffron, approximately 40–50 constituents have already been identified (17–41). Based on these data, we can conclude that saffron contains three main pharmacologically active metabolites: 1.) Saffron-colored compounds are crocins, which are unusual water-soluble carotenoids (mono and diglycosyl esters of a polyene dicarboxylic acid, named crocetin). The digentiobiosyl ester of crocetin - α-crocin is the major component of saffron. 2.) Picrocrocin is the main substance responsible of the bitter taste in saffron. 3.) Safranal is the volatile oil responsible of the characteristic saffron odor and aroma. Furthermore, saffron contains proteins, sugars, vitamins, flavonoids, amino acids, mineral matter, gums, and other chemical compounds (4, 13, 17).

Animal studies indicate that the oral LD_{50} of saffron was 20.7 g/kg administered as a decoction (42). Our experi-

This work was partially supported by Research Grant 28513-N from the Consejo Nacional de Ciencia y Tecnología.

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Received May 14, 2001.
Accepted August 15, 2001.

1535-3702/01/2271-0020$15.00

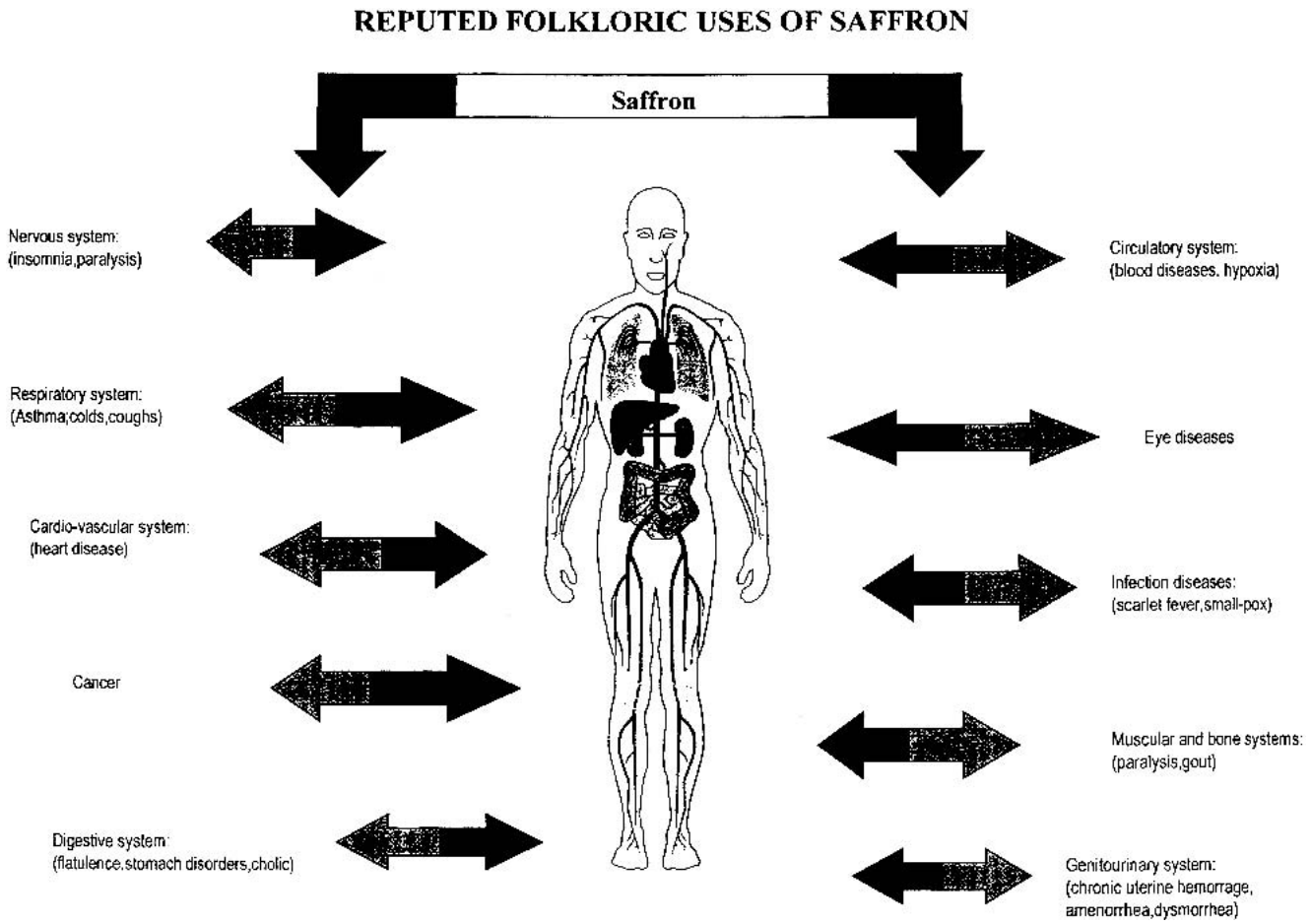

Figure 1. Reputed folkloric uses of saffron.

ments demonstrated that oral administration of saffron extract at concentrations from 0.1 to 5 g/kg was nontoxic in mice (Abdullaev F, *et al*., unpublished data).

Some reports about the stability of saffron (13, 17, 36, 37) mention that two factors (temperature and humidity) exert a strong influence on the degradation of the main pharmacologically active ingredients of saffron under different storage conditions, but the developed HPLC assay can be utilized as a quality control method for saffron (39). On the other hand, when saffron is stored under −20°C, its pharmacological activities as a supplement remain unaltered for at least 2 years or even longer (43). Further studies to elucidate the structure and to characterize the biologically active ingredients of saffron are now in progress in different laboratories. The scientific evidence on the cancer chemopreventive effects of saffron extract and its main ingredients are outlined here, updating previous reviews on this topic (4, 44–46).

Cancer Preventive and Tumoricidal Effects of Saffron and Its Ingredients

Research into the effect of saffron on neoplastic cells has seen a renaissance in the last decade, and a growing body of evidence indicates that saffron and its characteristic components possess anticarcinogenic and antitumor activities *in vivo* and *in vitro* (47–71).

Saffron extract has been shown capable of inhibiting and/or retarding tumorigenesis in a variety of experimental models *in vivo* (47–56). Topical application of saffron extract (100 mg/kg body wt) inhibited two-stage initiation/promotion dimethylbenz[a]anthracene (DMBA)-induced skin carcinogenesis and oral administration of saffron extract in the same dose restricted 20-methylchloanthrene (MCA)-induced soft tissue sarcomas in mice (47–49). Later, it was demonstrated that saffron extract significantly prolonged (almost 3-fold) the life spans of cisplatin-treated (2 mg/kg body wt) mice and partially prevented the decrease in body weight, hemoglobin levels, and leukocyte counts (50).

Another study (51) examined the protective effect of concurrent administration of cysteine (20 mg/kg body wt) together with vitamin E (2 mg/kg body wt) and saffron extract (50 mg/kg body wt) against cisplatin-induced (3 mg/kg body wt) toxicity in rats. It was shown that treatment of animals with protective (saffron together with vitamin E and cysteine) agents significantly reduces blood urea nitrogen, serum creatinine levels, and blood glucose levels, as well as partially prevents many changes in the activities of different serum enzymes (51). Taken together, these studies indicated

that saffron may be a promising agent for reducing cisplatin-toxic side effects, including nephrotoxicity.

Oral administration of saffron extract (200 mg/kg body wt) induced a dose-dependent inhibition of the growth in mice of ascite tumors derived from sarcoma-180 (S-180), Ehrlich ascites carcinoma (EAC), Dalton's lymphoma ascites (DLA), and significantly increased (2- to 3-fold) life spans of treated tumor-bearing mice (52). Later, these authors reported that oral administration of saffron extract significantly suppressed the growth of DLA and S-180 tumor cells, but did not affect the growth of EAC tumor cells in mice (53). The authors suggested that increase in the levels of β-carotene and vitamin A in the serum of the experimental animals receiving saffron might be one explanation for this antitumor effect of saffron. Interestingly, when liposome-encapsulated saffron extract was injected i.p. into mice, the increasing of antitumor effect of this extract towards several solid cells was observed, including the EAC tumor cells, which were insensitive to orally administered extract (54). These authors suggested that enhancement in antitumor activity of saffron extract could be due to site-directed drug delivery or to carrier-mediated increased drug solubility. More recently, it was reported (55) that crocin, a carotenoid isolated from saffron, increased the survival time and decreased tumor (colon adenocarcinoma) growth in female rats without any significant effects in male animals. The authors suggested that the selective antitumor action of crocin in female rats compared with male might be related to hormonal factors.

In another study (56), crocetin at nontoxic doses inhibited genotoxic effect and neoplastic transformation in C3H1OT1/2 cells induced by benzo(a)pyrene (BP). Thus, studies *in vivo* showed that saffron extract and its purified constituent significantly increased the life span of animals with different types of tumor, but the mechanism of anticarcinogenic effect of saffron has not been elucidated.

A number of studies have demonstrated an antitumor effect of saffron and its constituents on different malignant cells *in vitro* (Table I). Observed differences in sensitivity to saffron and its ingredients between different cultured malignant cells (57–71) could be due to the existence of distinct cell surface receptors, intracellular retention transport, differences in the drug uptake, or differences in the methods of extraction and determination of cytotoxicity.

By using trypan-blue dye exclusion as a criterion of cell viability, the IC_{50} of saffron extract was found to range from 7 to 30 μg/ml, dependent upon the type of tumor cells, whereas there was no significant effect on normal mouse spleen cells (47, 52, 53). Utilizing the method of colony formation as a measure of cell viability, it was demonstrated that the IC_{50} of saffron extract ranged from 100 to 200 μg/ml upon the type of human malignant cells, but had no significant effect on normal human lung cells (57, 58). It was shown that the saffron extract inhibited cellular nucleic acid synthesis and had no effect on protein synthesis in tumor cells (54, 57, 58). Interestingly, there was a stimulatory or supporting effect of saffron extract on nonspecific proliferation of immature and mature lymphocytes *in vitro* and colony formation of normal human lung cells (54, 57, 58). It was also observed that saffron increased the intracellular levels of reduced glutathione and glutathione-related enzymes and suggested a possible antioxidant activity of saffron (54, 59). It was shown that saffron extract and its purified characteristic compounds crocin, safranal, picrocrocin, and β-carotene (Table I) inhibited different types of tumor cell growth (55, 59–61). Interestingly, in two stud-

Table I. Cytotoxic (IC_{50}) Effect of Saffron and Its Components on Tumor Cells *in vitro*

Agents	Cells	IC_{50}	References
Saffron extract	S-180; EAC; DLA; P388 osteosarcoma; and ovarian sarcoma	7–30 μg/ml	44, 48, 52, 54
Saffron extract	HeLa; A549; WI-38VA	100–250 μg/ml	57–58
Saffron extract	A-204; HEPG-2; SW-480	150–200 μg/ml	Abdullaev F.I. *et al.,* unpublished data
Saffron extract	HeLa	2.3 mg/ml	62
Crocetin	HL-60; K562	2 μM	60, 61
Dimethyl crocetin	HL-60; K562	0.8 μM	60, 61
Crocin	HL-60; K562; HeLa; and HT-29 DHD/K12-PROb	2 μM, 3 mM, 0.4 mM, and 1 mM, respectively	55, 60–62
-Carotene	K562	3 μM	60
Safranal	HeLa	0.8 mM	62
Picrocrocin	HeLa	3 mM	62
All-trans retinoic acid	HL-60	0.12 μM	61
Saffron corm callus extract	HeLa	100–150 μg/ml	64–68
Saffron proteoglycan	HeLa, fibrosarcoma, and breast carcinoma	7 μg/ml, 9 μg/ml, and 22 μg/ml, respectively	64, 66
Glucoconjugate from saffron corms	Tobacco BY-2 cells, protoplasts	0.5 μg/ml and 2 μg/ml, respectively	68

ies (60, 61), crocetin isolated from saffron had a cytotoxic activity on tumor cells, but in another study, it was shown that crocetin did not show any cytotoxic effect (62). Our study (63) demonstrated that crocetin had no cytotoxic effect on colony formation of different tumor cells, but had a dose-dependent inhibitory effect on DNA, RNA, and protein synthesis in these human malignant cells. We also reported that treatment of tumor cells with saffron extract in combination with well-known antitumor agents such as selenium compounds caused a more effective inhibition of colony formation and nucleic acid synthesis relative to the effects of these agents alone (59).

It was reported that a novel glucoconjugate isolated from corms and callus of saffron possessed cytotoxic activity against different tumor cells (64–68). These authors demonstrated that glucoconjucate from corms of *C. sativus L.* possessed cytotoxic activity on human tumor cells derived from fibrosarcoma, cervical epithelioid carcinoma, and breast carcinoma (Table I). This compound was about eight times more cytotoxic for malignant cells than for their normal counterparts and it caused plasma membrane damage in these cells. Interestingly, that analysis of DNA fragmentation indicated that cell death was not mediated by apoptosis. Thus, extracts of saffron stigmas, corm, and callus and its ingredients possessed both anticarcinogenic and antitumor activities *in vivo* and *in vitro* (64–68).

Only one study (48) using the Ames assay had indicated that crocin and dimethyl-crocetin from saffron were nonmutagenic and nonantimutagenic. In our laboratory, it has been demonstrated that saffron extract was nontoxic, nonmutagenic, and nonantimutagenic (69).

Thus, saffron and its constituents are suggested as alternative anticancer agents, which alone and in combination with other synthetic substances may have the potential for the prevention and the treatment of certain forms of cancer.

Proposed Mechanisms for Cancer Preventive and Tumoricidal Effects of Saffron

Different hypotheses for the modes of anticarcinogenic and antitumor actions of saffron and its components have been proposed. One of the mechanisms for the antitumor or anticarcinogenic action of saffron and its components is the inhibitory effect on cellular DNA and RNA synthesis, but not on protein synthesis (44, 52, 57–59, 63). A second suggested mechanism for the antitumor action of saffron and its constituents is the inhibitory effect on free radical chain reactions, because most carotenoids are lipid-soluble and might act as membrane-associated high-efficiency free-radical scavengers, which is connected with their antioxidant properties (44, 46, 53, 70–75). A third proposed mechanism by which the saffron extract exerts its antitumor effect is the metabolic conversion of naturally occurring carotenoids to retinoids (61, 71), but recently, it was reported that conversion carotenoids to vitamin A is not a prerequisite for anticancer activity (76). A fourth suggested mechanism is that the cytotoxic effect of saffron is connected with interaction of carotenoids with topoisomerase II, an enzyme involved in cellular DNA-protein interaction (44, 50, 76).

Recently, several other mechanisms for the antitumor effect of saffron and its constituents have also been proposed. It was demonstrated that a novel glucoconjugate, isolated from corm and callus extract of saffron, caused swelling and local plasma membrane evagination, and it was suggested that cytotoxicity is mediated via extracellular fluid uptake (64–66). It was also reported that saffron contains lectins (77, 78), and it might also be suggested that antitumor activity of saffron is mediated via lectins (45, 79). The literature also contains reports that saffron extract and/or its components inhibited activities of different cellular enzymes, and it was suggested that the antitumor effect of these agents might be associated with the effect on enzyme functions (45, 51, 54, 80). Treatment of tumor cells with saffron resulted in an increase in the level of intracellular sulphydryl compounds (51, 59), and this could be one explanation for the potentiation of saffron cytotoxicity. Another suggested mechanism is that cytotoxic effect of carotenoids from saffron is mediated via apoptosis (60).

Interesting studies (54, 81) indicate that encapsulation in amorphous polymer matrices of saffron extracts or saffron carotenoid greatly improves their stabilities and enhances their antitumor effects. More recently, it was shown that γ-irradiation, necessary for microbial decontamination, did not produce significant qualitative changes of volatile essential oil constituents of saffron, but induced a slight decrease in glycosides and an increase in aglycon content in carotene constituents of saffron (82). This relative stability of saffron to irradiation should also be taken to account in the search for an explanation of the chemopreventive potential of this spice.

Thus, although several hypotheses have been put forward, the exact mechanism(s) of anticarcinogenic and antitumor effects of saffron and its main constituents are not clear at present.

Conclusion

Chemoprevention involves pharmacological intervention with naturally occurring and synthetic agents alone or in combination to reverse, suppress, or prevent the cancer in human beings, and today it plays a key role in the fight against this terrible disease. Considerable scientific evidence has suggested that plant-based dietary agents can inhibit the process of carcinogenesis effectively (2).

In the last decade, much attention has been focused on the biological and medical properties of an ancient spice—saffron and its ingredients. Recent scientific findings have been encouraging, uniformly showing that saffron and its components can affect carcinogenesis and currently have been studied extensively as the most promising cancer chemopreventive agents.

Because the relationship between saffron and cancer is an important concern, comprehensive, in-depth studies need

to be conducted further along the following lines: 1.) Define the mechanism(s) involved in the therapeutic properties of saffron; 2.) Investigate the mechanism(s) involved in saffron cancer chemoprevention; 3.) Determine the biologically active components of saffron; and 4.) Perform human studies to define efficacy of saffron in cancer treatment and prevention.

The scarcity and expense in obtaining large quantities of saffron may provide impediments to human chemoprevention and cancer treatment using this agent; however, an indoor cultivation method is advantageous in achieving the highest quality of saffron and for decreasing its price.

The results of each of these researches provide parts of the scaffolding to construct a logical platform for the appearing of a new scientific discipline to be called saffronology.

The author is indebted to the many his colleagues who gave him information on saffron *Crocus sativus* L.

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