Anticarcinogenic effect of saffron (*Crocus sativus L.*) and its ingredients

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ABSTRACT

Conventional and newly emerging treatment procedures such as chemotherapy, catalytic therapy, photodynamic therapy and radiotherapy have not succeeded in reversing the outcome of cancer diseases to any drastic extent, which has led researchers to investigate alternative treatment options. The extensive repertoire of traditional medicinal knowledge systems from various parts of the world are being re-investigated for their healing properties *Crocus sativus L.*, commonly known as saffron, is the raw material for one of the most expensive spice in the world, and it has been used in folk medicine for centuries. Chemical analysis has shown the presence of more than 150 components in saffron stigmas. The more powerful components of saffron are crocin, crocetin and safranal. Studies in animal models and with cultured human malignant cell lines have demonstrated antitumor and cancer preventive activities of saffron and its main ingredients, possible mechanisms for these activities are discussed. More direct evidence of anticancer effectiveness of saffron as chemo-preventive agent may come from trials that use actual reduction of cancer incidence as the primary endpoint. This review discusses recent literature data and our results on the cancer chemopreventive activities of saffron and its main ingredients.

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INTRODUCTION

Cancer development has been considered as a major threat for health in the world that varies in the age of onset, invasiveness, the response to treatment.[1] Conventional and newly emerging treatment procedures such as chemotherapy, catalytic therapy, photodynamic therapy and radiotherapy have not succeeded in reversing the outcome of cancer diseases to any drastic extent, which has led researchers to investigate alternative treatment options.[1] Fortunately, in recent years, naturopathic medicine functions most often as supportive or complementary care is involved both the prevention and treatment of the disease has helped to dramatically decrease the mortality of cancer. Naturopathic therapies with conventional cancer treatment lead to enhance recovery time from surgery, reduce the side effects of conventional treatments, improve appetite and the quality of sleep in order to aid healing, protect healthy cells and tissues when possible post chemotherapy or radiation, detoxify after use of potent conventional drugs and regenerate the body post conventional treatments. Recent research suggests that many edible fruits, vegetables, herbs and spices contain chemicals that may reduce the incidence of cancer.[2] From purple foxglove, Digitalis purpurea (Plantaginaceae), which produce digitalins, to the Pacific yew, Taxus brevifolia (Taxaceae), from which taxanes were isolated docetaxel), plants have been a source of research material for useful drugs. Commercial saffron and is one of the most expensive spices in the world that has been considered as alternative treatment options from ancient to present times.[3] In recent times, *in vivo* and *in vitro* studies are in progress to find new biomedical activity of saffron and its ingredients. There are several reviews published in the past years about the phytochemical and biomedical uses of the saffron (*Crocus sativus Linn.*). Anticarcinogenic activity of saffron was reported in the beginning of 1990 and research on this subject has increasingly continued during the past decade.[4] There are established documents of *in vivo* and *in vitro* studies on the anticarcinogenic and antitumor actions of saffron and its main components.[5] The present study provides an updated overview of experimental *in vitro* and *in vivo* investigations on the biological activities of saffron (*Crocus sativus L.*)

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and its principal ingredients, especially focusing on their anticancer effect.

HISTORICAL AND TRADITIONAL APPLICATION OF SAFFRON

Saffron is the dried stigmas of *Crocus sativus L. Crocus sativus L* belongs to the family of Iridaceas, the line of liliaceas and is mainly cultivated in several countries of mild and dry climate.[6] Although the source of saffron is unknown, it apparently originated in the area of Iran, Turkey and Greece, but now it is also successfully cultivated in such European countries as Spain, Italy, France and Switzerland, as well as in Morocco, Egypt, Israel, Azerbaijan, Pakistan, India, New Zealand, Australia and Japan. While the world's total annual saffron production is estimated to be 190 tons, Iran produces about 90% of the total with a commercial cost.[7,8] The main reason for its great cost is that saffron is still cultivated and harvested as it has been for millennia by hand.[9] Saffron's name is derived from the Arab word for yellow, a name reflecting the high concentration of carotenoid pigments present in the saffron flowers' stigmas which contribute most to the color profile of this spice. From ancient times, the saffron is widely used as drug to promote health and fight disease and it is also valued as a food additive for tasting, flavoring and coloring, as well as for its therapeutic properties.[7] In the traditional medicine, saffron is used as a diaphoretic, eupeptic, tranquilizer, expectorant, aphrodisiac, abortifacient, emmenagogue and in the treatment of hepatic disorders, flatulence, spasm, vomiting, dental and gingival pain, insomnia, depression, seizures, cognitive disorders, lumbago, asthma, cough, bronchitis, colds, fever, cardiovascular disorders and cancer. Saffron is recognized as an adaptogen in Indian ayurvedic medicine.[10]

CHEMISTRY OF SAFFRON AND ITS INGREDIENTS

Saffron contains more than 150 volatile, non-volatile and aroma-yielding compounds which consist of lipophilic and hydrophilic carbohydrates, proteins, amino acids, minerals, musilage, vitamins (especially riboflavin and thiamine) and pigments including crocin, anthocianin, carotene, lycopene, zigzantin, flavonoids, starch, gums and other chemical compounds.[1] Based on chemical analyses of dry stigma of saffron extracts, carotenoids, namely crocin and crocetin and the monoterpene aldehydes picrocrocin and safranal are the most important active carotenoid secondary metabolites of saffron. Crocin, with elementary composition $C_{44}H_{64}O_{24}$ and molecular weight 976.96, is 8 a hydrophilic carotenoid (8'-diapocarotene-8,8'-dioic acid), constitutes approximately 6 to16% of saffron's total dry matter depending upon the variety, growing conditions, and processing methods.[11] This is the diester formed from the disaccharide gentiobiose and the dicarboxylic acid crocetin. Deep red color of crocin produces the color of saffron. Crocin 1 (or α-crocin), a digentiobioside, is the most abundant crocin with a high solubility being attributed to these sugar moieties. Crocin widely used as a natural food colorant.[12] In addition to crocin, saffron contains crocetin as a free agent and small amounts of the pigment anthocianin, α-carotene, β– carotene, and zegxantin.[12] The structure of crocetin is presented in [Figure 1]. Crocetin, with elementary composition ($C_{20}H_{24}O_4$), melting point 285°C and molecular weight 328.4, is an amphiphilic low molecular natural carotenoid (8, 8'-diapo-8, 8'-carotenoic acid) and consists of a C-20 carbon chain with seven double bonds and a carboxylic acid group at each end of the molecule. This compound present in the central core of crocin and responsible for the color of saffron, constitutes approximately 14% of saffron's total dry matter depending upon the variety, growing conditions and processing methods. It is soluble in organic bases and slightly soluble in aqueous solution (20 μM at pH 8.0) (12). The structure of crocetin is presented in [Figure 1]. Picocrocin, with elementary composition ($C_{16}H_{26}O_7$) and molecular weight 330.37 g/mol is the main bitter crystalline terpene-glucoside of saffron. The actual taste of saffron is derived primarily from picrocrocin which is the second most abundant component (by weight), accounting for approximately 1 to 13% of saffron's dry matter.[13] Action of β-glucosidase on picrocrocin liberates the aglycone4-hydroxy-2, 6, 6-trimethyl-1-cyclohexene-1-carboxaldehyde (HTCC, $C_{10}H_{16}O_2$), which is transformed to safranal by dehydration during the drying process of the plant material. Natural de-glycosylation of picrocrocin will yield another important aroma factor, safranal, ($C_{10}H_{14}O$) which comprises of about 60% of the volatile components of saffron. Dehydration is not only important to the preservation of saffron but is actually critical in the

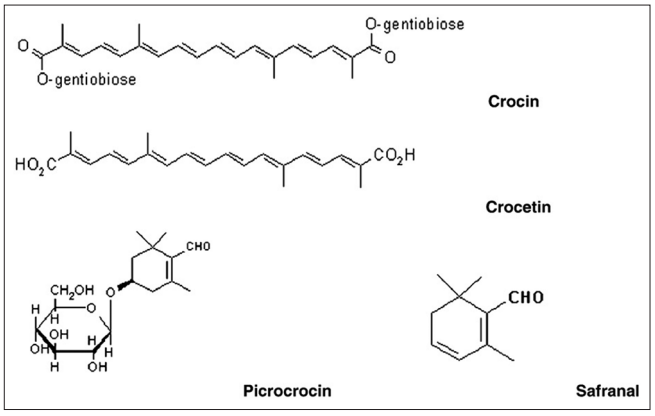

Figure 1: Chemical structures of crocin, crocetin, picrocrocin, and safranal

release of safranal from picrocrocin *via* enzymatic activity, the reaction yielding D-glucose and safranal, the latter being the volatile oil in saffron. Safranal, with, elementary composition (C10H14O) and molecular weight 150. 21 g/mol is the major volatile oil responsible for the aroma.[12] The stability of saffron and its ingredients is also dependent upon temperature, light and humidity on degradation of potency under storage conditions. Ingredients of saffron can be stored under -20°C and pharmacological activities as a supplement remain unaltered for at least 2 years or even longer.[9]

TOXICITY OF SAFFRON AND ITS INGREDIENTS

Toxicological studies have been identified that the toxicity of saffron has been found to be quite low and oral LD_{50} of saffron in animal was 20.7 g/kg administrated as a decoction. It has been demonstrated that oral administration of saffron extract at doses from 0.1 to 5g/kg was non-toxic in mice. Ames/Salmonella test system was revealed that crocin and dimethyl-crocetin isolated from saffron were non-mutagenicand non-toxic.[14] Saffron should always be obtained from a reputable source that observes stringent quality control procedures and industry-accepted good manufacturing practices. People with chronic medical conditions should consult with their physician before taking the herb. Pregnant women should never take the herb for medicinal purposes, as saffron can stimulate uterine contractions.[5]

MODERN BIOMEDICAL FINDING OF SAFFRON AND ITS INGREDIENTS

Biomedical findings have been demonstrated that saffron and its ingredients may be useful as a treatment for neurodegenerative disorders and related memory impairment, ischemic retinopathy and/or age-related macular degeneration, coronary artery disease, blood pressure abnormalities, acute and/or chronic inflammatory disease, mild to moderate depression, seizure, Perkinsonism. Furthermore, antioxidant, antimutagenic, antigenotoxic, tumoricidal and antioxidant activity of saffron and its ingredient have been found.[5]

ANTICANCER ACTIVITY OF SAFFRON AND ITS INGREDIENTS

Recent scientific findings have been encouraging, uniformly showing that saffron and its derivatives can affect carcinogenesis in a variety of *in vivo* and *in vitro* models particularly crocin and crocetin have significant anticancer activity in breast, lung, pancreatic and leukemic cells.[15] Most of the *in vivo* studies were interested in the isolated bioactive compounds of saffron. Little research has been done to examine anticancer properties of saffron in its natural form. Therefore, *in-vitro* studies have been designed to evaluate the exact mechanism and effective derivate of saffron against each type of cancer. Observed differences in sensitivity to saffron and its ingredients between different cultured malignant cells 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.[1]

Skin cancer

Oral administration of saffron extract (200 mg/kg) inhibited the growth of ascite tumors derived from sarcoma-180 (S-180), Ehrlich ascites carcinoma (EAC), Dalton's lymphoma ascites (DLA) in dose-dependent manner, and significantly elevated (2- to 3-fold) life spans of treated tumor-bearing Swiss albino mice. At this time, the authors did not identify the exact nature of the active compound from saffron stigmas, but suggested that this compound showed the presence of glycosidic linkage.[16] Furthermore, intraperitenoal injection of liposome encapsulation of saffron effectively enhanced its antitumor activity against Sarcoma-180 (S-180) and Ehrlich as cites carcinoma solid tumors in mice. On the other hand, in the presence of the T-cell mitogen phytohaemagglutinin, saffron stimulated non-specific proliferation of lymphocytes *in vitro* suggesting that the antitumor activity might be immunologically mediated.[17] These authors have also showed that oral administration of saffron extract significantly induce inhibition the growth of DLA and S-180 tumor cells, but did not affect the growth of EAC tumor cells in mice. The authors suggested that explanation for this antitumor effect of saffron is related to increase in the levels of carotene and vitamin A in the serum. It was suggested that saffron carotenoids possessed provitamin activity according to the hypothesis that the action of carotenoids was dependent upon its conversion to retinal (Vitamin A), because most of the evidence supporting the anticancer effects of carotenoids were referred to β-carotene.[18,19] However, other molecules with no provitamin A activity, such as lycopene, also show protective effects, and conversion of carotenoids into retinoids seems not to be a prerequisite for their anticancer-action.[20-22] Saffron extract has been also shown capable of inhibiting induction of tumorigenesis by chemical compound in a variety of experimental models *in vivo*. Topical application of saffron extract (100 mg/kg body wt) inhibited two-stage initiation/promotion dimethylbenz[a] anthracene (DMBA)-induced skin carcinogenesis,[23] and oral administration of the same dose of saffron extracts restricted tumour incidence of 20-methylcholanthrene (MCA)-induced soft-tissue

sarcomas in mice.[24] This observation perfectly agrees with those of Das and co-workers that demonstrated oral administration of saffron extract in mice after, before topical applications of[7,12] dimethylbenz[a] anthracene (DMBA) inhibited the formation of skin papillomas in animals, simultaneously reduced their size and DMBA-induced skin carcinoma in mice when treated early. These authors related this effect of saffron to the induction of cellular antioxidant systems.[25] In addition dose dependent inhibitory effects of aqueous extract of saffron was found on the growth human transitional cell carcinoma (TCC) and mouse non-neoplastic fibroblast cell lines (L929).[26] It was suggested that saffron rich in carotenoids might exert its chemopreventive effects by the modulation of lipid peroxidation, antioxidants, and detoxification systems.

Administration of crocetin delayed the onset of skin tumor initiation and decreased its tumor formation initiated with dimethylbenz[a] anthracene (DMBA) and promoted by croton oil in Swiss-Webster mice.[27] Similar antitumor activity of crocetin also observed in hairless mice with skin tumors developed by the application of DMBA and croton oil.[28] However, these studies showed that crocetin significantly increased the life span of animals with different types of tumor, but the exact mechanism of anticarcinogenic effect of crocetin has not been revealed. Later, Giaccio demonstrated that crocetin inhibits skin tumor promotion in mice (i.e. with benzopyrene a); it has an inhibitory effect on intracellular nucleic acid and protein synthesis in malignant cells, as well as on protein kinase C (PKC) which is most likely due to its antioxidant activity.[29]

Lukemia

The most *in vitro* experimental on anticarcinogenic effect of saffron has been focused on crocin as important anticancer compound in several described inhibition of growth of human chronic myelogenous leukaemia K562 and promyelocytic leukaemia HL-60 cells by dimethyl-crocetin, crocetin and crocin, with 50% inhibition (ID_{50}) reached at concentrations of 0.8, 2 and 2 mM, respectively.[30] Cytotoxicity of dimethylcrocetin and crocin to various tumour cell lines (L1210 leukaemia and P388 leukaemia) has been reported,[17] with concentrations producing 50% cytotoxicity ranging from 7 to 30 mg/ml for dimethyl-crocetin and from 11 to 39 mg/ml for crocin. These authors detected significant inhibition in the synthesis of nucleic acids, and suggested that dimethyl-crocetin could disrupt DNA–protein interactions (e.g. toposiomerases II) important for cellular DNA synthesis. Oral administration of saffron extract significantly inhibited genotoxicity induced by cisplatin (CIS), mitomycin-C (MMC) and urethane (URE) in the mouse bone marrow micronucleus test.[31] Premkumar and co-workers (2001) assessed the effects of aqueous extracts of saffron (composed mainly by carotenoids) in Swiss albino mice, and suggested that pre-treatment with saffron can significantly inhibit the genotoxicity of cisplatin, cyclophosphamide, mitomycin and urethane.[32] In an experiment to evaluate its protective effect on cisplatin-induced toxicity in rats (3 mg/kg body wt), el Daly (1998) showed that treatment of animals with cysteine (20 mg/kg body wt) together with saffron extract (50mg/kg body wt) significantly reduced the toxic effects caused by cisplatin, such as nephrotoxicity and changes in enzyme activity.[33]

Cervical cancer

HeLa cells (derived from a cervical epitheloid carcinoma) were more sensitive than the normal cells to the inhibitory effects of saffron on both DNA and RNA synthesis.[34] It was shown that synthesis of cellular nucleic acid was inhibited by the saffron extract in HeLa cells (derived from a cervical epitheloid carcinoma).[35] In another study ROS did not play effective role in the cytotoxic effect of saffron extract on HeLa cell lines, in which apoptosis or programmed cell death plays an important role.[36] The effect of crocin and its nano liposomal was examined on HeLa cells by measuring apoptotic cell *via* flow cytometry method. Results indicated that crocin and its liposomal form induced a sub-G1 peak in flow cytometry histogram of treated cells indicating apoptosis is involved in this toxicity. Liposomal encapsulation enhances apoptogenic effects of crocin on cancerous cells. It might be concluded that crocin and its liposomes could cause cell death in HeLa cells, in which liposomal encapsulation improved cytotoxic effects.[37]

Escribano and co-workers (1996) demonstrated that the inhibitory activity on the *in vitro* growth of HeLa cells produced by saffron extracts (ID_{50} ¼ 2, 3 mg/ml) was mainly due to crocin (ID_{50} of 3mM), whereas picrocrocin and safranal, with an ID_{50} of 3 and 0.8 mM, respectively, played a minor role in the cytotoxicity of saffron extracts. These results suggested that sugars might play a role in saffron's cytotoxic effect, since crocetin (the deglycosylated carotenoid) did not cause cell growth inhibition even at high doses.[38] These findings are in accordance with the results of Abdullaev (1994), who found no effect of crocetin on colony formation in HeLa cells and two other solid tumour cell lines, but are, however, in disagreement with other authors who reported cytotoxicity for crocetin against a cell line derived from a nonsolid tumour,[19,39] various tumour cell lines and human primary cells from surgical specimens.[14] Microscopic studies revealed that Hela cells treated with crocin showed vacuolated areas, size reduction, cell shrinkage and piknotic nuclei. Changes of HeLa cells after exposure to crocin has indicated the activation of program cell death pathways.[38,40] At one study cervical cancer cell line (HeLa), non-small cell lung

cancer cell line (A549) and ovarian cancer cell line (SKOV3) were treated with crocetin alone or in combination with vincristine. Data of this study has shown that crocetin has antiproliferation effect a concentration-dependent manner. Crocetin significantly induced cell cycle arrest through p53-dependent and -independent mechanisms accompanied with p21 (WAF1/Cip1) induction. Therefore, crocetin caused anticancer effect in the 3 types of cancer cells by enhancing apoptosis in a time-dependent manner. Furthermore, crocetin may be able to enhance vincristine cytotoxicity.[41] Later, Abdullaev and Frenkel also indicated that the malignant cells: A549 cells (derived from a lung tumour), WI-38 cells (normal lung fibroblasts) and VA-13 cells (WI-38 cells transformed by SV40 virus) were more sensitive than the normal cells to the inhibitory effects of saffron on both DNA and RNA synthesis.[34] However, saffron extract induce nonspecific proliferation of immature and mature lymphocytes *in vitro* and colony formation of normal human lung cells.[17,34,35] Moreover, other findings also supported pro-apoptotic effects of saffron extract on alveolar basal epithelial cells (A549). These results indicated that saffron-induced apoptosis of the A549 cells in a concentration-dependent manner, as determined by flow cytometry histogram of treated cells that induced apoptotic cell death, is involved in the toxicity of saffron. It might be concluded that saffron could cause cell death in the A549 cells, in which apoptosis plays an important role.[42] Another *in vivo* study reported that crocetin has antitumor activity in a lung cancer animal model by scavenging free radicals and increasing the activity of drug metabolizing enzymes.[43] Crocetin scavenged free radicals as evidenced by inhibiting lipid peroxidation and increase of the activity of GST, GSH-Px, catalase, and superoxide dismutase due to crocetin treatment. Crocetin also decreased marker enzymes such as arylhydrocarbon-hydroxylase (AHH), lactate dehydrogenase (LDH), GGT, adenosine deaminase (ADA) and 5-nucleotidase related to carcinogen following administration of benzo[*a*] pyrene (B[*a*] P) in lung tissues.[43] Furthermore, Magesh *et al.,*[44] also demonstrated that crocetin was capable of inhibiting proliferation of lung cancer cells as determined by proliferating cell nuclear antigen (PCNA), glycoproteins and polyamine synthesis. This study strongly suggested that the protective effect of crocetin on B[*a*] P-induced lung carcinogenesis in Swiss albino mice are likely due to the inhibitory effects on polyamine synthesis and glycoprotein alterations. Crocetin has shown inhibitory effects on intracellular nucleic acid synthesis and colony formation of A549 (lung carcinoma) and VA13 (SV-40 transformed fetal lung fibroblast) cells.[39]

Breast cancer

It was reported that saffron and crocetin induced apoptosis on human breast cancer cell (MCF-7) *via* p53-mediated stimulation of apoptosis. Results indicated that caspase-dependent pathway was induced by saffron in MCF-7 cells and Bax protein expression was also increased in saffron-treated cells.[45] Samarghandian and co workers (2011) investigated the potential of the ethanolic extract of saffron to induce antiproliferative and cytotoxic effects in cultured carcinomic human alveolar basal epithelial cells (MCF-7) in comparison with non-malignant (L929) cells. They showed that even higher concentrations of saffron is safe for L929, but the extract exerts pro-apoptotic effects in a lung cancer-derived cell line and could be considered as a potential chemotherapeutic agent in lung cancer.[46] Parallel to this study, the effect of crocin and its nano liposomal was examined on MCF-7 cells by measuring apoptotic cell *via* flow cytometry method. Results indicated that crocin and its liposomal form induced a sub-G1 peak in flow cytometry histogram of treated cells indicating apoptosis is involved in this toxicity. Liposomal encapsulation enhances apoptogenic effects of crocin on cancerous cells. It might be concluded that crocin and its liposomes could cause cell death in MCF-7 cells, in which liposomal encapsulation improved cytotoxic effects.[37] MCF-7 and MDA-MB-231 breast cancer cells showed concentration-dependent inhibition of proliferation by crocetin and this effect was independent of estrogen receptor. This study also suggested that crocetin can be used as chemopreventive agent in breast cancer.[47] Study on the effect of crocetin on breast cancer cells MCF-7 and invasive MDA-MB-231 indicted that crocetin, the main metabolite of crocins, inhibits MDA-MB-231 cell invasiveness via downregulation of MMP expression.[47]

Colorectal cancer

Another study has been investigated the p53-dependency of saffron's mechanism of action in two p53 isogenic HCT116 cell lines (HCT wildtype and HCT p53-/-) and shown induction of DNA-damage and apoptosis in both cell lines. However, autophagy has delayed the induction of apoptosis in HCT116 p53-/- cells. Considering the fact that most tumors show a functional p53 inactivation.[48] Furthermore, the anti-proliferative effects of saffron and its major constituent crocin was evaluated on three colorectal cancer cell lines (HCT-116, SW-480, and HT-29) compared to that of non-small cell lung cancer (NSCLC) cells by MTS assay. Results from this study showed that Crocus sativus extract and its major constituent, crocin, significantly inhibited the growth of colorectal cancer cells while not affecting normal cells.[49] Another study was reported that interaperiteneoal injection of crocin (400mg/kg body) for long term enhances survival selectively and decreases tumor growth in female rats with colon cancer, induced

by rat adenocarcinoma DHD/K12-PROb cells injected subcutaneously. Hormonal factors might be have effective role in the selective antitumor action of crocin in female rats compared with male.[50]

Liver cancer

The other study provided evidence that saffron exerts a significant chemopreventive effect against diethylnitrosamine (DEN)-induced liver cancer (HepG2) through inhibition of cell proliferation *via* induction apoptosis, modulating oxidative damage and suppressing inflammatory response. These findings showed inhibition of nuclear factor-kappa B activation, increased cleavage of caspase-3, as well as DNA damage and cell cycle arrest upon saffron treatment.[50] In another study ROS did not play effective role in the cytotoxic effect of saffron extract on HepG2 cell lines, in which apoptosis or programmed cell death plays an important role.[36] In one study was focused on antiproliferative effects of crocin on HepG2 cells. At this study melting temperature of a synthetic telomeric oligonucleotide was measured in the presence of crocin by using FRET analysis to examine two probably mechanisms of crocin inhibition. Two mechanisms include of the interaction of crocin with telomeric quadruplex sequences and down regulation of hTERT expression. Data indicated that telomerase activity of HepG2 cells decreases after treatment with crocin, which is probably caused by down-regulation of the expression of the catalytic subunit of the enzyme.[51] The cytotoxicity and DNA-adduct formation of rat liver microsomes activated by aflatoxin B1 (AFB1) in the C3H10T1/2 fibroblast cells are significantly inhibited by pretreatment of crocetin.[52] Crocetin treatment resulted in a decrease in AFB1-DNA adduct formation *in vitro* that suggested the protective effect of crocetin on the AFB1-cytotoxity due to the elevation of cytosolic glutathione (GSH) following the activities of GSH-S-transferase (GST) formation as cellular defense mechanisms. Crocetin pretreatment in rats protected hepatic AFB1-induced hepatic damage and AFB1-DNA adducts formation due to the elevation hepatic GSH, activities of GST and glutathione peroxidase (GSH-Px).[53] In another study, significant suppression of AFB1-induced hepatotoxic lesions was observed as indicated by reduction of activities of serum aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase and gamma-glutamyl transpeptidase (GGT) by crocetin in rats.[54] Inhibitory effect of crocetin on benzo[*a*] pyrene-induced genotoxicity and neoplastic transformation in C3H1OT1/2 cells is due to a mechanism that increased the activity of GSH and reduced the formation of benzo[*a*] pyrene-DNA adducts.[55] Crocetin also inhibited the formation of malondialdehyde (MDA), a marker for lipid peroxidation, induced by reactive oxygen species (ROS) generated by the activity of xanthine oxidase (XO) in primary hepatocytes and protected against oxidative damage.[56] Therefore, these studies indicated that crocetin displayed protective action against the ROS due to direct scavenging that inhibited free radical production following neoplastic transformation.[55,56]

Pancreatic cancer

One study was designed to investigate apoptotic effect of crocin, a main component of Crocus sativus in a human pancreatic cancer cell line (BxPC-3). In this study Hoechest33258 staining was used to detect the chromatin condensation characteristic of apoptosis, and DNA fragmentation was assessed by gel electrophoresis and cell cycle analysis by flow cytometry. According these results, cancer cell line is highly sensitive to crocin-mediated growth inhibition and apoptotic cell death.[57]

Table 1: Anticarcinogenic effects of saffron and proposed mechanisms

Types of cancers	Cell lines/ animal models	Mechanism of action	References
Skin cancer	Swiss albino mice	Immunologically mediated	Nair *et al.*[17]
	Swiss albino mice	Metabolic conversion of carotenoids to retinoids	Nair *et al.*[19]; Tarantilis *et al.*[18]
	Swiss albino mice	Induction of cellular antioxidant systems and modulation lipid peroxidation	Salomi *et al.*[24]; Das *et al.*[25]; Feizzadeh *et al.*[26]
Lukemia	Mice	Inhibition of genotoxicity	Premkumar *et al.*[32]
cervical cancer	HeLa	Inhibition of DNA and RNA synthesis	Abdullaev and Frenkel.[34]
	HeLa	Induction of apoptosis	Tavakkol-Afshari *et al.*[36]
Lung cancer	A549, WI-38, VA-13	Inhibition of DNA and RNA synthesis	Abdullaev and Frenkel.[35]
	A549	Induction of apoptosis	Samarghandian *et al.*[46]
Breast cancer	MCF-7	Induction of apoptosis;	Mousavi *et al.*[45]
	MCF-7	Induction of cytotoxicity and inhibition of cell proliferation	Samarghandian *et al.*[42]
Colorectal cancer	HCT116	Induction of apoptosis;	Bajbou *et al.*[48]
	HCT-116, SW-480, and HT-29	Induction of cytotoxicity and inhibition of cell proliferation	Aung *et al.*[49]
Liver cancer	HepG2	Induction of cytotoxicity and inhibition of cell proliferation	Amin *et al.*[50]
	HepG2	Induction of apoptosis	Tavakkol-Afshari *et al.*[36]

At the series of experiments was shown that crocetin has a significant antitumorigenic effect on both the *in vitro* pancreatic cancer cells and *in vivo* athymic nude mice tumor *via* induction apoptosis. At the *in vitro* studies, pancreatic cancer cells (MIA-PaCa-2), crocetin were significantly altered cell cycle proteins (Cdc-2, Cdc-25C, Cyclin-B1) and epidermal growth factor receptor (EGFR) and the *in vivo* results showed significant regression in tumor growth with inhibition of proliferation as determined by proliferating cell nuclear antigen and epidermal growth factor receptor expression in the crocetin-treated animals compared with the controls. Both the *in vitro* pancreatic cancer cells and *in vivo* athymic nude mice tumor, apoptosis was significantly stimulated as indicated by Bax/Bcl-2 ratio.[58]

CONCLUSION

Scientists worldwide are more attracted to indicate that consumption of saffron positively correlates with a lower risk of many types of cancers, and they also investigated the attribution of the large number of phytochemicals in saffron. Among these phytochemicals, crocins, crocetin, picrocrocin, and safranal are considered the most medicinally bioactive and the most frequently examined in many *in vitro* and *in vivo* studies.[59] Different hypotheses for the mode of anticancer action of saffron and its ingredients have been proposed [Tables 1, 2 and 3] and in detail discussed in our review. To date, the exact mechanism of anticancer effect of saffron is not clear.

Table 2: Anticarcinogenic effects of crocin and proposed mechanisms

Types of cancers	Cell lines/animal models	Mechanism of action	References
Lukemia	L1210, HL-60	Induction of cytotoxicity and inhibition of cell proliferation	Morjani *et al.*[30]; Tarantilis *et al.*[18]
	L1210, P388	Inhibition DNA synthesis	Nair *et al.*[17]
Cervical cancer	HeLa	Induction of apoptosis	Mousavi *et al.*[37]; Escribano *et al.*[38], Garcı×a-Olmo *et al.*[40]
Breast cancer	MCF-7	Induction of apoptosis;	Mousavi *et al.*[37]
Colorectal cancer	HCT-116, SW-480, and HT-29	Induction of cytotoxicity and inhibition of cell proliferation	Aung *et al.*[49]
Liver cancer	HepG2	Induction of cytotoxicity and inhibition of cell proliferation	Noureini *et al.*[51]
Pancreatic cancer	BxPC-3	Induction of cytotoxicity and inhibition of cell proliferation	Bakshi *et al.*[57]

Table 3: Anticarcinogenic effects of crocetin and proposed mechanisms

Types of cancers	Cell lines/Animal models	Mechanism of action	References
Skin cancer	Mice		Gainer *et al.* [27]
	Mice		Mathews-Roth *et al.* [28]
	Mice	Inhibition intracellular nucleic acid and protein synthesis and protein kinase C (PKC)	Giaccio *et al.* [29]
Lukemia	L1210, P388	Inhibition DNA synthesis	Nair *et al.* [17]
	K562, HL-60	Induction of cytotoxicity and inhibition of cell proliferation	Morjani *et al.*[30] Tarantilis *et al.* [18]
Cervical cancer	HeLa	Induction of apoptosis	Zhong *et al.* [41]
Lung cancer	Swiss albino mice (B[a] P)	Inhibition lipid peroxidation and increase of the activity of antioxidant enzyme	Magesh *et al.* [43]
	Swiss albino mice (B[a] P)	Induction of cytotoxicity and inhibition of cell proliferation	Magesh *et al.* [44]
	A549 lung carcinoma	Inhibition DNA, RNA and protein synthesis	Abdullev. [39]
Breast cancer	MCF-7	Induction of apoptosis;	Mousavi *et al.*[45]
	MCF-7, MDA-MB-231	Inhibition of proliferation by downregulation of MMP expression	Chryssanthi *et al.*[47]
Liver cancer	Wistar rat (AFB1)	Inhibition lipid peroxidation increase of the activity of antioxidant enzyme	Wang *et al.*[54]
	C3H1OT1/2 cells		Chang *et al.*[55], Tseng *et al.*[56]
Pancreatic cancer	MIA-PaCa-2, BxPc3, Capan-1 and ASPC-1 cells; Athymic mice xenograft model (MIA-PaCa-2)	Inhibition of proliferation and induction apoptosis	Dhar et la. [58]

PKC=Protein kinase C

However, the most medicinally bioactive of saffron belong to carotenoids. Carotenoids exhibit biological activities as antioxidants, affect cell growth regulation, and modulate gene expression and immune response.[60-62] Several studies have pointed out the use of some of them, such as β-carotene, α-carotene, lycopene, zeaxanthin or canthaxantine, in cancer prevention and therapy.[20,21,63-67] Because most carotenoids are lipid-soluble and might act as membrane-associated. Free-radical scavengers, the antioxidant properties of these compounds could prevent DNA, RNA and protein damages induced by free radicals and free radical chain reactions.[1] This review suggests that the anticancer activity of saffron and its compounds related to antioxidant properties of carotenoids of saffron. However, present findings have not yet been verified by clinical trials in humans and in-depth studies need to define efficacy of saffron in cancer treatment and prevention. However, the scarcity and expense in obtaining large quantities of saffron may provide impediments to human chemoprevention and cancer treatment using this agent.

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