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Review

Saffron and natural carotenoids: Biochemical activities and anti-tumor effects

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ABSTRACT

Saffron, a spice derived from the flower of *Crocus sativus*, is rich in carotenoids. Two main natural carotenoids of saffron, crocin and crocetin, are responsible for its color. Preclinical studies have shown that dietary intake of some carotenoids have potent anti-tumor effects both in vitro and in vivo, suggesting their potential preventive and/or therapeutic roles in several tissues. The reports represent that the use of carotenoids without the potential for conversion to vitamin A may provide further protection and avoid toxicity. The mechanisms underlying cancer chemo-preventive activities of carotenoids include modulation of carcinogen metabolism, regulation of cell growth and cell cycle progression, inhibition of cell proliferation, anti-oxidant activity, immune modulation, enhancement of cell differentiation, stimulation of cell-to-cell gap junction communication, apoptosis and retinoid-dependent signaling. Taken together, different hypotheses for the antitumor actions of saffron and its components have been proposed such as a) the inhibitory effect on cellular DNA and RNA synthesis, but not on protein synthesis; b) the inhibitory effect on free radical chain reactions; c) the metabolic conversion of naturally occurring carotenoids to retinoids; d) the interaction of carotenoids with topoisomerase II, an enzyme involved in cellular DNA-protein interaction. Furthermore, the immunomodulatory activity of saffron was studied on driving toward Th1 and Th2 limbs of the immune system. In this mini-review, we briefly describe biochemical and immunological activities and chemo-preventive properties of saffron and natural carotenoids as an anticancer drug.

Contents

1. Introduction

Natural products are of particular interest as chemo-preventive agents because of their low toxicity and potent efficacy [1]. They have long been used to prevent and treat various diseases including cancers (e.g., renal cell cancer) [2,3]. Several reports have shown that low intake of fruits and vegetables form a risk factor for chronic diseases such as cancer, coronary heart disease (CHD), stroke and cataract formation [4]. Indeed, high consumption of carotenoid-rich fruits and vegetables could offer a protective effect by increasing LDL-oxidation resistance, lowering DNA damage and inducing higher repair activity in European

Abbreviations: DMC, dimethylcrocetin; CHD, coronary heart disease; LDL, low density lipoprotein; ROS, reactive oxygen species; PCa, prostate cancer; COX-2, cycloxygenase-2; NF-κB, nuclear factor-κB; RAR, retinoid-like receptors; PPARγ, Peroxisome proliferator-activated receptors; SXR/PXR, steroid/xenobiotic receptor/pregnane X receptor; CAR, constitutive androstane receptor; IGF-1, insulin growth factor-1; SOD, superoxide dismutase; CAT, catalase; GPx, glutathione peroxidase; GJC, gap junctional communication; CD, circular dichroism; AGS, gastric adenocarcinoma; ctDNA, calf thymus DNA; MHC-I, major histocompatibility complex class I; NK-cells, natural killer cells; Th, T-helper; NO, nitric oxide; HepG-2, hepatocellular carcinoma cell line; Hep-2, laryngeal carcinoma cell line; COPD, chronic obstructive lung disease

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subjects [4]. The studies suggested that a diet characterized by a large quantity of vegetables and a great variety of both fruit & vegetable intake is associated with a reduced risk of type 2 diabetes [5]. In contrast, some studies indicated that higher intakes of specific dietary carotenoids, vitamin C, vitamin E as well as multi-vitamins were not associated with reduced risk of MS among women [6]. Intake of certain antioxidant micronutrients, particularly β-cryptoxanthin and supplemental zinc, and possibly diets high in fruits and cruciferous vegetables, could be protective against the development of rheumatoid arthritis; but, there was no association with total carotenoids, α- or β-carotene, lycopene and lutein/zeaxanthin [7]. Some studies indicated that natural products could be more effective than a dietary supplement. For example, black tea polyphenols significantly inhibited rat ovarian and human placental aromatase activities. These compounds also suppressed the proliferation in MCF-7 cancer cells [8]. On the other hand, there is a growing body of literature on the role of β-carotene and other carotenoids in human chronic diseases including cancer. Epidemiological evidence showed that a high dietary intake of fruits and vegetables rich in carotenoids is associated with a reduced risk for cancer [9]. For instance, lycopene in tomato is a powerful antioxidant which can neutralize the free radicals thereby conferring protection against macular degenerative disease; lung, bladder, cervix, skin, prostate and breast cancers; atherosclerosis and associated coronary artery diseases along with oral leukoplakia [10]. Lycopene reduces low density lipoprotein (LDL) oxidation thus reducing cholesterol levels in the blood. Its treatment has been shown to cause a 73% suppression of cellular cholesterol synthesis in J-774A.1 macrophage cell line and augment the activity of macrophage LDL receptors [10]. New reports suggest that the lycopenoids are biologically active and may reduce the risk for chronic diseases as well as influence androgen metabolism in rodent models [11]. The studies have shown that the use of lycopene in combination with other dietary agents, are the most promising treatments against cancer [12]. For example, lycopene enhances the anti-proliferative and apoptotic effects of capsaicin, the active compound in chili peppers, in in vitro prostate cancer (PCa) model. Capsaicin can induce apoptosis through the generation of reactive oxygen species (ROS), dissipation of the mitochondrial inner transmembrane potential and downstream activation of the caspase-3 cascade [12]. In addition, flavonoids are polyphenolic compounds that are abundant in fruits and vegetables. These components have anti-oxidant properties as well as anti-viral, anti-allergic, anti-inflammatory and anti-tumor activities. Flavonoids are generally classified as flavonols, flavones (e.g., apigenin and luteolin in green leafy spices), flavanones, flavanols, isoflavones or anthocyanidins based on their chemical structure [13]. Epidemiologic investigations and human clinical trials showed that flavonoids have important effects on cancer chemoprevention and chemotherapy including the treatment of mammary and prostate cancer. Flavonoids play a major role by interacting between different types of genes and enzymes. Many mechanisms of action have been identified including carcinogen inactivation, anti-proliferation, cell cycle arrest, induction of apoptosis, inhibition of angiogenesis, anti-oxidation and reversal of multi-drug resistance or a combination of these mechanisms [14]. For example, flavonoids exert their anti-inflammatory activities by inhibiting cycloxygenase-2 (COX-2) in colon cancer cells. Furthermore, they induce apoptosis and suppress the growth of colon cancer cells by inhibiting the COX-2 and Wnt/epidermal growth factor receptor/nuclear factor-κB (NF-κB) signaling pathways [13]. Recent studies have shown that some flavonoids are modulators of pro-inflammatory gene expression, thus leading to the attenuation of the inflammatory response [15].

In general, the medicinal plants are playing an important role in cancer prevention and therapy in several ways:

1. Plants represent a potential source for anti-cancer compounds. Their anti-tumor activity may result via some mechanisms such as a) effects on cytoskeletal proteins which play a key role in cell division, b) inhibition of DNA topoisomerase enzymes, c) anti-protease or antioxidant activity and d) stimulation of the immune system [16]. In this regard, natural compounds with strong anti-oxidative, hepatoprotective and anti-inflammatory effects are good candidates to evaluate their ability of influence on the initiation and growth of tumors [17].
2. Plants can delay or prevent cancer onset [16].
3. Plants can support the immune system, improving body resistance to the disease [16].
4. Plants can prevent and decrease side effects of conventional treatments [16].
5. Plants can provide nutritional and psychological support [16].

As known, several hundred theories have proposed to explain aging phenomenon. One of the most popular is the “oxidative stress theory”. The endocrine system seems to have a role in the modulation of oxidative stress; however, much less is known about the role of oxidative stress in the aging of the endocrine system and the induction of age-related endocrine diseases [18]. The mechanisms such as cell senescence, mitochondrial dysfunction and microRNA dysregulation, as well as inflammation itself, can be considered to elucidate the effects of oxidative stress on aging of endocrine glands as well as the antioxidant effects of natural compounds [18]. Among natural products, saffron (*Crocus sativus* L.) stigmas are included secondary metabolites such as terpenes, flavonoids, anthocyanins and carotenoids [19]. Between them, carotenoids are the most important molecules possessing potent chemopreventive properties [20]. *C. sativus* possesses a number of medicinally important activities such as antihypertensive, anticonvulsant, antitussive, antigenotoxic and cytotoxic effects, anxiolytic aphrodisiac, antioxidant, antidepressant, antinociceptive, anti-inflammatory and relaxant activity. It also improves memory and learning skills and increases blood flow in retina and choroid [21]. Herein, we have concentrated on properties of saffron (*C. sativus* L.) and its major components especially carotenoids, as an anti-tumor compound associated with short description of other main components.

2. Saffron and its components

Saffron (*C. sativus* L.) is a species belonging to the Iridaceae family cultivated in Iran, Europe, Turkey, Central Asia, India, China and Algeria and has a wide range of activities including: a) oxytocic, b) anti-carcinogenic, c) exhilarant, d) anti-depressant and e) anti-asthma effects [22]. Saffron has been widely used as an herbal medicine, spice, food coloring and a flavoring agent since ancient times. It can increase the bioavailability and enhance absorption of other drugs [22].

Chemical analysis has shown the presence of more than 150 components in saffron stigmas [23]. *C. sativus* stigmas are characterized by the presence of sugars, minerals, fats, vitamins and secondary metabolites including terpenes, flavonoids, anthocyanins and carotenoids. Between them, carotenoids are the most important molecules because they determine color and taste of the spice [19]. From these compounds, we can mention lycopene, α- and β-carotene, zeaxanthin, crocetin (liposoluble) and crocins (hydrosoluble) derived by crocetin esterification with sugars. Crocins are *trans*-crocetin di-(β-D-gentiobiosyl) ester (named *trans*-4-GG), *trans*-crocetin (β-D-glucosyl)-(β-D-gentiobiosyl) ester (named *trans*-3-Gg), *trans*-crocetin (β-D-gentiobiosyl) ester (named *trans*-2-G), *cis*-crocetin di-(β-D-gentiobiosyl) ester (named *cis*-4-GG), *trans*-crocetin di-(β-D-glucosyl) ester (named *trans*-2-gg) and *cis*-crocetin (β-D-glucosyl)-(β-D-gentiobiosyl) ester (named *cis*-3-Gg) [19]. Crocetin is a natural carotenoid dicarboxylic acid that forms brick red crystals with a melting point of 285 °C. Its chemical structure is the central core of crocins [24]. Saffron has three main chemical constituents, which are so-called as crocin, picrocrocin and safranal. The color of saffron is due to the presence of crocin(s), which have glycoside carotenoid structure [22,25]. Crocin has a deep red color and it forms crystals with a melting point of 186 °C [24]. The bitter taste of saffron is attributed to picrocrocin [22,25].

Picrocrocin is a degradation product of the zeaxanthin carotenoid and also a monoterpene glycoside precursor of safranal [24]. Safranal is an aromatic aldehyde which is the main component of plant volatile oil [22]. It composes ~70% of total volatiles from saffron flowers. There are also some compounds synthesized from extracts of *C. sativus*, e.g., dimethylcrocetin (DMC) [24]. Fig. 1 shows the structures of main saffron components.

Carotenoids are terpenoid and ubiquitous in nature which can be synthesized in vivo through two different pathways: 1) mevalonic acid (MVA) pathway in the cytoplasm and 2) non-mevalonic acid pathway (2-C-methyl-D-erythritol 4-phosphate pathway: MEP) in plastids that provides the precursors for carotenoids. The MVA pathway starts with synthesis of mevalonate through three molecules of acetyl CoA and then continues with production of isopentenyl diphosphate (IPP) molecules, geranyl geranyl pyrophosphate (GGPP), color less phytoene, colored lycopene, β-carotene and zeaxanthin [26,27]. This pathway possesses many enzymes which catalyzed the reactions and coded by related key genes such as *PSY*, *LYC*, *CCD*, *BCH* and *ZCD*. β-carotene with two rings is built up via cyclization of lycopene with lycopene-β-cyclase (*LYC*). The hydroxylation of β-carotene in MVA pathway is catalyzed by β-carotenoid hydroxylase that coded by *BCH* gene to yield zeaxanthin. The biogenesis of the color and odor active compounds of saffron are derived by bio-oxidative cleavage of zeaxanthin at the points 7, 8 (7′, 8′) by zeaxanthin cleavage dioxygenase to produce crocetin dialdehyde and picrocrocin. In *C. sativus* stigmas, the final step involves glucosylation of the generated zeaxanthin cleavage products by glucosyltransferase enzyme in chromoplast of stigmas [26,27]. Fig. 2 shows the biosynthesis pathway of saffron components.

Saffron tablets (200–400 mg) changed some hematological and biochemical parameters in normal ranges that they were not important clinically. Saffron (200 mg) showed a positive effect on sexual function with increased number and duration of erectile events in patients with erectile dysfunction after taking it for ten days [28]. Furthermore, *C. sativus* L. and its constituents were effective in different models of psychiatric disorders including anxiety and depression. Crocins are among the active components of saffron. A study indicated that crocins might play a role in compulsive behavior and support a functional interaction between crocins and the serotonergic system [29].

There is a growing interest in the effects of saffron carotenoids on human health due to their high antioxidant capacity, e.g., many natural and synthetic compounds present in *C. sativus* flowers [30–32]. Reactive oxygen species such as hydrogen peroxide, superoxide anion radical, peroxyl radical, hydroxyl radical, are the most important sources of oxidative damage in human body. Various mechanisms of action may be related with the antioxidant effect of saffron extracts: a) interaction with enzymes (superoxide dismutase: SOD, peroxidase) or with signal transduction of free radicals in the monocytes. This is possible because the presence of sugar moieties attached to the terminal COOH groups of the crocetin skeleton (crocin structure) plays a role in the penetration of cell membranes. Free radicals are produced in hypoxic conditions after stimulation by the addition of insulin. Incubation of insulin-stimulated monocytes with saffron extracts results in a marked reduction of free radicals production, almost counteracting the negative effect of insulin; b) direct suppression of ROS that varies depending on the compounds [30–32]. Free radical scavenging activity of crocetin is superior to dimethylcrocetin. The reasons for that are the structural features of the two carotenoids. The length of the conjugated double bonds is the same. The difference between the two carotenoids is the presence of hydroxyl moiety of the carboxylic group in crocetin and the presence of methyl ester group on the termini of unsaturated hydrocarbon chain in dimethylcrocetin. Free radicals tend to react with H-donors; thus, the presence of hydroxyl in carboxylic group in crocetin makes it more effective than dimethylcrocetin. Furthermore, the antioxidant activity in crocetin enhances as the concentration increases; but in dimethylcrocetin, it reaches a certain point and then it starts to decrease [30–32]. Moreover, safranal has lower antioxidant activity than crocetin and dimethylcrocetin. Therefore, treatment with crocin present in saffron can reduce brain ischemia volume, increase SOD & glutathione peroxidase (GPx) activities and decrease malondialdehyde (MDA) content. MDA is a biomarker of oxidative stress, because it was generated from ROS during poly-unsaturated lipids degradation [30–32].

Generally, the studies in animal models and with cultured human malignant cell lines have demonstrated anti-tumor and anti-cancer activities of saffron [33]. Saffron extracts were shown to inhibit papilloma growth, decline the incidence of squamous cell carcinoma and soft tissue sarcoma in mice [17]. In fact, high levels of carotenoids may enhance clearance of HPV infection and avoid persistent infection. Different hypotheses for anti-tumor effects of saffron and its ingredients have been proposed including: a) inhibition of DNA and RNA synthesis, but not protein; b) ability to scavenge free radicals; c) involvement in the metabolic conversion of carotenoids to retinoids; d) mediation of interactions of carotenoids with topoisomerase II (an enzyme involved in cellular DNA–protein interaction) [33]. Despite the accumulating evidence demonstrating that saffron may be a promising cancer therapy agent, mechanisms of its anti-cancer actions are still largely unknown.

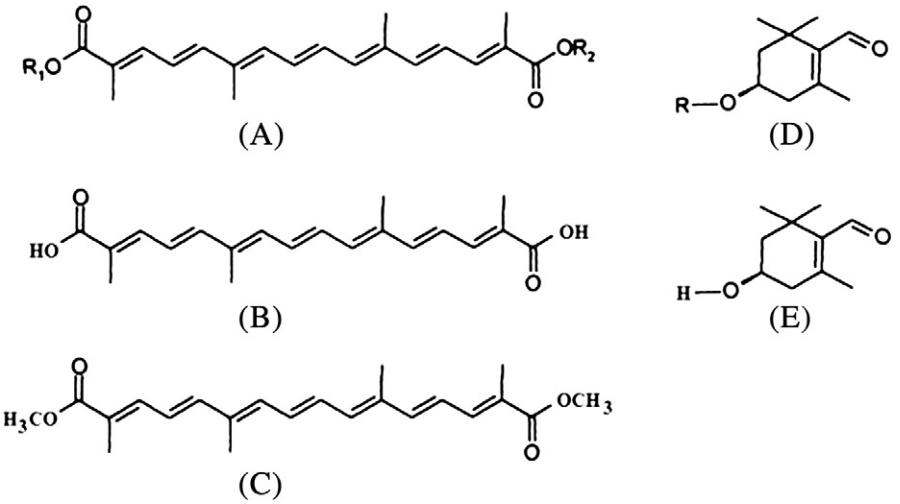

Fig. 1. Structures of various saffron ingredients: A) crocin: R1 = R2 = β-D-gentiobiosyl and/or glucose; B) crocetin: R1 = R2 = H; C) dimethylcrocetin: R1 = R2 = CH_3; D) picrocrocin: R = β-D-gentiobiosyl and/or glucose; E) safranal is the aglycone dehydrated derivative of picrocrocin: R = H.

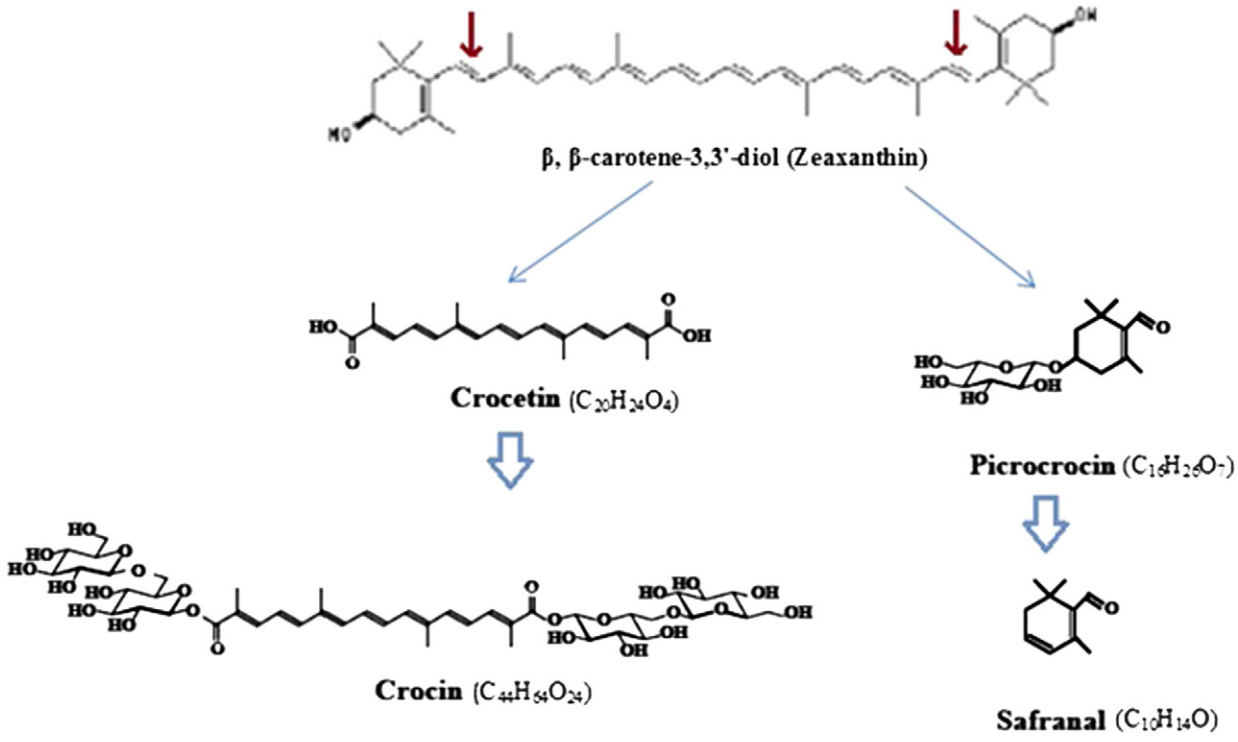

Fig. 2. The biosynthesis of the crocin and picrocrocin derived from the bio-oxidative cleavage of zeaxanthin at points 7, 8 (7′, 8′) as shown by arrows.

3. Carotenoids

Carotenoids are synthesized in sub-cellular organelles (plastids, i.e. chloroplasts and chromoplasts) of plants, but not in animals [Table 1; 34]. Up to now, more than 600 types of carotenoid have so far been isolated from natural sources. About 40 types are present in a typical human diet and ~20 carotenoids have been determined in human blood and tissues [34]. Carotenoids are polyisoprenoid compounds and can be divided into two major groups: a) hydrocarbon carotenoids (known as carotenes) and b) oxygenated hydrocarbon derivatives (known as xanthophylls). Their structural characteristic is a conjugated double bond system (natural isoprenoid pigments), which influences their chemical, biochemical and physical properties [34]. The carotenoid biosynthetic pathway has been extensively studied in a range of organisms providing an almost complete pathway for carotenogenesis. Developmental and environmental signals can regulate carotenoid gene expression thereby affecting carotenoid accumulation [35]. The most abundant carotenoids in human serum are β-carotene (vitamin A precursors; male: 0.47 μmol/L, female: 0.41 μmol/L), α-carotene (male: 0.065 μmol/L, female: 0.081 μmol/L), β-cryptoxanthin (partial vitamin A activity; 0.13 μmol/L), lycopene (male: 0.47 μmol/L, female: 0.41 μmol/L), lutein and zeaxanthin (non-vitamin A precursors; 0.35 μmol/L) [Fig. 3; 36–39]. Indeed, vitamin A can either be obtained from preformed vitamin A, such as retinol and retinyl esters or from pro-vitamin A carotenoids (i.e., β-carotene, α-carotene and β-cryptoxanthin) which are converted to vitamin A in the body [37]. For dietary carotenoids to be absorbed intestinally, they must be released from the food matrix and incorporated into mixed micelles (mixtures of bile salts and several types of lipids). Therefore, carotenoid absorption requires the presence of fat (~3–5 g) in a meal. Because they do not need to be released from the plant matrix, carotenoids supplements (in oil) are more efficiently absorbed than carotenoids in foods [35]. Within the cells of intestine (enterocytes), carotenoids are incorporated into triglyceride-rich lipoproteins called chylomicrons and released into the circulation. Triglycerides are depleted from circulating chylomicrons through the activity of an enzyme called lipoprotein lipase, resulting in the formation of chylomicron remnants. Chylomicron remnants are taken up by the liver, where carotenoids are incorporated into lipoproteins and secreted back into the circulation. In the intestine and the liver, provitamin A carotenoids may be cleaved to produce retinal (a form of vitamin A) [35]. The conversion of provitamin A carotenoids to vitamin A is influenced by the vitamin A status of the individual. Although the regulatory mechanism is not yet clear in humans, cleavage of provitamin A carotenoids appears to be inhibited when vitamin A stores are high [35].

In general, carotenoids possess potent cancer chemo-preventive properties [20,38,40]. The mechanisms underlying cancer chemo-preventive activities of carotenoids include modulation of carcinogen metabolism; regulation of cell growth and cell cycle progression [e.g., cell-cycle delay in the G1 phase, reduction in cyclin D1 protein levels, inhibition of both Cdk4 and Cdk2 kinase activity, hypo-phosphorylation of pRb], inhibition of cell proliferation; anti-oxidant activity; immune modulation; enhancement of cell differentiation [e.g., activation of differentiation-related proteins: retinoid-like receptors (RAR), nuclear receptors effective in the differentiation of adipocytes (PPARγ), xenobiotic and orphan nuclear receptors such as the steroid/xenobiotic receptor/pregnane X receptor (SXR/PXR)/or the constitutive androstane receptor (CAR), reduction of growth factor-induced stimulation of AP-1

Table 1
Sources of main carotenoids.

Carotenoids	Dietary sources
Alpha-carotene	Yellow-orange vegetables, dark-green vegetables
Beta-carotene	Green leafy vegetables and orange and yellow fruits and vegetables
Beta-cryptoxanthin	Orange fruits, corn, peas, egg yolks
Lycopene	Tomatoes, water melon, apricot, peaches
Lutein/zeaxanthin	Dark green leafy vegetables, red peppers, maize, tomatoes, corn, egg yolks
Astaxanthin	Green algae, salmon, trout, Crustacea
Canthaxanthin	Salmon, Crustacea
Fucoxanthin	Brown algae, heterokonts

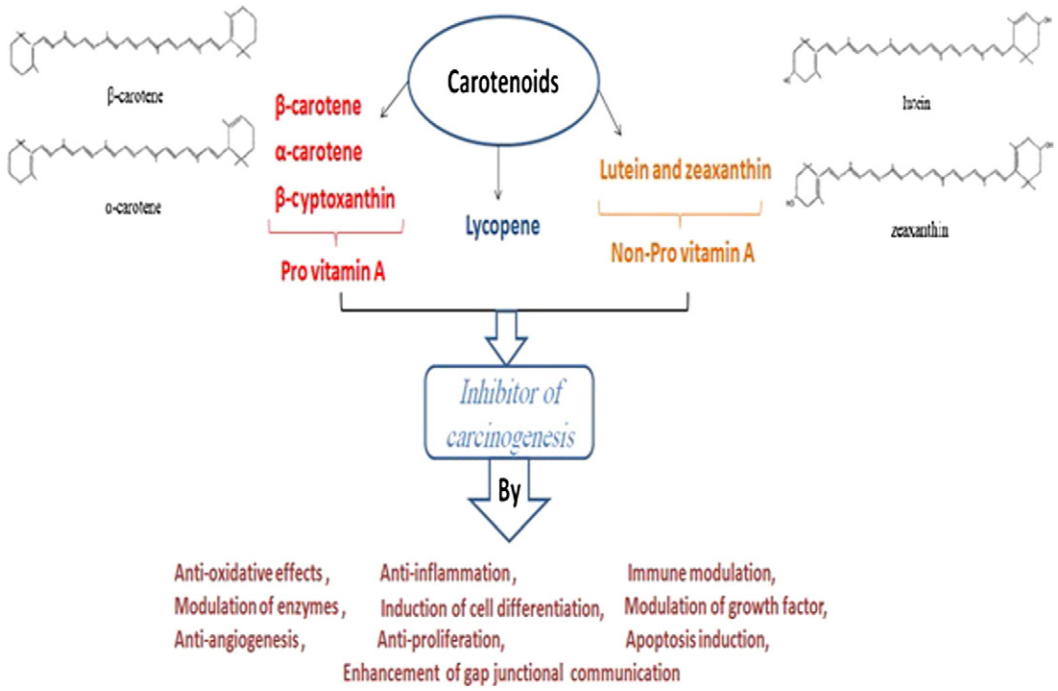

Fig. 3. Inhibitory effects of carotenoids (provitamin A and/or non-provitamin A) on carcinogenesis.

transcriptional activity by altering the composition of AP-1 complexes bound to DNA, inhibition of Wnt/β-catenin signaling via the connection along the Akt/GSK3β/β-catenin]; hormone and growth factor signaling [e.g., inhibition of insulin growth factor (IGF)-1]; stimulation of cell-to-cell gap junction communication; apoptosis and retinoid-dependent signaling [34,38,41]. Fig. 3 shows the suggested mechanisms of carotenoids on carcinogenesis. The findings suggest that the use of carotenoids without the potential for conversion to vitamin A may provide further protection and avoid toxicity. For example, saffron carotenoid, crocin, is of considerable interest because of its potent anti-inflammatory, anti-carcinogenesis and anti-oxidant activities, which are distinctly different from those of β-carotene and other carotenoids [20]. The studies showed that the use of a high-dose supplemental β-carotene can generate a protective activity against lung cancer, but it failed to demonstrate a sufficient protective effect in clinical trial. In contrast, any clinical or histopathological toxicity was not observed after crocin treatment. Furthermore, crocin had stronger antioxidant activity compared to α-tocopherol [35]. Indeed, the anti-inflammatory effects of crocin are suggested to be based on its anti-oxidant activity. Many in vitro studies have demonstrated that extracts of saffron and its components are able to inhibit the growth of several types of human cancer cells as reported in the next sections. However, there have already been few in vivo studies conducted to demonstrate the anti-cancer effects of saffron and its constituents [35]. Altogether, the data indicate that the anti-proliferative effect of carotenoids in different cancer cell lines (breast, colon, prostate …) was cellular type, time and dose-dependent [42].

4. Biochemical properties of saffron carotenoids

Dietary anti-oxidants are a substance present in food that significantly decreases the adverse effect of reactive species such as reactive oxygen and nitrogen on normal physiological function [43]. Recently, the chemistry, structure and pharmacological roles of dietary anti-oxidants in the management of free radicals associated diseases have further been studied. Free radicals can be formed in three ways: a) by the hemolytic cleavage of a covalent bond of a normal molecule, with each fragment retaining one of the paired electrons; b) from the loss of single electron from a normal molecule; c) by addition of single electron to a normal molecule [43]. The classification of dietary anti-oxidants is included as following [Fig. 4]:

A) Non-enzymatic and hormonal anti-oxidants (nutritional) such as vitamins [e.g., A, E (tocopherols and tocotrienols), C (ascorbic acid)], flavonoids, polyphenol anti-oxidants, vitamin co-factor and minerals, non-flavonoid phenolics and other antioxidants; B) enzymatic and hormonal antioxidants (non-nutritional) such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), melatonin and C) food anti-oxidants [43–45]. Lebanese saffron significantly decreased lipid peroxidation and increased superoxide dismutase activity in all tissues as compared to control, thus strongly protecting vital organs against oxidative stress [46]. Apolar carotenoids, such as lycopene and β-carotene, disordered the membrane bilayer and showed a potent pro-oxidant effect [>85% increase in lipid hydroperoxide (LOOH) levels, 47].

Both β-carotene and lycopene are recognized as dietary chemo-preventive agents, protecting against cancerogenesis [44]. Carotenoids are known for anti-oxidant activities including a) quenching free radicals, b) reducing damage from reactive oxidant species and c) inhibiting lipid peroxidation. In addition, these compounds facilitate cell-to-cell communication which regulates cell growth, differentiation and apoptosis [36]. The efficacy of individual carotenoids may depend on concentrations of other carotenoids. Indeed, carotenoids may interact synergistically and supplementation with a single carotenoid may be ineffective [36].

Carotenoids influence the strength and fluidity of membranes, thus affecting its permeability to oxygen and other molecules. For example, β-carotene, canthaxanthin, 4-hydroxy-β-carotene and the synthetic retro-dehydro-β-carotene show an efficient induction of the gap junctional communication (GJC) in murine fibroblasts [34]. In addition, lutein and zeaxanthin have been considered as protective agents against aging macular degeneration and senile cataracts. It has been suggested that β-carotene suppresses the increment of hormones related to stress syndrome [34]. As mentioned above, saffron is rich in carotenoids. Among many different probable mechanisms of anti-proliferative effect of saffron extract, a significant inhibition in the synthesis of nucleic acids but not of proteins [by dimethylcrocetin as a semi-synthetic product] was related to retardation of papilloma growth and disruption of DNA-protein interactions, e.g. topoisomerases II [2,17]. In

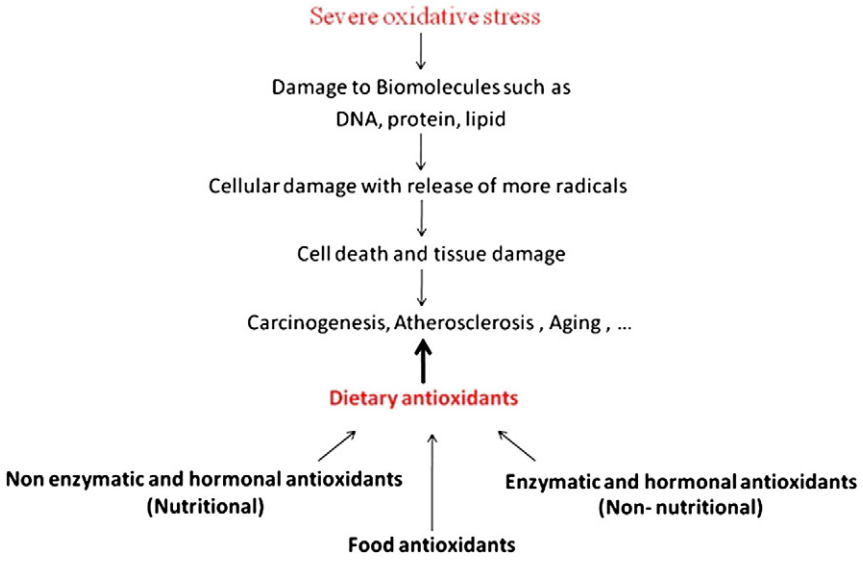

Fig. 4. Dietary antioxidants: the antioxidants decrease the adverse effects of reactive species, such as reactive oxygen and nitrogen on normal physiological function in humans. They delay or prevent the oxidation of substrate.

addition, the cardio-protective effects of saffron and its active components (e.g., crocetin and crocin) have been reported that are related to modulation of endogenous anti-oxidant enzymatic activities [48]. The methanolic extract of *C. sativus* flowers showed a significant inhibitory effect on tyrosinase activity of 28.22% [45].

Many studies have shown a variety of pharmacological effects of crocin [20]. Crocin derived from stigma of *C. sativus* has been confirmed as a powerful antioxidant, stronger than α-tocopherol [44]. Moreover, the aqueous extract of *C. sativus* stigmas and its constituents, crocin and safranal repressed the genotoxic potency of methyl methane sulfonate (MMS)-induced DNA damage in multiple mice organs (liver, lung, kidney and spleen) [28]. The anti-inflammatory effects of crocin are suggested to be based on its antioxidant activity [20]. Another possible mechanism of crocin in its neuroprotective properties could be inhibition of protein aggregation and fibrillar formation [17]. Crocin protects oxidative damage to the hippocampus and reduces plasma level of corticosterone after the end of stress [17]. The studies showed 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 (hTERT). In fact, telomerase activity in 0.5 μg protein extract of HepG2 cells treated with 3 mg/mL crocin was reduced to about 51% as compared to untreated control cells [17]. Indeed, high concentrations of crocin remarkably inhibited the enzyme in a dose-dependent manner. Thus, anti-proliferative effect of crocin in cancer cells is probably due to strong inhibition of telomerase activity [49]. Crocin revealed a dose- and time-dependent cytotoxic effect against the gastric adenocarcinoma (AGS) cell line. The increased sub-G1 population and activated caspases in the treated AGS cells confirmed its anticancer effect. Apoptosis was significantly stimulated by increasing the Bax/Bcl-2 ratio after crocin treatment [50]. The studies suggested a differential response between male and female rats with colon adenocarcinomas to crocin indicating the potential involvement of hormonal factors in tumorigenesis [51].

In a study, the possibility for application of the saffron components as sequence-specific drugs was investigated. Circular dichroism (CD) data indicated that crocetin has high affinity (a minor groove binding) for the I-motif and G-quadruplex, unique secondary structures of telomeric DNA [52]. Our group studied the possible molecular mechanisms of saffron and its purified components as an anti-cancer compound (e.g., interaction with DNA, H1 and H1-DNA complex). The existence of three types of picrocrocin and four types of crocin in Iranian saffron was proved using TLC and HPLC techniques [24,53]. As known, some anticancer drugs have direct interaction with calf thymus DNA (ctDNA) and GC and AT oligonucleotides. Our studies showed that carotenoids of Iranian saffron directly bind to DNA minor grooves and induce conformational changes of targeted DNA (a transition from B- to C-DNA) [33,54]. The potency of interaction with DNA is as following:

$$\text{Crocetin} > \text{Dimethylcrocetin(DMC)} >> \text{Crocin}.$$

Furthermore, structural results showed the non-intercalative/minor groove binding of monoterpene aldehydes family (picrocrocin and safranal) to ctDNA [55]. In fact, picrocrocin interacts with DNA and oligonucleotides at lower concentrations than safranal (a transition from B- to C-DNA). At higher concentrations, picrocrocin induces more conformational changes including un-stacking of DNA and oligonucleotide bases similar to saffron carotenoids. However, similar results are obtained for safranal interaction with ctDNA (56% AT) and oligo $(AT)_{15}$, but after adding safranal to oligo $(GC)_{15}$, the CD spectra indicated a characteristic of triple-helix DNA, i.e., a mechanism for B- to H-DNA transition [56]. Moreover, our data showed that both carotenoids and monoterpene aldehydes of saffron quenched the fluorescence emission of H1. These ligands demonstrated a shift in the precipitation curve to the left, which is due to the reduction on H1-DNA interaction between 5 to 22%. These results confirm that the anti-cancer effect of saffron and its components is due to their interaction with DNA and reduction in the H1-DNA complexes [24,53]. These observations suggest a mechanism in which the H1 depletion may promote transcription [57].

Another study also showed that crocetin reduces in vitro histone H1-DNA interaction and interfered with transcription. Recently, crocetin showed significant reduction of cell proliferation in both MCF-7 and MDA-MB-231 breast cancer cells through apoptosis mechanism [58]. In addition, the induction of apoptosis by saffron has been reported to play an essential role in the death of human hepatocellular carcinoma cells and HeLa cells [33]. Considering the fact that most tumors show a functional p53 inactivation, the effect of saffron in HCT116 colorectal cancer cells with different p53 status (+/−) indicated the induction of DNA-damage and apoptosis in both cell lines. However, further research is needed to elucidate the long-term effects of saffron in p53 −/− tumors [33]. Moreover, the reduction of spermatogenesis and its index in rat testis is the result of saffron orally administration probably via the reducing of blood testosterone level [59]. The current studies have demonstrated that crocetin can enhance the oxygen diffusivity through plasma. As a consequence of this property, crocetin increases alveolar oxygen transport and also pulmonary oxygenation.

It improves cerebral oxygenation in hemorrhaged rats and positively acts in the atherosclerosis and arthritis treatment. It inhibits skin tumor promotion in mice induced by benzo-α-pyrene through inhibitory effect on intracellular nucleic acid and protein synthesis in malignant cells, as well as on protein kinase-C in INNIH/3T3 cells. This is likely due to its antioxidant activity. Furthermore, crocetin suppresses aflatoxin B1-induced hepatotoxic lesions and has a modulatory effect on aflatoxin B1 cytotoxicity and DNA adduct formation on C3H10/T1/2 fibroblast cells. It also has a protective effect on the bladder toxicity, induced by cyclophosphamide [60].

Recently, several reports showed that lycopene can induce cell cycle arrest at the G1 phase in prostate and breast cancer cell lines after 48 h of its treatment. In breast cancer models, reduced expression of cell cycle regulatory proteins, such as cyclins D1 and E and the cyclin-dependent kinases 2 and 4, as well as suppression of insulin-like growth factor (IGF-I) action have been correlated with lycopene's effects on proliferation [42]. Some studies reveal that lycopene has been useful in lowering of mild hypertension and improving eyesight due to the production of vitamin A. Lycopene increases the High Density Lipid Levels (HDL) which is useful for the absorption of low density lipoprotein (LDL), thereby preventing atherosclerosis and hypercholesterolemia [10].

A study showed that lycopene can synergize with other phytonutrients in the inhibition of cancer cell growth. The mechanism underlying the anti-tumor effects of lycopene and other carotenoids may be related to the changes in the expression of many proteins participating in these processes including connexins, cyclins, cyclin-dependent kinases and their inhibitors. These changes in protein expression suggested that the initial effect involves modulation of transcription by ligand-activated nuclear receptors or by other transcription factors [61]. Considering the current studies, the beneficial effects of carotenoid-rich vegetables and fruits in relation to cancer risk have been found in many epidemiological studies. Provitamin A carotenoids (α-carotene, β-carotene and β-cryptoxanthin) combined with other antioxidants (ascorbic acid, α-tocopherol and lycopene) limit the oxidative cleavage products of carotenoids, formed in large quantities in the highly oxidative conditions of the smoke-exposed lung and enhance retinoid signaling, by blocking the activation of MAP kinase. It seems that the combination of provitamin A carotenoids and antioxidants could be employed as a chemopreventive strategy against certain human cancers [38]. As known, reactive oxygen species are important mediators to induce pancreatitis. Lycopene functions as a very potent antioxidant to suppress the induction of inflammatory cytokines, in pancreatic acinar cells. The data indicated that serum levels of antioxidant enzymes and carotenoids including lycopene are lower in patients with pancreatitis than those of healthy subjects [62].

5. Immunological functions of saffron carotenoids

Carotenoids, pro-vitamins A or not, have shown two beneficial effects to human health: a) enhancement of the immune response and b) reduction of the risk of degenerative diseases such as cancer, cardiovascular diseases, cataract and muscular degeneration [34]. The modulation of immune responses using carotenoids includes increasing natural killer cell (NK-cell) activity in the elderly, increasing the lymphocyte response to mitogens, protection of immune cells from their own bactericidal production of reactive species, increasing total white blood cells and CD4/CD8 ratio in HIV infected humans [43]. For example, β-carotene increased the number of receptor on white blood cells for a molecule known as major histocompatibility complex class I (MHC-I) [26]. The reports showed that saffron's ability to protect cells from oxidative stress by scavenging free radicals comes from the two main chemical components of saffron (i.e., crocin and crocetin) [63]. A clinically useful effect of crocin was observed in stimulation of dendritic cells that can particularly enhance the proliferation of T cells in mononuclear cells isolated from bone marrow of leukemia children [17].

Cancer frequently develops in inflamed tissues, suggesting that the inflammatory condition is closely related to carcinogenesis. Examples of this relationship are: chronic hepatitis (HBV and HCV infection) and liver cancer; Barrett dysplasia and esophageal cancer; chronic gastritis (*Helicobacter pylori* infection) and gastric cancer; and inflammatory bowel disease and colorectal cancer [38]. The nuclear factor-κB (NF-κB) signaling pathway has a major role in inflammation-associated carcinogenesis. Thus, the natural compounds that suppress NF-κB expression may be useful for cancer chemoprevention [20]. As known, inflammatory genes including COX-2, iNOS, tumor necrosis factor-α (TNF-α), IL-6 and IL-1β, are the most common target genes participating in the activation of NF-κB associated with a number of chronic inflammatory diseases [20,38]. The data showed that dietary crocin suppresses chemically induced colitis and colitis-related colon carcinogenesis in mice, by inhibiting inflammation, the mRNA expression of certain pro-inflammatory cytokines and inducible inflammatory enzymes [20]. A recent study demonstrated that astaxanthin also suppressed the expression of inflammatory cytokines and NF-κB, and inhibited inflammation-associated colon carcinogenesis in mice [38]. Recent studies have shown that a humoral immune response decreased circulating carotenoids and carotenoid-based coloration. For example, the intraperitoneal injection of lipopolysaccharide (LPS) of *Escherichia coli*, which promotes an inflammatory response followed by antibody production decreases plasma carotenoids and carotenoid-based coloration. These studies support the hypothesis that mounting an immune response may drain carotenoids from the blood stream [64].

A laboratory has used a transplantable mammary tumor model in BALB/c mice to study on the mechanism of action of dietary lutein against tumor growth. Dietary lutein increased IFN-γ mRNA expression but decreased the expression of IL-10 in splenocytes of tumor-bearing mice, suggesting the inhibitory action of lutein against tumor growth. IFN-γ has several immuno-regulatory actions. It is produced by activated T cells and NK cells, and is a potent inducer of macrophage activation and MHC class II molecules. In contrast, IL-10 inhibits IFN-γ production, antigen presentation and IL-1, IL-6 and TNFα production by macrophages. The anti-tumor and immuno-modulatory actions of lutein suggest the involvement of subcellular events such as apoptosis, angiogenesis and gene regulation [65]. Dietary lutein decreased apoptosis in blood leukocytes from tumor-bearing mice compared to unsupplemented mice, suggesting an increased immune status. On the other hand, apoptosis in tumor cells was increased by dietary lutein, suggesting increased death of tumor cells. These results demonstrate a selective action of lutein by decreasing apoptosis in immune cells but increasing apoptosis in tumor cells [65]. In addition, dietary lutein increased the mRNA expression of the proapoptotic genes p53 and BAX, decreased the expression of the anti-apoptotic gene Bcl-2, and increased the BAX: Bcl-2 ratio in tumors. The p53 tumor suppressive gene can induce cell cycle arrest to allow DNA repair or apoptosis. Bcl-2 functions as a suppressor of apoptotic death and is negatively regulated by wild type p53 [65]. The predominance of BAX over Bcl-2 accelerates apoptosis. Bcl-2 resides in the outer mitochondrial membrane and prevents cytochrome c release. BAX is inactive until it is translocated to the mitochondria where it binds to Bcl-2 to induce cytochrome c release. Once released, cytochrome c activates caspases to bring about apoptosis. Uncontrolled cell proliferation can lead to cancer and autoimmune diseases whereas excessive cell death can lead to neurodegenerative diseases and AIDS [65]. Cytochrome c located between the inner and outer mitochondrial membranes plays a critical role in the apoptotic process. The release of cytochrome c is regulated by the pro-apoptotic proteins BAX, BID and BIM, and by the anti-apoptotic proteins Bcl-2, Bcl-XL and BFL-1. The presence of these carotenoids in subcellular organelles can protect the immune cells against oxidative injury, and ensure optimal cellular functions, including apoptosis, cell signaling and gene regulation [65]. Carotenoids can also influence immune function through their ability to regulate membrane fluidity and gap-junctional communication. Of course, all these actions are most likely interrelated

in their modulation of an immune response. Evidence has suggested that the action of carotenoids on immunity and diseases may be mediated by their ability to quench ROS. The action of carotenoids on immune response depends not only on the type and concentration of the carotenoid but also on the cell type and animal species involved in experiment [65].

One theory of immune regulation involves homeostasis between T-helper 1 (Th1) and T-helper 2 (Th2) activity. Several nutrients and hormones influence Th1/Th2 balance including plant sterols/sterolins, oligodeoxynucleotides, probiotics, estrogen and the minerals (e.g., zinc) [66]. The immuno-modulatory activity of an Indian saffron was studied on Th1and Th2 limbs of the immune system. Oral administration of alcoholic extract of *C. sativus* (ACS) potentiated a significant elevation of CD19 + B cells and IL-4 cytokine, a signature cytokine of Th2 pathway as well as increased levels of IgG-1 and IgM antibodies. However, ACS showed no significant expression of the Th1 cytokines such as IL-2 (growth factor for CD4 T-cells) and IFN-γ (signature cytokine of Th1 response). This data represents the selective up-regulation of the Th2 response of ACS and suggests its use for subsequent selective Th2 immuno-modulation [66]. Some reports suggest that the cytotoxic effect of saffron extract may be related to a decrease in nitric oxide (NO) concentration produced by the hepatocellular carcinoma cell line (HepG-2) and laryngeal carcinoma cell line (Hep-2) [67].

Circulating monocytes differentiate into macrophage (MQ) subpopulations in response to environmental signals. Carotenoids may reduce MQ oxidant stress and alter the inflammatory response led to lower risk of atherosclerosis. A study was performed to determine the effects of β-carotene, lycopene, astaxanthin and lutein on differentiation of human monocytes into MQs (M1 and M2) phenotypes. Monocyte-to-MQ differentiation was driven by treatment for 6 days with GM-CSF or M-CSF ± individual carotenoids [68]. M1 MQs (+GM-CSF) were activated with LPS + IFN-γ; M2 MQs (+M-CSF) were activated with IL-4. M1 and M2 cells showed distinct morphologies and expression patterns of scavenger receptors (SR-A, LDLR, CD36, SR-B1) and cytokines (IL-10, IL-12). Analysis of M1 and M2 markers (CD14, CD16, CD36, CD80, CD163) and cytokines (PGE2, IL-6, IL-8, IL-10, IL-12, TNF-α, IFN-γ) showed that expression of CD14, CD16, CD36, CD163 and IL-10 was higher in M2 than M1 MQs. In addition, lycopene and astaxanthin decreased SRA, CD36 and LDLR mRNA levels in M1 cells but had no significant effect on M2 cells. Lutein also reduced SRA, CD36 and IL-10 expression in M2. Conversely, β-carotene increased the expression of SRA, CD36, IL-10 and IL-12 in M1 cells and decreased their levels in M2 cells. Results showed individual carotenoids can regulate MQ scavenger receptor and cytokine expression. It seems that carotenoids influence MQ polarization, thereby inhibiting cholesterol accumulation in MQ-derived atherogenic foam cells [68].

6. Anti-tumor activities of saffron carotenoids

Chemoprevention is the use of dietary or pharmaceutical agents to prevent the development or progression of a disease [69]. For example, the oxidative stress and inflammation play an important role in pulmonary carcinogenesis. Therefore, anti-oxidants [e.g., vitamins, selenium, green tea extracts and isothiocyanates] or anti-inflammatory agents [e.g., glucocorticoids, NSAIDs, statins and peroxisome proliferator-activated receptor gamma (PPARγ) agonists] may be particularly effective in preventing the development of lung cancer [69].

C. sativus has been used to treat several medical conditions such as gastrointestinal disorders, urological infections as well as in treating malignancies [2]. For instance, saffron aqueous extract (SAE) inhibits the progression of gastric cancer in rats, in a dose dependent manner [70]. Herein, apoptosis is an important mechanism led to cell growth reduction. This mechanism is reported to be induced by *C. sativus* in different cancer types (e.g., colorectal, pancreatic, and bladder cancer) [2,71,72]. Stigmas of *C. sativus* (saffron) contain crocin, anthocyanin, carotene and lycopene. These constituents are known to have anti-tumor effects by inhibition of cell growth [45,46]. Crocin exhibits a variety of pharmacological effects in mice such as a) inhibition of skin tumor growth (anti-tumor effects), b) improvement of learning behavior previously impaired by ethanol (memory-improving effects), c) prevention of long-term potentiation inhibition caused by ethanol, d) anti-hyperlipidemic effects, e) therapeutic efficacy for colon adenocarcinomas in rats, f) anti-atherosclerotic effects and g) anti-oxidant and radical scavenging effects [25,71,72]. A study elucidated apoptosis induction and G1-phase cell cycle arrest by crocin (~10 μg/L) in a human pancreatic cancer cell line (BxPC-3), while decreasing cell viability in a dose and time dependent manner [71]. In addition, the results showed that crocin inhibited HL-60 cell proliferation and induced apoptosis and cell cycle arrest at G0/G1phase and regulated Bcl-2/Bax ratio of expression, in a concentration and time-dependent manner [72]. Interestingly, crocin significantly inhibits the growth of cancer cells but has no effects on normal cells. However, higher dose of crocin at 10 mg/mL induced cell necrosis, suggesting the toxic effect of crocin at high dose. In general, these results suggest that crocin inhibits tumor growth by modulating the expression of apoptosis-related molecules [72].

A study indicated the cytotoxic effect of crocin (IC_{50} = 3 mg/mL) in hepatocarcinoma HepG2 cells after 48 h treatment [17]. Furthermore, a neuroprotective effect of crocin on acrylamide-induced cytotoxicity in PC12 cells has been reported due to its antioxidant properties [17]. Another study has shown improvement of cytotoxic and apoptogenic properties of crocin in cancer cell lines, HeLa and MCF-7, using its nano-liposomal form, which make it more efficient with lower IC_{50} values [17]. Another study demonstrated that crocin at 1.0 mM, significantly reduces proliferation of three colorectal HCT-116, SW-480 and HT-29 cells up to 2.8%, 52%, and 16.8%, respectively. Since 3.0 mg/mL *C. sativus* extract contained approximately 0.6 mM crocin, the observed effects suggest that crocin is a major responsible constituent in the extract. Significant anti-proliferative effects were also observed in non-small cell lung cancer (NSCLC) cells [73]. In addition, treatment of SW480 cells with the major ingredient of saffron, crocetin (0.2, 0.4, 0.8 mmol/L), for 48 h significantly inhibited their proliferation in a concentration-dependent manner. Crocetin (0.8 mmol/L) significantly induced cell cycle arrest (S arrest) through p53-independent mechanisms associated with P21 induction. Indeed, there was a clear decrease in the levels of cyclin A and cdk2 protein in SW480 cells treated with crocetin. Crocetin (0.8 mmol/L) caused cytotoxicity in the SW480 cells by enhancing apoptosis and decreasing DNA repair capacity in a time-dependent manner [74]. Altogether, two distinct anticancer functions for crocetin can be referred including:

1) Inhibition of cell proliferation at early time by inducing cell cycle arrest via p53-dependent or p53-independent P21 mediated mechanisms. P21 is an inhibitor of cyclin-dependent kinases (Cdks) as well as proliferating cell nuclear antigen (PCNA) led to G1 arrest in crocetin-treated cancer cells [74].
2) Killing of cancer cells via apoptosis at late time [74].

Recently, crocetin has shown significant potential as an anti-tumor agent in animal models and cell culture systems. Crocetin affects the growth of cancer cells by inhibiting nucleic acid synthesis, enhancing anti-oxidative system, inducing apoptosis and hindering growth factor signaling pathways [75].

Moreover, the researchers reported that ethanolic extract of saffron is selectively cytotoxic against epithelial-like human hepatocellular carcinoma cells (HepG-2) as well as human cervical carcinoma cells (HeLa) but non-toxic towards normal mouse fibroblast cells (L929). These findings indicated that HepG2 cells treated with the saffron extract (especially crocin and safranal) increased cleavage of caspase-3 as well as DNA damage and cell cycle arrest [74]. In addition, saffron aqueous extract has inhibitory effects on the growth of both TCC 5637 (50–200 μg/mL) and normal L929 (>200 μg/mL) cell lines. This effect is dose dependent [76]. Abdullaev and Frenkel detected a dose-

dependent decrease in colony formation of A549 lung adenocarcinoma, cervical epithelioid carcinoma and HeLa cells using saffron extract. The IC_{50} values against the A549 cell lines were determined as 1.2–1.5 and 0.565–0.65 mg/mL after 24 and 48 h treatment with the ethanolic extract of saffron, respectively [77,78]. Moreover, the reports indicated that carotenoids from saffron either as crocins or purified derivatives (dimethyl-crocetin) were very effective in inhibiting the proliferation of HL-60 leukemia cells. The concentrations that produced 50% inhibition in cell growth were 1.2, 5.0, and 6.6 mM for dimethyl crocetin, crocetin and crocins, respectively during three days in culture [79]. In addition, the doses inducing 50% cell growth inhibition on HeLa cells were 2.3 mg/mL for an ethanolic extract of saffron dry stigmas, 3 mM for crocin, 0.8 mM for safranal and 3 mM for picrocrocin [80]. Recently, cell proliferation inhibition of different *Crocus* species was shown in MCF-7 and MDA-MB-231 breast cancer cells [81]. We found that the concentration inducing 50% cytotoxicity (IC_{50}) on TC-1 cells was 4 mg/mL, 1.5 mM and 3 mM at 48 h after treatment with saffron, crocin and picrocrocin, respectively [submitted data, 2013]. Although, picrocrocin is capable of inhibiting the growth of TC-1 cells in vitro, its high IC_{50} along with low percentage of apoptotic effects, indicated that the cytotoxic activity detected in saffron extract is mostly due to crocin. Our data confirmed that saffron extract and its ingredients have cytotoxic activity against TC-1 cell line more than non-malignant cells (COS-7) which is consistent with previous studies indicating anti-tumor and anti-carcinogenic activities of saffron and its ingredients. In addition, the cytotoxic effect of saffron and its components is partly mediated via apoptosis [submitted data, 2013].

Safranal (2, 6, 6-trimethyl-1, 3-cyclohexadiene-1-carboxaldehyde, $C_{10}H_{14}O$) is an active ingredient in the saffron, which is used in traditional medicine. It has been reported to have anti-oxidant effects, but its anti-tumor effects remain uncertain [2]. Safranal showed antioxidant activity and reduced oxidative damages in different organs such as skeletal muscle, kidney and hippocampus [2]. As known, a balance between cell proliferation and apoptosis controls normal organ development. Pre-clinical study demonstrated a neuroblastoma cell line (N2A) to be highly sensitive to safranal-mediated growth inhibition and apoptotic cell death in a dose- and time-dependent manner [2].

Lycopene, an antioxidant carotenoid, is also associated with prevention of some human chronic diseases [e.g., chronic obstructive lung disease (COPD), 82]. Lycopene inhibited cell invasion, angiogenesis and metastasis [83]. Lycopene metabolites circulate in serum and accumulate in tissues at concentrations equivalent to bioactive retinoids [11]. Men with serum lycopene concentrations higher than 0.19 μmol/L had a 45% lower risk for total cancer than did men with lycopene under 0.08 mol/L [84]. Recent studies reported that lycopene metabolites reduce the proliferation of cancer cells (e.g., prostate cancer), induce apoptosis, enhance gap junction communication between cells, alter normal cell cycle progression and modulate androgen signaling pathways [11,85,86]. For example, the data showed Lycopene promoted cell cycle arrest followed by decreased cell viability as well as an increase in apoptosis in four cell lines (T-84, HT-29, MCF-7 and DU145) [42].

Previous studies have demonstrated that natural and synthetic agents such as saffron and sodium selenite, or sodium arsenite alone, inhibited the development of human tumor cells in vitro. In contrast to saffron extract, the synthetic agents used alone exhibited cytotoxic effects on normal human cells. The results demonstrated that natural extract of saffron in combination with synthetic anti-cancer agents such as selenium and arsenic compounds enhanced inhibitory effect of these agents on the development of different human malignant cells in vitro [87]. It was reported that selenium alone and in combination with β-carotene and vitamin E was effective in reducing the incidence of malignant tumors in vivo and in combination with Adriamycin or Taxol inhibited different cancer cells in vitro. Inhibition of nucleic acid synthesis in tumor cells by these natural and synthetic agents (alone or in combination) might explain their cytotoxic activities. Moreover, the pre-treatment of tumor cells with saffron resulted in increased levels of intracellular SH-compounds. It seems that the marked difference in the inhibition of colony formation in tumor cells by saffron in combination with sodium selenite or arsenite may be explained by an increase in the levels of intracellular SH-compounds in malignant cells or modulation of the GSH-redox system [87].

Cancer cells often develop cross-resistance to structurally and functionally unrelated cytostatic drugs, a finding which has been termed multidrug resistance (MDR). MDR is a major cause of chemotherapy failure. The activity of ATP-dependent multidrug transporters, which belong to the superfamily of ATP-binding cassette (ABC) proteins, is important in this process. P-glycoprotein (MDR1) is the most widely known ABC-transporter associated with clinical MDR. The enhanced activity of ABC transporters prevent that a toxic concentration of chemotherapeutic drugs is built up in cancer cells. A combination of cytotoxic drugs with non-toxic ABC transporter inhibitors (chemosensitizers) represents a new approach to overcome drug resistance [88,89]. In a study, some carotenoids (e.g., β-carotene, crocin, retinoic acid, canthaxanthin and fucoxanthin) were investigated whether they are substrates of MDR1, and if they can reverse MDR in resistant Caco-2 and CEM/ADR5000 cells as compared to the sensitive parent cell line CCRF-CEM. The carotenoids increased accumulation of the MDR1 substrates in a dose-dependent manner indicating that they themselves also function as substrates. Carotenoids showed a low cytotoxicity in cells with MDR. The combination of carotenoids with eight structurally different cytotoxic agents synergistically enhanced their cytotoxicity in Caco-2 cells, probably by inhibiting the function of the ABC transporters. Fucoxanthin and canthaxanthin significantly decreased MDR1 levels to 12% and 24%, respectively as compared to untreated control levels [88]. In summary, all carotenoids, especially β-carotene, canthaxanthin and fucoxanthin were competitive inhibitors of ABC-transporter. They could exert MDR reversal and enhanced the cytotoxicity of chemotherapeutic drugs in human MDR1 expressing cells in a synergistic fashion. They increased drug accumulation in drug-resistant cells, in variable degrees depending on their chemical structures. This study suggested the use of carotenoids in combination with chemotherapeutics in cancer treatment to enhance the efficacy of chemotherapy and reduce the influence of MDR [88].

7. Conclusion

Cancer represents the largest cause of mortality in the world (over six million lives each year). A wide variety of natural substances has been recognized to have the ability to induce apoptosis in various tumor cells. It is thus important to monitor apoptotic inducers from plants, as crude extracts or as their isolated components (e.g., carotenoids). In fact, the search for new pharmacologically active compounds for drug development is an important issue. Recently, carotenoids, by their various properties e.g., anti-oxidant effect, show benefits in many diseases such as cancer and strokes. In the human body, oxidant–antioxidant balance is critical because it maintains cell membrane integrity and functionality, cell proteins and nucleic acids. Many herbs and spices are the subject of future scientific investigations related to antioxidant properties and health. Epidemiological evidence indicates there is a correlation between increased dietary intake of antioxidants and a lower incidence of morbidity and mortality. Among natural plants, saffron and its ingredients (especially its carotenoids) possess anti-tumor and anti-carcinogenic activities and have no cytotoxic effect on non-malignant cells. In addition, the suppressive effects of saffron are partly due to the anti-inflammatory properties of the crocin by the inhibition of several cytokines and inducible inflammatory enzymes. At present, the determination of intake doses of carotenoids suitable for the general population and without cytotoxicity is necessary.

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