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The toxicity of saffron (Crocus sativus L.) and its constituents against normal and cancer cells

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ABSTRACT

Background and aims: Saffron is a spice with preventive and curative effects. This study aimed to review the toxicity of saffron extract and its constituents on normal and cancer cells.
Methods: We searched the PubMed, Science direct, SID and Magiran databases up to November 2015 using the following key words: toxicity, saffron, crocin, crocetin, safranal, cancer. Finally, 73 English and 5 Persian articles were selected to be recruited to be reviewed.
Results: Saffron has selective toxicity against cancer cells, through inhibition of RNA and DNA synthesis and increasing apoptosis. Crocin has been considered as the most important anticancer agent of saffron that plays a role in gene expression and apoptosis in cancer cells. Crocetin has an inhibitory effect on the cancer cells growth that may be due to reduced synthesis of DNA, RNA and protein in neoplastic cells, RNA polymerase II inhibition, and interaction with histone H1 and H1-DNA structures. Saffron and its crocin and crocetin have also shown anticancer and cancer-preventive effects in animal models of cancer. Safranal also has shown antitumor activity with low toxicity. On the other hand, the lethal dose of 50% (LD50) for the saffron and its constituents against normal cells can be very high.
Conclusion: In conclusion, emerging evidence suggests that saffron extract and its crocin, crocetin and safranal have a selective toxicity effects against cancer cells and also may have cancer preventive functions. However, Saffron and its constituent's toxicity against normal cells is negligible and they are even non-toxic in oral administration.
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1. Introduction

Cancer remains as a major leading cause of mortality worldwide. It has been reported that more than 8 million people are diagnosed with cancer each year. Moreover, 1,530,000 new cases of cancer and nearly 570,000 cancer deaths occurred in United State in 2010 [1]. Various types of cancers have different prevalence in different communities [2]. Changes in cancer patterns due to immigration have decreased the role of genetics in the observed differences among communities [3]. Studies showed that majority of cancers may be prevented by changes in lifestyle and environment, including following a healthy diet [4]. Recently, chemoprevention is considered as an effective treatment for cancer. In this regard, there is a considerable attention to the roles of herbs and spices in tumor growth inhibition and cancer progression [5].

Dried Stigma of saffron (*Crocus sativus* L.) is considered as the most expensive traditional spice by weight all over the world [1–3]. It has been estimated that Iran accounts for about 76% of the total world saffron production annually [4,5]. Saffron has been used as a stomach pain soother, antispasmodic, digestion aid, renal colic pains reliever, antidepressant, and appetizer agent in Islamic-Persian traditional medicine [6,7]. The stigma is the most used part of the saffron. The observed beneficial effects of the dried stigma are contributed to three main secondary metabolites including soluble crocin (mono-glycosyl or di-glycosyl polyenesters) which is responsible for saffron special color, picrocrocin (mono-terpene glycosideprecursorof safranal) which is responsible for saffron bitter taste, and safranal which is responsible for its special odor. Saffron contains a considerable amount of lipophilic carotenoids and flavonoids, as well as trace amounts of B1 and B2 vitamins [8–17]. Conducted researches have contributed beneficial effects of saffron to its antioxidant and anti-inflammatory properties, which may be related to saffron active constituents [18,19].

Although, many studies have investigated the effect of saffron and its active constituents in the prevention and treatment of cancer, so far, the exact mechanism of action has not been cleared yet [20–25]. to the aim of the present study was to review the literature on anticancer and toxic effects of saffron and its constituents as a chemo-preventive herb and propose mechanisms of action.

2. Material and methods

We identified published studies using MEDLINE (PubMed) and Science direct, SID and Magiran databases by following keywords provided from MESH: 'saffron', 'crocin', 'crocetin', 'safranal', 'saffron constituent', 'colchicum', 'cytotoxicity', 'toxicity', 'cancer', 'neoplasm', 'tumor', 'tumoricidal effect', and 'detrimental effect' We included all studies from inception up to November 2015 with language restriction (English and Persian, only). A total of 276 papers were found through the preliminary searching, among which 20 papers were in Persian. The inclusion criteria namely were interventional studies in human populations, *in vitro* and *in vivo* animal studies. However, we could not find any clinical trial. The exclusion criteria were articles in which the active components of saffron were obtained from a source other than saffron, articles in which the used saffron were belonged to a very special race other than the conventional saffron, and finally articles in which saffron was used either as a component of a mix herbal medicine or as an ingredient of a drug. The quality of the articles was assessed by Strengthening the Reporting of Observational Studies in Epidmiology (STROBE) statement [28]. The search protocol is shown in Fig. 1. Finally, 73 English and 5 Persian articles were reviewed.

3. Results

3.1. *In vitro studies*

In vitro studies on toxicity effects of saffron and its constituents on tumor cells are shown in the Table 1.

3.1.1. *Saffron*

In an *in vitro* study which evaluated the effect of ethanol extract of saffron on macromolecules synthesis in three cell layers, including cells taken from lung tumors, normal lung fibroblasts and virus induced transformed fibroblast cells, it has found that malignant cells are more sensitive to the inhibitory effects of saffron on the synthesis of RNA and DNA compared to the normal cells [29]. The inhibition of RNA and DNA synthesis is regarded as one of the main mechanisms of anti-tumor and anti-carcinogenic effects of saffron [17]. In another study, ethanol extract of saffron was incubated on the Hela (human epithelial cell of epitheloid carcinoma) and Hep G2 (epithelial like hepatocellular carcinoma cells) cell layers in different doses (200–2000 μgr/mL) for about 24, 48 and 72 h. Inhibitory concentration 50% (IC_{50}) against Hela and Hep G2 cell layers were 800 and 950 μgr/mL after 48 h, respectively. Data indicated that apoptosis plays a major role in the toxicity effects of saffron, although, it is not the only determining factor. Therefore, other functions of saffron should be taken into account. Since the ROS (reactive oxygen species) did not change during the study period, it seems that ROS did not have a role in these effects. In this study, significant inhibition of colony formation as well as DNA and RNA synthesis (50% inhibition) was seen at doses of 100–150 μgr/mL, while, no effect regarding inhibition of protein synthesis was found, even at high doses [33]. Several other mechanisms for anticancer effects of saffron particularly reactions with nucleic acid chain and free radicals, and carotenoids interaction with topoisomerase II were also reported [17,26]. It is plausible that synergistic effects of saffron phytochemicals increase their anti-cancer effects [34]. Tavakkol-Afshari et al. reported that alcoholic extract of saffron induced selective cytotoxicity against Hep G2 and Hela cells; however, did not have any toxic effects on normal fibroblast cells in rats [35]. In another study, the effects of different doses of the saffron extract (100, 200, 400 and 800 μgr/mL) were investigated on human alveolar carcinoma cells (cells A549) for three days. The study indicated that the percentage of apoptotic cells increased in a dose dependent manner with the presence of saffron. The researchers also observed apoptosis in the presence of caspase-dependent pathways stimulation. Human lung fibroblast cells as control cells were less affected by the pathway. The study demonstrated that anti-cancer effects of saffron can be partly attributed to the inhibition of cell proliferation and stimulation of apoptosis through caspase-dependent pathways [36]. However, in another study it has been reported that the saffron extract in doses

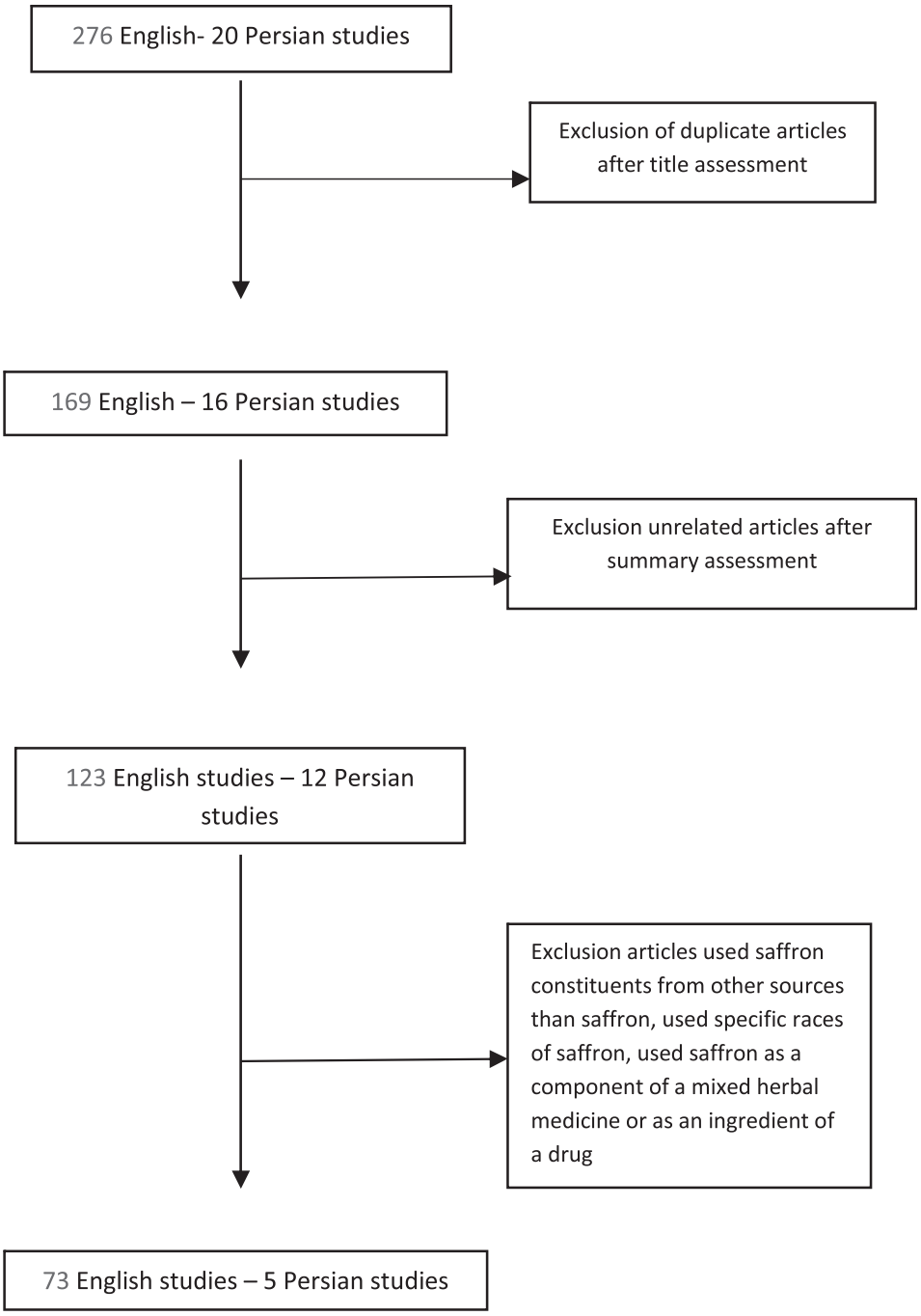

Fig. 1. Diagram of related studies search and extraction.

up to 1500 mg/area is not toxic, mutagenic or anti-mutagenic [37]. *In vitro* studies on saffron induced non-specific proliferation of lymphocytes in the presence of T cell mitogen phytohaemagglutinin [30], indicated that antitumor activity of saffron may be mediated immunologically. However, the constituents of saffron (ginsenosides and cannabinoid) could not reverse multi drug resistance in lymphoma cells [38]. Two other studies have separated a new bioactive substance from the saffron stem [40,39]. Lethal dose 50% (LD_{50}) for the substance against HeLa cells was 9 μgr/mL. The toxicity of this substance was also studied in the human malignant cell layers, a non-malignant cell layer, blood cells, and hair follicles. Calculated LD_{50} for tumor cells was 7 to 22 μgr/mL, and 100 μgr/mL for fibroblasts normal cells. On the other hand, it seems that this substance toxicity is 8 times higher in tumor cells compared with the non-tumor types [33].

In conclusion, it seems that saffron may have selective toxicity against cancer cells through various mechanisms such as inhibition of RNA and DNA synthesis and increasing apoptosis, and protein synthesis. So, it seems that some gene pathways related to cell apoptosis induction and proliferation inhibition is the major anti-tumor mechanism of saffron.

3.1.2. Crocin

Spanish researchers investigated the effects of four constituents

Table 1
Saffron toxicity against cancer cells at *in vitro*.

Study	Target cells	Intervention	Results
Abdullaev F et al. 1992 [29]	Lung tumor cells, normal lung fibroblasts and transformed fibroblasts	Saffron alcoholic extract	Saffron inhibited the growth of malignant cells by blocking the synthesis of RNA and DNA
Abdullaev F et al. 1992 [33]	Human embryonic epithelial cells of the cervix (Hela) and liver carcinoma cells (Hep G2)	Saffron alcoholic extract, and crocetin like substances	Saffron and crocetin like substances inhibited the growth of tumor cells by apoptotic and non-apoptotic mechanisms
Tavakkol-Afshar et al.2008 [35]	Hepatocellular carcinoma epithelial-like cells (Hep G2) and human cervical carcinoma cells	Saffron alcoholic extract	Saffron had selective cytotoxicity against tumor cells
Samarghandian et al. 2013 [36]	Human alveolar carcinoma cells (cells A549)	Saffron aqueous extract	Apoptotic malignant cells percentage increased dose dependently in the presence of saffron
Abdullaev Fet al. 1992 [33]	HeLa cells and human malignant cell layer	Isolated substance from saffron	ID50 against Hela cells was 9μgr/ml, and 7 to 22 μgr/ml against malignant cells
Escribano J et al. 1996 [41]	HeLa cervical carcinoma cells	Crocin, crocetin, picrocrocin, and safranal	All the constituents inhibited cancer cells growth. The effect of crocin was more considerable.
Molnar J et al. 1999 [38]	Human cells infected with adenovirus	Crocin and di glucosyl crocetin	Crocin and di glucosyl crocetin had inhibitory effects on primary expression of tumor antigen at adenovirus infected cells
Garc-Olmo DC et al. 1999 [44]	HT-29 cells and DHD/k12 (adenocarcinoma cells in rat and human colon carcinoma cells)	Crocin	Crocin exhibited strong selective cytotoxicity against HT-29 and DHD/k12 cells
Noureini et al. 2012 [45]	Hep G2 cells	Crocin	Crocin exhibited selective cytotoxicity against cancer cells
Aung Hal et al. 2007 [46]	Three layers of colorectal cancer cells (HCT-116, SW-480 and HT-29)	Crocin	Crocin significantly inhibited these three cells layers with a dose dependent manner
Mehri et al. 2012 [47]	PC12 Cells	Crocin	Crocin had neuro protective effect on acrylamide induced cytotoxicity in PC12 cells with a dose dependent manner
Abdullaev FI et al. 1994 [35]	HeLa cells, lung cancer cells modified fetal lung fibroblast	Crocetin	Crocetin inhibited cancer cells growth through dose dependent inhibition of protein and nucleic acid synthesis
Jagadeeswaran R et al. 2000 [51]	Human rhabdomyosarcoma cells	Crocetin	Crocetin had selective cytotoxicity against human rhabdomyosarcoma compared with cisplatin
Chryssanthi DG et al. 2007 [52]	MDA-MB-231 and MCF-7 breast cancer cells	Crocetin and its analogs	Crocetin and its analogs dose dependently inhibited breast cancer cells growth
Mousavi et al., 2009 [53]	MCF-7 cells	Crocetin	Crocetin had a pro-apoptotic effect on cancer cells
Tarantilis P et al. 1994 [31], Morjani H et al. 1990 [43]	Promyelocytic leukemia cells (HL60) and human myelogenous leukemia cells (K562)	Crocetin	Crocetin had cytotoxic effects against leukemia cells
Wang C-J et al., 1991 [55]	Fibroblast cells C3H10T1/2	Crocetin	Pretreatment with crocetin significantly inhibited aflatoxin induced cytotoxicity in fibroblast cells
Abdullaev F 1992 [33]	A549 (lung carcinoma) and VA13 (SV-40 transformed fetal lung fibroblasts) Cells	Crocetin	Crocetin had inhibitory effects on nucleic acid synthesis and lung cancer cells colony formation
Dhar A 2008 [59]	MIA-PaCa-2, BxPC3, Capan1 and Ascpc1 pancreatic cancer cells	Crocetin	Crocetin had anti-tumorigenic effects on pancreatic cancer cells

of saffron (crocin, crocetin, picrocrocin and safranal) on the HeLa cells of cervix carcinoma. Crocin showed the strongest inhibitory effect. The inhibitory effects of ethanol extract of saffron on *in vitro* growth of the HeLa cells (ID_{50} = 2.3 mg/ml) and apoptosis inhibition were mainly attributed to crocin (ID_{50} = 3 μM). However, picrocrocin and safranal had minor effects (ID_{50} was 3 and 0.8 mM, respectively). Moreover, crocetin did not show any cytotoxicity effect in the study. Therefore, crocin may be considered as the most important anti-cancer constituents of saffron [41]. Since, crocin is the glycated form of saffron constituents, it seems that glucose added to the crocin structure may increase its antitumor properties. It may be due to glucose ability to interact with DNA structure. Another study found that saffron isolated crocin and dimethyl crocetin did not have mutagenic effects [42]. Likewise, Molnar et al. reported that crocin and di glucosyl crocetin have inhibitory effects at different doses on the primary expression of tumor antigens in the adenovirus infected cells [38]. It also seems that sugars play a major role in the potential toxicity effect of the crocin, since crocetin (which is deglycosilated derivative of crocin) could not inhibit cell growth, even at high doses [43]. In another study, crocin was regarded as the most important anti-cancer constituent of saffron [17]. Garc-Olmo et al. reported that crocin exhibited potent cytotoxic effects against HT-29 and DHD/k12 cells (adenocarcinoma cells of rats and human colon) with a LD_{50} of 0.4 and 1 mM, respectively. Crocin also significantly decreased cytoplasm and wide cytoplasmic vacuole-like areas of the cells [44]. This low LD50 and the observed morphogenic changes in colon tumor cell layers may indicate that crocin can be more toxic against gastrointestinal cancer cells than other tumors cells. Microscopic studies have demonstrated that vacuolated areas and reduction in the size of the cells and the pyknotic nuclei of crocin treated HeLa cells may be attributed to stimulation of programmed cell death [43,44]. A study which investigated the crocin effects on Hep G2 cells, reported that after 48 h, crocin exhibited cytotoxicity effects on these cells at dose of 3 mg/mL. Telomerase activity and catalytic section of telomerase gene expression, decreased by 51% and 60% with a dose-dependent manner, respectively. Since, the transcription of the telomerase gene is higher in cancer cells compared to the normal ones, it seems that crocin has selective effects on cancer cells [45]. Telomerase concentration did not change during the study, therefore, crocin probably has interact only with telomerase gene area and may increase the enzyme stability rather than protein synthesis. In the same line with this data, another study found that saffron derived crocin significantly inhibited the growth of three layers of colorectal cancer cells (HCT-116, SW-480 and HT-29) with a dose-dependent manner [46] Dose dependent neuro-protective effect of crocin (at the doses of 10, 20 and 50 μmol) against cytotoxicity of acrylamide in PC12 cells has been attributed to its antioxidant properties [47]. Other possible mechanisms of this effect can be inhibition of protein aggregation and fibrillar formation [48].

In conclusion, crocin is considered as the most important anticancer constituent of saffron. It seems that crocin exhibits this effect through changes in gene expression and induction of apoptosis in cancer cells.

3.1.3. Crocetin

While some studies have not considered crocetin as an anticancer constituent of saffron [43,41], other studies have demonstrated anticancer effects of it [56–49,46]. In one study, inhibitory effects of saffron isolated crocetin on the synthesis of nucleic acid and protein in three human malignant cell layers, including HeLa cells, lung adenocarcinoma, and embryonic deformed lung fibroblast cells, have been investigated. Crocetin inhibited protein and nucleic acid synthesis dose dependently, but had no effect on colony formation [32]. Other studies have also shown growth inhibition of the cancer cells by dimethyl crocetin, crocetin, and crocin at doses of 0.8 and 2 μM for 50% inhibition (ID_{50}) [43,31]. Some other studies have demonstrated that dimethyl crocetin and crocin had toxic effects on different cell layers (DLA, EAC, S-180, L1210 leukemia and P388 leukemia) and human stem cells isolated from surgical samples (Osteosarcoma, fibrosarcoma and ovarian carcinoma). Several studies have indicated that crocetin can significantly inhibit the nucleic acids synthesis, and also, dimethyl crocetin can inhibit DNA-protein interactions (e.g. topoisomerase II) which are crucial for DNA synthesis [49,30]. Furthermore, some studies have reported the toxic effects of crocetin against a non-solid tumor isolated cell layer, different layers of tumor cells, and human stem cells (derived from surgical samples) [50,31]. In another study it has been found that crocetin at doses of 5–20 μg/ml exhibited selective cytotoxic effects against human rhabdomyosarcoma cells and less deteriorative effects on normal cells compared with Cispltin [51]. The effects of crocetin on some types of cancers are explained below:

1. **Breast cancer:** Chryssanthi et al. reported that crocetin and its analogs inhibited breast cancer cells proliferation. In this study, a dose-dependent inhibition of MDA-MB-231 and MCF breast cancer cells proliferation was observed in the presence of crocetin. This effect was independent of the estrogen receptors function [52]. Hence, the antitumor mechanism of crocetin is independent from hormone regulation. Another study also investigated the pro-apoptotic effects of crocetin on MCF-7 cells, indicating that crocetin leads to induction of a caspase-dependent pathway through elevation of the BAX protein expression [53].
2) **Cervices cancer:** It has been reported that crocetin like components of saffron may cause significant reduction in the colony formation and DNA and RNA synthesis at doses of 1,200 μg/mL in Hela cells. Crocetin inhibits DNA-dependent RNA polymerase II and RNA synthesis [35]. UV spectroscopy showed an interaction between crocetin and tRNA. Accordingly, it seems that crocetin may have binding activity at the molecular level [54].
3) **Leukemia:** Two studies have found that crocetin at low doses, even 0.8 μg, can exhibit cytotoxic effects against promyelocytic leukemia (HL60) and human myelogenous leukemia (K562) cells [43,31]. Crocetin cytotoxicity in other cell leukemia layers (L1210 and P388) has also been reported [43]. Very potent anti-leukemia properties of crocetin may be related to its polyesters that may act as an antigen for leukemic cells.
4) **Liver cancer:** Pre-treatment with crocetin in aflatoxin B_1 (AFB_1) activated fibroblasts C3H10T1/2 cells, significantly inhibited cytotoxicity and DNA-adduct formation [55]. This protective effect is probably due to the increase in cytosolic glutathione (GSH) following elevated GSH-S-transferase (GST) formation. According to one study, the inhibitory effect of crocetin on genotoxicity of benzo (α) pyrin and neoplastic formation in C3H10T1/2 cells is due to the increased activity of GSH and reduced formation of benzo (α) Pyrin- DNA adducts [57]. The study also indicated that crocetin inhibits ROS induced malondialdehyde (MDA) formation, produced by xanthine oxidase (XO), and thus, inhibits the oxidative damages [58]. Therefore, crocetin may induce protective effects through scavenging free radicals following neoplastic transformation [58,57].
5) **Lung cancer:** It has been shown that crocetin (at doses of 100 to 150 μgr/mL) exhibits inhibitory effects on nucleic acid synthesis and colony formation of A549 (lung carcinoma) and VA13 (transformed SV-40 of fetal lung fibroblasts) cells [33].
6) **Pancreatic cancer:** A study in 2009 for the first time investigated anti-cancer potential of crocetin on pancreatic cancer using different pancreatic cancer cells as well as a Xenograftathymic mice model. Crocetin (at doses of 50–200 μmol/L for 72 h), inhibited proliferation of MIA-PaCa-2, BxPC3, Capan1, and Ascpc1 cells. The study also indicated that DNA synthesis was inhibited in pancreatic cancer cells in the presence of crocetin. Moreover, it has been suggested that a reduction in the imbalance between anti-apoptotic (Bcl-2) and pro-apoptotic (Bax) proteins may be the main mechanism of crocetin antitumor formation activity [59]. Recent data demonstrated that crocetin along with low-doses of Paclitaxel or Cisplatin can inhibit proliferation and stimulate apoptosis of pancreatic cancer cells [60]. Therefore, crocetin can act safely along with the common chemotherapy drugs.

Based on the aforementioned findings, crocetin inhibits the growth of some types of cancer cells. This effect may be due to the reduced synthesis of DNA, RNA and protein. Crocetin also inhibits RNA polymerase II in neoplastic cells [32]. Similarly, it has been reported that crocetin interacts with the structure of histone H1 and the H1-DNA, which suggests that anti-cancer mechanisms of crocetin may be different, and epigenetic effects can also be one of them [61]. Crocetin inhibits protein and nucleic acid synthesis and then interact with some protein cascades.

3.1.4. Safranal

Safranal is the major coloring constituent of saffron [13]. Samarghandian et al. study has investigated antitumor activity of safranal in different doses (0, 10, 15, 20, 50 μg/ml) in cultured neuroblastoma cells. Safranal dose dependently inhibited cell proliferation and induced cell apoptosis with an IC50 of 11.1 and 23.3 μg/ml after 24 and 48 h, respectively [62]. Moreover, Malaekeh-Nikouei et al. study has shown dose dependent antitumor activity of safranal (0.01–3 mM) against Hela and MCF7 cells [63]. It seems that apoptosis induction is the most important antitumor mechanism of safranal. Safranal can be also considered as the most antitumor saffron constituent against Hela cell layer tumor [63]. Another study by Samarghandian and Shabestari found dose dependent antitumor activity of safranal (5, 10, 15, 20 μg/ml) in a prostate cancer cell layer. Apoptotic effect of safranal is also expressed in this study, that even approved by DNA analysis [64].

According to the mentioned studies, safranal exhibits tumorocidal activity even at low doses. Because of dose dependent effects and low doses tumorocidal activity, it can be considered as the most potent toxic constituent of saffron with selective toxicity.

3.2. In vivo studies-animal

Animal *in vivo* studies regarding the effects of saffron and its constituents against cancer are shown in the Table 2.

Table 2
Saffron toxicity against cancer in *in vivo* animal studies.

Study	Animal	Intervention	Dose	Time	Results
Salomi M et al. 1991 [49]	Mice	Saffron extract	100 mg/kg	12 weeks	Saffron extract inhibited onset and progression of induced skin tumors and delay papilloma onset in rats
Bathaie SZ et al. 2013 [69]	Rat	Saffron aqueous extract	100, 150, and 175 mg/kg	50 days	Saffron inhibited cancer progression dose dependently
El Daly E et al. 1997 [68]	Rat	Saffron extract + cystein	50 mg/kg	5 days	Saffron extract along with cysteine significantly reduced cisplatin toxicity
Premkumar K et al. 2001 [73]	Swiss albino mice	Saffron aqueous extract	20, 40, and 80 mg/kg	5 days pretreatment	Saffron significantly reduced genotoxicity of anti-cancer drugs
Garc-Olmo DC et al. 1999 [43]	Rat	Crocin – crocetin	Crocin at a dose of 400 mg/kg– crocetin at doses of 50–200 μmol/liter	13 weeks	Crocin treatment significantly increased survival of rats with colorectal tumor. Crocetin reduced tumor growth in female rats.
Hariri et al. 2011 [76]	Rat	Crocin - safranal	Crocin at doses of 50, 100, and 200 mg/kg – safranal at doses of 0.25, 0.05, and 0.1 mg/kg	4 weeks	Crocin and safranal reduced Diazinon cytotoxicity in rat blood, but did not inhibit genotoxicity
Wang CJ et al. 1996 [77]	Rat	Crocetin	60 and 120 μmol	15 min	Crocetin inhibited induced skin cancer in rat
Wang C-J et al. 1991 [56]	Rat	Crocetin	2 to 6 mg/kg	3 days preteatment	Crocetin protected liver against aflatoxin induced carcinogenicity
Wang C-J et al. 1991 [78]	Rat	Crocetin	0.1 mg	45 weeks	Crocetin significantly inhibited aflatoxin induced hepatotoxic ulcers
Magesh V et al. 2006 [79]	Swiss albino mice	Crocetin	20 mg/kg	4 weeks pretreatment	Crocetin had anti-tumor effect against lung cancer
Magesh V et al. 2009 [80]	Swiss albino mice	Crocetin	50 mg/kg	18 weeks	Crocetin inhibited lung cancer cells proliferation
Dhar A et al. 2009 [59]	Athymic mice	Crocetin	4 mg/kg	30 days	Crocetin had anti-tumor effect against pancreatic cancer

3.2.1. saffron

Salomi et al. have conducted a 12-weeks study to assess the effect of saffron extract on induced cancer in albino rats. Two to seven papilloma was observed in the 90% of rats in the control group, while, the saffron group (100 mg/kg) had only 0.26 to 1 papilloma. Saffron extract inhibited formation and progression of induced skin tumors in rats and also delayed papilloma attack [49]. Oral intake of the same dosage extract induced sarcoma in soft tissues of rats, and limited tumor formation [49,42]. In one study the chemopreventive effects of the carotenoids rich extract have been related to lipid peroxidation, antioxidants and detoxification systems regulation [65]. Previous studies have shown that anti-tumor effects of saffron are more considerable at oral than intravenous receiving. Therefore, it seems that some structural transformations may occur during saffron ingestion. Moreover, liposome encapsulated saffron is more effective at oral administration. The liposome encapsulated saffron significantly inhibited implanted tumor cells growth in rats [66]. Oral administration with alcoholic extract of saffron (200 mg/kg) increased Lifetime of the sarcoma cells in transplanted albino rats [30,67]. Furthermore, oral administration of the extract increased serum β-carotene and vitamin A levels in the animals [68]. Therefore, it seems that saffron carotenoids may have vitamin A precursor function and their anti-cancer effects may be related to this function, as some studies have regarded B-carotene (a precursor of vitamin A) as an antioxidant [31]. Moreover, B-carotene transformation to vitamin A may be the major cause of higher effectiveness of oral administration. Another study investigated the effects of saffron aqueous extract on induced gastric cancer in rats. Aqueous extract (100, 150, and 175 mg/kg) inhibited cancer progression after 50 days in a dose-dependent manner. Hence, 20% of rats treated with higher doses of the extract were completely normal and none of the rats in the extract group was affected by adenoma at the end of the study. In this study, increased apoptosis and proliferation confirmed the pro-apoptotic effect of saffron. In addition, saffron renormalized serum levels of enzymes and antioxidants after induction of cancer [69]. The antitumor activity of saffron against gastric cancer at such low doses may indicate local and systemic effects on the gastrointestinal cells.

Although, the mentioned studies have found anti-carcinogenic effects of saffron, a majority of *in vivo* studies have investigated the effects of saffron constituents rather than saffron extracts [34].

On the other hand, different studies have found that saffron extract (at a low dose such as 2 mg/kg) increased lifetime and reduced the drug side effects in Cisplatin treated mice [70–72]. Likewise, pre-treatment with aqueous extract of saffron (at doses of 20, 40 and 80 mg/kg) in Swiss albino mice, significantly reduced genotoxicity of anticancer drugs [73]. Animals treatment with cysteine (20 mg/kg) and saffron extract (50 mg/kg) significantly reduced the Cisplatin induced toxicity [68]. In the study by Mohajeri et al. alcoholic extract of saffron at a dose of 80 mg/kg for 30 days, reduced Rifampin induced hepatotoxicity in rats, which was similar to the silymarin effects [74].

In conclusion, saffron also exhibits anti-cancer effects in animal models of cancer which may be attributed to its antioxidant and pro-apoptotic actions in the cancer cells. Oral administration of saffron is more effective, that may be attributed to its B-carotene precursor properties. Saffron also induced antioxidant enzymes. Moreover, it seems that saffron may also reduce the toxic effects of cancer drugs, although, the mechanism has not been cleared yet.

3.2.2. Crocin

One study investigated the effects of long-term treatment with crocin (400 mg/kg) on tumor growth and lifetime of adenocarcinoma cell subcutaneous injection of induced colorectal tumor in rats. Crocin treatment significantly increased survival of the animals and reduced tumor growth, specially in females [44]. This selective effect on the females suggested that crocin performance may partly be related to hormonal factors. Another study has also indicated that crocin reduced Diazinon cytotoxicity in rat blood samples, but did not inhibit genotoxicity [75]. In Naghizadeh et al. study, crocin at doses of 100, 200 and 400 mg/kg attenuated acute renal toxicity induced by Cisplatin [76].

3.2.3. Crocetin

A study on the frog embryo demonstrated that saffron isolated crocetin was effective in the treatment of some cancers which are treatable with all-trans retinoic acid (ATRA). The study suggested that crocetin may be a safer alternative therapy for treatment of ATRA sensitive cancers in pregnancy [50]. Dhar et al. have shown that crocetin exhibits anti-tumorigenic effect in pancreatic cancer both *in vitro* and *in vivo* [59]. Similarly, Wang et al. indicated that crocetin at doses of 60 and 120 μmol can inhibit induced skin cancer through inhibiting protein kinase C activity by 50% and 66%, respectively in rats [77]. Garcia-Olmo et al. also reported that supplementation with crocetin (at the doses of 50–200 mol/L) slowed tumor growth in female rats, but not significantly in male rats [44]. Accordingly, it seems that at least one of the female hormones may play a role in this effect. A study has reported that pre-treatment with crocetin (2–6 mg/kg) protected liver against damages caused by AFB1 and inhibited formation of AFB1-DNA adducts by increasing liver GSH and activating GST and glutathione peroxidase (GSH-Px) in rats [56]. In another study, significant suppression of AFB1 induced hepatotoxic ulcers has observed due to the reduction in the activity of aspartate amino transferase (AST), alanine amino transferase (ALT), alkaline phosphatase and gamma glutamyl transpeptidase (GGT) after pretreatment with crocetin (1.0 mg) in rats [78]. An *in vivo* study reported that crocetin (20 mg/kg) has antitumor activity against a lung cancer animal model. Crocetin exhibits these actions through scavenging free radicals and increasing activity of drug metabolizing enzymes. The study also found that crocetin inhibited lipid peroxidation and increased GST, GSH-Px, catalase, and superoxide dismutase (SOD) activities. Similarly, crocetin reduced carcinogen related marker enzymes such as aryl hydrocarbon hydroxylase (AHH), lactate dehydrogenase (LDH), GGT, adenosine deaminase (ADA) and 5′ nucleotidase in lung tissue after Benzo (α) Pyrin induction [79]. Magesh et al. study showed that 50 mg/kg crocetin inhibited lung cancer cells proliferation. The study strongly suggested that the protective effect of crocetin on lung carcinogenesis induced by benzo (α) Pyrin in Swiss albino mice was most likely due to the inhibitory effects on polyamine synthesis and glycoproteins changes [80]. In an animal model study, crocetin (at the dose of 4 mg/kg) had anti-tumorigenic effects on pancreatic cancer through inhibiting the proliferation (by inhibiting phosphorylation and expression of EGFR) in athymic mice [59]. Therefore, crocetin may intervene with gene expression and phosphorylation at higher doses. In the same line with these findings, another study found that crocetin (600 mg/kg) delayed onset of skin tumors, tumor formation in skin induced by Dimethyl Benz [α] Anthracene (DMBA), and its progression by castor oil in Swiss Webster rats [81]. In a similar study, antitumor activity of crocetin was observed in DMBA and castor oil induced skin cancer in hairless rats [82]. Moreover, a study in mice has reported that crocetin at a dose of 50 mg/kg attenuated the bladder toxicity of cyclophosphamide anti-cancer compounds without altering their antitumor activity [71].

In conclusion, crocetin may exhibit anti-cancer and cancer-preventive effects even in the presence of cancer inducing factors. These effects can be attributed to the antioxidant potential and internal enzymes regulation by crocetin. Crocetin also regulate liver enzymes.

3.2.4. Safranal

The DNA protective effect of safranal that observed in *in vitro* studies, has also previously found in Hosseindzadeh and Sadeghnia study. The protective effect was dose dependently [83]. According to the study, we can assume DNA migration inhibition along with apoptosis induction as the safranal protective mechanisms.

3.3. Toxicity against normal cells

Compelling evidence suggests that saffron toxicity is quite low [17,30,67,84,85]. In animal studies, calculated LD_{50} for oral intake of saffron is 20.7gr/kg [17]. Studies have reported controversial results regarding the harmful effects of saffron. Some studies have reported that the injection of 1.2 to 2gr/ABW2 saffron may cause nausea, vomiting, diarrhea and bleeding [15], while, other studies even with a high dose of 4gr per day for several days did not observe adverse effect, even in the pregnant women. However, all of these studies have conducted in Germany and it is not clear whether the used saffron is a common type or native wild saffron of Germany [15,27]. Furthermore, it seems that some toxicities such as gastrointestinal symptoms and bleeding only occur with injection. According to a study, high doses of saffron (more than 10 gr) may stimulate absorption and induce previously reported side effects including loss of appetite, insomnia, nausea, vomiting and dizziness [15]. One study also reported an allergy case to saffron [86]. Determined ID_{50} of saffron in these studies is high (20gr/kg), therefore, researchers have considered saffron as a safe herb for human consumption [84]. In the *in vivo* studies in animals, a very low or even zero toxicity of saffron and its constituents is reported [30,67,85]. A study has investigated the toxicity of safranal in daily intakes on 2nd and 21^{th}days in rats and mice, respectively. LD_{50} for intraperitoneal injection has determined as 1.48 ml/kg in male mice, 1.88 ml/kg in female mice, and 1.50 ml/kg in male rats. LD_{50} for Oral intake was 21.42 ml/kg in male mice, 11.42 ml/kg in female mice, and 5.53 ml/kg in male rats. According to the results, safranal toxicity in mice and rats at intraperitoneal injection is low, and it is non-toxic at oral administration. Moreover, safranal did not cause death at oral doses of 0.1, 0.25, and 0.5 mg/kg for 21 days in rats [87]. Ziaee et al. study has found that saffron and safranal co-treatment may inhibit safranal alone treatment toxicity. However, safranal toxicity was only limited to triglyceride, blood urea nitrogen, and alanine amino transferase. Saffron was not toxic in this study even at a dose of 10 mg/kg [88]. However, a recent study by Riahi-Zanjani et al. could not find hematologic, histopathologic or immune system toxic effects by safranal in mice. It seems that safranal doses used in this study were higher (0.1, 0.5 and 1 mg/kg) compared to the similar studies [89]. Hence, it seems that despite public belief about safranal toxicity that considers safranal as the most toxic constituent of saffron, available studies have reported low LD50 for safranal [88,89].

Moreover, Hariri et al. study have shown that crocin at doses of 50, 100 and 200 mg/kg and safranal at doses of 0.025, 0.05 and 0.1 have reduced diazinon toxicities include hematologic changes [90]. In Hariri et al. study safranal at doses of 0.025, 0.05 and 0.1 mg/kg and crocin at doses of 50, 100 and 200 mg/kg have moderated hematologic toxicities of diazinon, but not by genotoxicity inhibition [76].

A study found that oral intake as well as intraperitoneal injection of crocin (3gr/kg) did not cause death within 2 days in mice

Table 3
Saffron toxicity against normal cells.

Study	Study condition	Intervention	Administration	Dose	سمیت
Abdullaev FI et al. 2002 [48]	In vivo- animal	saffron	oral	20.7 gr/kg	The dose was equivalent to LD50 and was non-toxic
Schmidt M et al. 2007 [15]	In vivo- animal	saffron	injection	1.2 to 2 gr/ABW[a]	Nausea, vomiting, diarrhea, and bleeding were observed.
Melnyk JP et al. 2010 [27]	In vivo- human	saffron	oral	4 gr/day	Non-toxic
Hosseinzadeh et al. 2013 [87]	In vivo – rats and mice	safranal	Oral- injection	1.48 ml/kg in male mice, 1.88 ml/kg in female mice, 1.50 ml/kg in male rat intraperitoneal, 21.42 ml/kg in male mice, 11.42 ml/kg in female mice, and 5.53 ml/kg in male rat in oral intake	Non-toxic
Modaghegh et al. 2008 [92]	In vivo- human	saffron	oral	200 to 400 mg/day	Biochemical and hematologic parameters were changed in the normal ranges in healthy adults

[a] Actual Body Weight.

[91]. Another study found that safranal and saffron reduced hematocrit, hemoglobin, and erythrocytes, while, they did not cause significant damage in any body organs [41]. Saffron pill altered biochemical and hematological parameters in high doses (200–400 mg/day) in healthy adults; however, these changes were in the normal ranges and were clinically non-considerable [92]. A study found that crocin did not cause death or major organ damages at pharmacologic doses [91]. However, a Persian study by Mohajeri et al. has shown that saffron extract in very high intraperitoneal injected doses (0.35, 0.7 and 1.05 g/kg) induced anemia and hepato-renal toxicity in rats [93]. Furthermore, it has been shown that pregnant women should not consume saffron for medical purposes, since it can cause uterus contraction [26] (Table 3). Bahmani et al. study has determined saffron toxicity in different doses (500, 100 and 2000 mg/kg/day) in newborn mice. LD50 for 3 weeks oral administration was determined as 556 mg/kg. Histopathology tests have found only slight changes in mice kidney. Moreover, 21 days oral administration of saffron to lactating mothers only increase blood urea nitrogen [94]. Although, saffron toxicity is very low in this study, hematologic changes indicate that pregnant and lactating women should avoid at least from consumption of high doses of saffron.

In conclusion, due to the high LD_{50} of saffron and its constituents in normal cells, it seems that their toxic effect against non-cancer cells on the body is reasonably low. Moreover, it seems that some toxicities only occur with injection of saffron and its constituents. Furthermore, safranal is low toxic, and even, may reduce toxic effects of some drugs.

4. Discussion

According to the aforementioned findings, saffron and its constituents appropriately act against cancer formation and exhibit selective toxicity against tumors. In addition, saffron and its constituents can inhibit anti-cancer drugs toxicity. However, this effect occurs with high doses of saffron. Although, anti-cancer mechanisms of saffron and its constituents have not been cleared yet, many probable mechanisms have been proposed. For instance, saffron may directly target DNA and regulate gene expression. Although, RNA synthesis inhibition has been observed, protein synthesis did not change significantly in various studies. Thereofore, it seems that some gene pathways related to cell apoptosis induction and proliferation inhibition such as caspase pathway moderation is the major antitumor mechanism of saffron.

Bathaie et al. has shown that saffron carotenoids (crocin, crocetin, and dimethyl crocetin) directly link to the DNA small grooves and consequently, cause changes in the DNA structure. Saffron may also stimulate apoptosis in tumor cells [69]. It seems that apoptosis stimulation by saffron plays an important role in human liver (Hep G2) and human cervical (Hela) carcinoma cells death [35]. Moreover, saffron oral administration maybe more effective than intraperitoneal injection that can be attributed to its B-carotene precursor properties. Saffron also induced antioxidant enzymes. Anti-cancer effect of crocetin may be due to reduced synthesis of DNA, RNA and protein. Potent antitumor activity of crocin may be due to sugar in its structure that may interact with DNA bases to regulate some pathways such as telomerase pathway. Crocetin also inhibits RNA polymerase II in neoplastic cells. It also has been found that crocetin interact with the structure of histone H1 and H1-DNA. These findings suggest that anticancer mechanisms of crocetin are different, and epigenetic mechanisms can also be one of to them.

Antioxidant and anti-inflammatory properties of saffron and its constituents may be regarded as other anti-cancer mechanisms. Interestingly, in addition to the vitamin A precursor activity of saffron, crocetin may play a role similar to retinoic acid. Since crocetin and saffron can regulate serum levels of liver enzymes in the fatty liver disease, a major worldwide problem, it can be considered as a suitable clue for future studies.

Safranal also induced antitumor activity with low toxicity. Although, some studies have found safranal toxicities at high doses [88,89]. Apoptosis induction is the most mentioned antitumor mechanism of safranal. Safranal exhibits tumorocidal activity even at low doses. Because of dose dependent effects and low doses tumorocidal activity, it maybe considered as the most potent toxic constituent of saffron with selective toxicity.

In addition, the impact of saffron and its constituents on the gene expression and enzymes regulation are the recently discussed mechanisms. However, there are rare clinical trials regarding anticancer effects of saffron. Moreover, further studies are warranted to determine the effective dose of saffron and conclusive mechanisms of it. Besides, the absence of human studies has made it difficult to generalize the results of laboratory and animal studies to humans and also, to determine the best dose for human consumption, hence, clinical trials are recommended for future researches. Furthermore, it seems that anti-cancer effects of saffron constituents synergistically increase, and thus, saffron extract may exhibit strongest anticancer activity compared to its constituents, alone.

Interestingly, it seems that saffron toxicity is selective and target only cancer cells. Since, according to the international classification, LD_{50} between 1 to 5gr/kg is practically considered as low toxicity and more than 5 gr/kg as non-toxic [95,96], saffron and its constituents, at least can be regarded as “with low toxicity” substances.

Moreover, it seems that some toxicities such as gastrointestinal symptoms and bleeding only occur with injection of saffron and its constituents. Hence, they can be considered as “non-toxic” at oral intakes [76,88–90]. However, Vulnerable groups such as pregnant and lactating women should avoid high doses consumption of safranal, and saffron.

On the other hand, the actual amount of saffron used in daily dietary intakes is very lower than doses used in the studies that reported side effects (including nausea, vomiting, diarrhea, bleeding, changes in hematologic and hepato-renal toxicity) [15,41,92,93]. Moreover, observed beneficial effects of saffron are in low doses which are fairly similar to doses used in daily dietary intakes.

5. Conclusion

Saffron and its constituents have cancer preventive effects and selective toxicity against cancer cells, while, they do not have toxic effects against normal cells. However, the results of conducted studies are inconclusive, and further researches are needed.

References

[1] A. Jemal, R. Siegel, J. Xu, E. Ward, Cancer statistics, 2010, CA a cancer J. Clin. 60 (5) (2010) 277–300.
[2] R. Doll, R. Peto, The causes of cancer: quantitative estimates of avoidable risks of cancer in the United States today, J. Natl. Cancer Inst. 66 (6) (1981) 1192–1308.
[3] J. Peto, Cancer epidemiology in the last century and the next decade, Nature 411 (6835) (2001) 390–395.
[4] P. Anand, A.B. Kunnumakara, C. Sundaram, K.B. Harikumar, S.T. Tharakan, O.S. Lai, et al., Cancer is a preventabledisease that requires major lifestyle changes, Pharm. Res. 25 (9) (2008) 2097–2116.
[5] B.M. Lee, K.-K. Park, Beneficial and adverse effects of chemopreventive agents, Mutat Res/Fundamental Mol. Mech. Mutagen. 523 (2003) 265–278.
[6] W.C. Evans, Trease and Evans' Pharmacognosy, Elsevier Health Sciences, 2009.
[7] T. Fleming, PDR for Herbal Medicines, Medical Economics Company, 1998.
[8] K.C. Huang, The Pharmacology of Chinese Herbs, CRC press, 1998.
[9] J.A. Duke, Handbook of Medicinalherbs, CRC press, 2010.
[10] S. Sweetman, I. Martindale, The Complete Drug Reference, Pharmaceutical Press, London, UK, 2002.
[11] K. Abe, H. Saito, Effects of saffron extract and its constituent crocin on learning behaviour and long-term potentiation, Phytotherapy Res. 14 (3) (2000) 149–152.
[12] F.S. D'Amelio Sr, Botanicals: a Phytocosmetic Desk Reference, CRC Press, 1998.
[13] A.R. Gohari, S. Saeidnia, M.K. Mahmoodabadi, An overview on saffron, phytochemicals, and medicinal properties, Pharmacogn. Rev. 7 (13) (2013) 61.
[14] M. Kamalipour, S. Akhondzadeh, Cardiovascular effects of saffron: An evidence-based review, J. Tehran Heart Cent. 6 (2) (2011) 59.
[15] M. Schmidt, G. Betti, A. Hensel, Saffron in phytotherapy: pharmacology and clinical uses, Wien. Med. Wochenschr. 157 (13–14) (2007) 315–319.
[16] F. Abdullaev, Biological effects of saffron, BioFactors Oxf. Engl. 4 (2) (1993) 83–86.
[17] F.I. Abdullaev, Cancer chemopreventive and tumoricidal properties of saffron (Crocus sativus L.), Exp. Biol. Med. 227 (1) (2002) 20–25.
[18] J. Rios, M. Recio, R. Giner, S. Manez, An update review of saffron and its active constituents, Phytotherapy Res. 10 (3) (1996) 189–193.
[19] P. Winterhalter, M. Straubinger, Saffron—renewed interest in an ancient spice, Food Rev. Int. 16 (1) (2000) 39–59.
[20] P.A. Tarantilis, M.G. Polissiou, Isolation and identification of the aroma components from saffron (Crocus sativus), J. Agric. Food Chem. 45 (2) (1997) 459–462.
[21] B. Deo, Growing saffron—the world's most expensive spice, Crop Food Res. 20 (1) (2003) 1–4.
[22] S. Sampathu, S. Shivashankar, Y. Lewis, A. Wood, Saffron (Crocus sativus Linn.)—Cultivation, processing, chemistry and standardization, Crit. Rev. Food Sci. Nutr. 20 (2) (1984) 123–157.
[23] M.J. Gregory, R.C. Menary, N.W. Davies, Effect of drying temperature and air flow on the production and retention of secondary metabolites in saffron, J. Agric. food Chem. 53 (15) (2005) 5969–5975.
[24] A. Assimopoulou, Z. Sinakos, V. Papageorgiou, Radical scavenging activity of Crocus sativus L. extract and its bioactive constituents, Phytother. Res. 19 (11) (2005) 997–1000.
[25] G.L. Alonso, M.R. Salinas, J. Garijo, M.A. Sánchez-fernández, Composition of crocins and picrocrocin from Spanish saffron (Crocus sativus L.), J. food Qual. 24 (3) (2001) 219–233.
[26] F. Abdullaev, J. Espinosa-Aguirre, Biomedical properties of saffron and its potential use in cancer therapy and chemoprevention trials, Cancer Detect. Prev. 28 (6) (2004) 426–432.
[27] J.P. Melnyk, S. Wang, M.F. Marcone, Chemical and biological properties of the world's most expensive spice: Saffron, Food Res. Int. 43 (8) (2010) 1981–1989.
[28] E. Von Elm, D.G. Altman, M. Egger, S.J. Pocock, P.C. Gotzsche, J.P. Vandenbroucke, The Strengthening the Reporting of Observational Studies in Epidmiology (STROBE) statement: guidelines for reporting observational studies, Prev. Med. 45 (4) (2007) 247–251.
[29] F. Abdullaev, G. Frenkel, The effect of saffron on intracellular DNA, RNA and protein synthesis in malignant and non-malignant human cells, BioFactors Oxf. Engl. 4 (1) (1992) 43–45.
[30] S.C. Nair, S. Kurumboor, J. Hasegawa, Saffron chemoprevention in biology and medicine: a review, Cancer Biother. Radiopharm. 10 (4) (1995) 257–264.
[31] P. Tarantilis, H. Morjani, M. Polissiou, M. Manfait, Inhibition of growth and induction of differentiation of promyelocytic leukemia (HL-60) by carotenoids from Crocus sativus L, Anticancer Res. 14 (5A) (1994) 1913–1918.
[32] F.I. Abdullaev, Inhibitory effect of crocetin on intracellular nucleic acid and protein synthesis in malignant cells, Toxicol. Lett. 70 (2) (1994) 243–251.
[33] F. Abdullaev, G. Frenkel, Effect of saffron on cell colony formation and cellular nucleic acid and protein synthesis, BioFactors Oxf. Engl. 3 (3) (1992) 201–204.
[34] R.H. Liu, Potential synergy of phytochemicals in cancer prevention: mechanism of action, J. Nutr. 134 (12) (2004) 3479S–3485S.
[35] J. Tavakkol-Afshari, A. Brook, S.H. Mousavi, Study of cytotoxic and apoptogenic properties of saffron extract in human cancer cell lines, Food Chem. Toxicol. 46 (11) (2008) 3443–3447.
[36] S. Samarghandian, A. Borji, S.K. Farahmand, R. Afshari, S. Davoodi, Crocus sativus L. (Saffron) stigma aqueous extract induces apoptosis in alveolar human lung cancer cells through caspase-dependent pathways activation, BioMed Res. Int. 2013 (2013).
[37] F. Abdullaev, L. Riveron-Negrete, H. Caballero-Ortega, J. Manuel Hernández, I. Perez-Lopez, R. Pereda-Miranda, et al., Use of in vitro assays to assess the potential antigenotoxic and cytotoxic effects of saffron (*Crocus sativus* L.), Toxicol. vitro 17 (5) (2003) 731–736.
[38] J. Molnar, D. Szabo, R. Pusztai, I. Mucsi, L. Berek, I. Ocsovszki, et al., Membrane associated antitumor effects of crocine-, ginsenoside-and cannabinoid derivates, Anticancer Res. 20 (2A) (1999) 861–867.
[39] J. Escribano, A. Piqueras, Jn Medina, A. Rubio, M. Alvarez-Ortı, J.A. Fernández, Production of a cytotoxic proteoglycan using callus culture of saffron corms (*Crocus sativus* L.), J. Biotechnol. 73 (1) (1999) 53–59.
[40] J. Escribano, M.J.M. Dıaz-Guerra, H.H. Riese, J. Ontañón, D. Garcıa-Olmo, D.C. Garcıa-Olmo, et al., In vitro activation of macrophages by a novel proteoglycan isolated from corms of *Crocus sativus* L, Cancer Lett. 144 (1) (1999) 107–114.
[41] J. Escribano, G.-L. Alonso, M. Coca-Prados, J.-A. Fernández, Crocin, safranal and picrocrocin from saffron (*Crocus sativus* L.) inhibit the growth of human cancer cells in vitro, Cancer Lett. 100 (1) (1996) 23–30.
[42] M. Salomi, S. Nair, P. Panikkar (Eds.), Cytotoxicity and Non-mutagenicity of Nigela Sativa and Saffron (Crocus Sativus) in Vitro. Proc Ker Sci Congr, 1991.
[43] H. Morjani, P. Tarantilis, M. Polissiou, M. Manfait, Growth inhibition and induction of crythroid differentiation activity by crocin, dimethylcrocetine and b-carotene on K562 tumor cells, Anticancer Res. 10 (1990) 1398–1406.
[44] D.C. Garc-Olmo, H.H. Riese, J. Escribano, J. Ontáón, J.A. Fernandez, M. Atiénzar, et al., Effects of long-term treatment of colon adenocarcinoma with crocin, a carotenoid from saffron (Crocus sativus L.): an experimental study in the rat, Nutr. cancer 35 (2) (1999) 120–126.
[45] S.K. Noureini, M. Wink, Antiproliferative effects of crocin in HepG2 cells by telomeraseinhibition and hTERT down-regulation, Asian Pac J. Cancer Prev. 13 (5) (2012) 2305–2309.
[46] H. Aung, C. Wang, M. Ni, A. Fishbein, S. Mehendale, J. Xie, et al., Crocin from Crocus sativus possesses significant anti-proliferation effects on human colorectal cancer cells, Exp. Oncol. 29 (3) (2007) 175.
[47] S. Mehri, K. Abnous, S.H. Mousavi, V.M. Shariaty, H. Hosseinzadeh, Neuroprotective effect of crocin on acrylamide-induced cytotoxicity in PC12 cells, Cell. Mol. Neurobiol. 32 (2) (2012) 227–235.
[48] M.B. Ebrahim-Habibi, M. Amininasab, A. Ebrahim-Habibi, M. Sabbaghian, M. Nemat-Gorgani, Fibrillation of α-lactalbumin: Effect of crocin and safranal, two natural small molecules from Crocus sativus, Biopolymers 93 (10) (2010) 854–865.
[49] M. Salomi, S.C. Nair, K. Panikkar, Inhibitory Effects of Nigella sativa and Saffron (Crocus sativus) on Chemical Carcinogenesis in Mice, 1991.
[50] G. Martin, E. Goh, A. Neff, Evaluation of the developmental toxicity of crocetin on *Xenopus*, Food Chem. Toxicol. 40 (7) (2002) 959–964.
[51] R. Jagadeeswaran, C. Thirunavukkarasu, P. Gunasekaran, N. Ramamurty, D. Sakthisekaran, In vitro studies on the selective cytotoxic effect of crocetin and quercetin, Fitoterapia 71 (4) (2000) 395–399.
[52] D.G. Chryssanthi, F.N. Lamari, G. Iatrou, A. Pylara, N.K. Karamanos, P. Cordopatis, Inhibition of breast cancer cell proliferation by style constituents of different Crocus species, Anticancer Res. 27 (1A) (2007) 357–362.
[53] S.H. Mousavi, J. Tavakkol-Afshari, A. Brook, I. Jafari-Anarkooli, Role of caspases and Bax protein in saffron-induced apoptosis in MCF-7 cells, Food Chem. Toxicol. 47 (8) (2009) 1909–1913.
[54] C.D. Kanakis, P.A. Tarantilis, H.-A. Tajmir-Riahi, M.G. Polissiou, Interaction of tRNA with safranal, crocetin, and dimethylcrocetin, J. Biomol. Struct. Dyn. 24 (6) (2007) 537–545.
[55] C.-J. Wang, H.-S. Shiah, J.-K. Lin, Modulatory effect of crocetin on aflatoxin B1

cytotoxicity and DNA adduct formation in C3H10T12 fibroblast cell, Cancer Lett. 56 (1) (1991) 1–10.
[56] C.-J. Wang, S.-J. Shiow, J.-K. Lin, Effects of crocetin on the hepatotoxicity and hepatic DNA binding of aflatoxin B1 in rats, Carcinogenesis 12 (3) (1991) 459–462.
[57] W. Chang, Y. Lin, M. Lee, S. Shiow, C. Wang, Inhibitory effect of crocetin on benzo (a) pyrene genotoxicity and neoplastic transformation in C3H10T1/2 cells, Anticancer Res. 16 (6B) (1995) 3603–3608.
[58] T.-H. Tseng, C.-Y. Chu, J.-M. Huang, S.-J. Shiow, C.-J. Wang, Crocetin protects against oxidative damage in rat primary hepatocytes, Cancer Lett. 97 (1) (1995) 61–67.
[59] A. Dhar, S. Mehta, G. Dhar, K. Dhar, S. Banerjee, P. Van Veldhuizen, et al., Crocetin inhibits pancreatic cancer cell proliferation and tumor progression in a xenograft mouse model, Mol. cancer Ther. 8 (2) (2009) 315–323.
[60] G. Gutheil, W. ReedG, A. Ray, S. Anant, A. Dhar, Crocetin: an agent derived from saffron for prevention and therapy for cancer, Curr. Pharm. Biotechnol. 13 (1) (2012) 173–179.
[61] M. Ashrafi, S. Bathaie, M. Taghikhani, A. Moosavi-Movahedi, The effect of carotenoids obtained from saffron onhistone H1 structure and H1–DNA interaction, Int. J. Biol. Macromol. 36 (4) (2005) 246–252.
[62] S. Samarghandian, M.E. Shoshtari, J. Sargolzaei, H. Hossinimoghadam, J.A. Farahzad, Anti-tumor activity of safranal against neuroblastoma cells, Pharmacogn. Mag. 10 (Suppl. 2) (2014) S419.
[63] B. Malaekeh-Nikouei, S.H. Mousavi, S. Shahsavand, S. Mehri, H. Nassirli, S.A. Moallem, Assessment of cytotoxic properties of safranal and nano-liposomal safranal in various cancer cell lines, Phytotherapy Res. 27 (12) (2013) 1868–1873.
[64] S. Samarghandian, M.M. Shabestari, DNA fragmentation and apoptosis induced by safranal in human prostate cancer cell line, Indian J. Urol. IJU J. Urol. Soc. India 29 (3) (2013) 177.
[65] K. Prem-kumar, S.K. Abraham, S.T. Santhiya, A. Ramesh, Protective effects of saffron (Crocus sativus L.) on genotoxin induced oxidative stress in Swiss albini mice, Phytother. Res. 17 (6) (2003) 614–617.
[66] S. Nair, M. Salomi, C. Varghese, B. Panikkar, K. Panikkar, Effect of saffron on thymocyte proliferation, intracellular glutathione levels and its antitumor activity, BioFactors Oxf. Engl. 4 (1) (1992) 51–54.
[67] S. Nair, B. Pannikar, K. Panikkar, Antitumour activity of saffron (*Crocus sativus*), Cancer Lett. 57 (2) (1991) 109–114.
[68] E. El Daly, Protective effect of cysteine and vitamin E, Crocus sativus and Nigella sativa extracts on cisplatin-induced toxicity in rats, J. de Pharm. de Belg. 53 (2) (1997) 87–93;
discussion -5.
[69] S.Z. Bathaie, H. Miri, M.-A. Mohagheghi, M. Mokhtari-Dizaji, A.-A. Shahbazfar, H. Hasanzadeh, Saffron aqueous extract inhibits the chemically-induced gastric cancer progression in the wistar albino rat, Iran. J. basic Med. Sci. 16 (1) (2013) 27.
[70] S.C. Nair, M. Salomi, B. Panikkae, K. Panikkar, Modulatory effects of *Crocus sativus* and *Nigella sativa* extracts on cisplatin-induced toxicityin mice, J. Ethnopharmacol. 31 (1) (1991) 75–83.
[71] S. Nair, K. Panikkar, R. Parthod, Protective effects of crocetin on the bladder toxicity induced by cyclophosphamide, Cancer Biotherapy Radiopharm. 8 (4) (1993) 339–343.
[72] S. Nair, C.D. Varghese, K. Paniker, S. Kurumboor, R. Parathod, Effects of saffron on Vitamin A levels and its Antitumour activity on the growth of solid tumours in mice, Pharm. Biol. 32 (2) (1994) 105–114.
[73] K. Premkumar, S.K. Abraham, S. Santhiya, P. Gopinath, A. Ramesh, Inhibition of genotoxicity by saffron (Crocus sativus L.) in mice, Drug Chem. Toxicol. 24 (4) (2001) 421–428.
[74] D. Mohajeri, Y. Doustar, A. Rezaei, M. Mesgari-Abbasi, Hepatoprotective effect of ethanolic extract of Crocus sativus L. (Saffron) stigma in comparison with silymarin against rifampin induced hepatotoxicity in rats, ZJRMS 12 (5) (2010) 53–59 (In Persian).
[75] B. Naghizade, M.T. Broshki, H. Moufidpoor, Protective effect of crocin on cisplantin induced acute renal toxicity in rats, J. basic Med. Sci. Iran 9 (4) (2006) 281–286 ([In Persian]).
[76] A.T. Hariri, S.A. Moallem, M. Mahmoudi, H. Hosseinzadeh, The effect of crocin and safranal, constituents of saffron, against subacute effectof diazinon on hematological and genotoxicity indices in rats, Phytomedicine 18 (6) (2011) 499–504.
[77] C.J. Wang, T.C. Cheng, J.Y. Liu, F.P. Chou, M.L. Kuo, J.K. Lin, Inhibition of protein kinase C and proto-oncogene expression by crocetin in NIH/3T3 cells, Molecularcarcinogenesis 17 (4) (1996) 235–240.
[78] C.-J. Wang, J.-D. Hsu, J.-K. Lin, Suppression of aflatoxin B1-induced hepatotoxic lesions by crocetin (a natural carotenoid), Carcinogenesis 12 (10) (1991) 1807–1810.
[79] V. Magesh, J.P.V. Singh, K. Selvendiran, G. Ekambaram, D. Sakthisekaran, Antitumour activity of crocetin in accordance to tumor incidence, antioxidant status, drug metabolizing enzymes and histopathological studies, Mol. Cell. Biochem. 287 (1–2) (2006) 127–135.
[80] V. Magesh, K. DurgaBhavani, P. Senthilnathan, P. Rajendran, D. Sakthisekaran, In vivo protective effect of crocetin on benzo (a) pyrene-induced lung cancer in Swiss albino mice, Phytotherapy Res. 23 (4) (2009) 533–539.
[81] J. Gainer, D. Wallis, J. Jones, The effect of crocetin on skin papillomas andRous sarcoma, Oncology 33 (5–6) (1976) 222–224.
[82] M. Mathews-Roth, Antitumor activity of β-carotene, canthaxanthin and phytoene, Oncology 39 (1) (1982) 33–37.
[83] H. Hosseinzadeh, H.R. Sadeghnia, Effect of safranal, a constituent of Crocus sativus (saffron), on methyl methanesulfonate (MMS)-induced DNA damage in mouse organs: an alkaline single-cell gel electrophoresis (comet) assay, DNA cell Biol. 26 (12) (2007) 841–846.
[84] M. Wichtl, Herbal Drugs and Phytopharmaceuticals: a Handbook for Practice on a Scientific Basis, Medpharm GmbH Scientific Publishers, 2004.
[85] G.R. Karimi, H. Hosseinzadeh, P.P. Khalegh, Study of antidepressant effect of aqueous and ethanolic extract of Crocus sativus in mice, Iran. J. Basic Med. Sci. (2001) 11–15.
[86] C.D. Lucas, J.B. Hallagan, S.L. Taylor, The role of natural color additives in food allergy, Adv. food Nutr. Res. 43 (2000) 195–216.
[87] H. Hosseinzadeh, S.S. Shakib, A.K. Sameni, E. Taghiabadi, Acute and Subacute Toxicity of Safranal, a Constituent of Saffron, in Mice and Rats, Iran. J. Pharm. Res. IJPR 12 (1) (2013) 93.
[88] T. Ziaee, B.M. Razavi, H. Hosseinzadeh, Saffron reduced toxic effects of its constituent, safranal, in acute and subacute toxicities in rats, Jundishapur J. Nat. Pharm. Prod. 9 (1) (2014) 3.
[89] B. Riahi-Zanjani, M. Balali-Mood, E. Mohammadi, H. Badie-Bostan, B. Memar, G. Karimi, Safranal as a safe compound to mice immune system, Avicenna J. Phytomedicine 5 (5) (2015) 441.
[90] A.T. Hariri, S.A. Moallem, M. Mahmoudi, B. Memar, H. Hosseinzadeh, Sub-acute effects of diazinon on biochemical indices and specific biomarkers in rats: protective effects of crocin and safranal, Food Chem. Toxicol. 48 (10) (2010) 2803–2808.
[91] H. Hosseinzadeh, M. Shariaty, A. Khadem-Sameni, M. Vahabzadeh, J. Ríos, M. Recio, Acute and sub-acute toxicity of crocin, a constituent of Crocus sativus L. (saffron), in mice and rats, Pharmacologyonline 2 (2010) 943–951.
[92] M.-H. Modaghegh, M. Shahabian, H.-A. Esmaeili, O. Rajbai, H. Hosseinzadeh, Safety evaluation of saffron (*Crocus sativus*) tablets in healthy volunteers, Phytomedicine 15 (12) (2008) 1032–1037.
[93] D. Mohajeri, M. Mesgari Abbasi, A. Delazar, Y. Doustar, Mousavi Gh, B. Amouoghli Tabrizi, Histopathological study of subacute toxicity of Crocus sativusL. (Saffron) stigma total extract on liver and kidney tissues in the rat, Pharm. Sci. 15 (2) (2009) 115–124 ([In Persian]).
[94] M. Bahmani, M. Rafieian, A. Baradaran, S. Rafieian, M. Rafieian-kopaei, Nephrotoxicity and hepatotoxicity evaluation of Crocus sativus stigmas in neonates of nursing mice, J. Nephropathol. 3 (2) (2014) 81.
[95] T.A. Loomis, A.W. Hayes, Loomis's Essentials of Toxicology, Academic Press, 1996.
[96] G.L. Kennedy, R.L. Ferenz, B.A. Burgess, Estimation of acute oral toxicity in rates by determination of the approximate lethal dose rather than the LD50, J. Appl. Toxicol. 6 (3) (1986) 145–148.