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African Journal of Pharmacy and Pharmacology Vol. 4(11), pp. 834-840, November 2010
Available online http://www.academicjournals.org/ajpp
ISSN 1996-0816 ©2010 Academic Journals

*Full Length Research Paper*

# Impact of saffron as an anti-cancer and anti-tumor herb

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Accepted 29 November, 2010

**Cancer continues to represent the largest cause of mortality in the world and claims over 6 million lives each year. An extremely promising strategy for cancer prevention today is chemoprevention, which is defined as the use of synthetic or natural agents (alone or in combination) to block the development of cancer in human beings. Plants, vegetables, herbs and spices used in folk and traditional medicine have been accepted currently as one of the main sources of cancer chemopreventive drug discovery and development. This review gives an overview from one of this herbs and spices that is saffron. The chemical composition of saffron has attracted the interest of several research groups during the last decades, and among the estimated more than 150 volatile and several nonvolatile compounds of saffron, approximately 40 – 50 constituents have already been identified. Oral administration of saffron extract inhibited the growth of mouse tumors that were derived from three different kinds of cancer cells and significantly increased the life spans of treated tumor-bearing mice.**

**Key words:** Chemoprevention, volatile, tumor-bearing.

## INTRODUCTION

A large and increasing number of patients in the world use medicinal plants and herbs for health purposes. Therefore, scientific scrutiny of their therapeutic potential, biological properties and safety will be useful in making wise decisions about their use. For example, one in three people in the United States has used at least one form of alternative medicine (Abdullaev and Frenkel, 1999). From ancient times to the present, saffron has been used as a spice for flavoring and coloring food preparations, as a perfume and also as a dye or ink. In folklore medicine, as well as in modern pharmacy, saffron has been reputed to be useful in the treatment of numerous human diseases (Abdullaev and Frenkel, 1992a; Abdullaev and Gonzalez de Mejia, 1995, 1996, 1997; Abdullaev et al., 2000, 2003a; Abdullaev, 1993, 1994, 2001, 2002; Hadizadeh et al., 2007). Commercial saffron is produced from dried stigmas of *Crocus sativus* L., a member of the large family *Iridaceae* and is cultivated in Azerbaijan, France, Greece, India, Iran, Italy, Spain, China, Israel, Morocco, Turkey, Egypt, and Mexico (Abe and Saito, 2000; Abdullaev-Jafarova and Espinosa-Aguirre, 2004; Akhondzadeh et al., 2004; Lage and Cantrell, 2009). Saffron is produced worldwide at an annual rate of 50 tons with a commercial cost of about $ 50 million (Akhondzadeh et al., 2005). The main reason for its great cost is that saffron is still cultivated and harvested as it has been for millennia by hand.

The chemical composition of saffron has attracted the interest of several research groups during the last decades and among the estimated more than 150 volatile and several nonvolatile compounds of saffron, approximately 40 - 50 constituents have already been identified (Alonso et al., 1998, 1996; Basker and Negbi, 1983; Castellar et al., 1993; Chang et al., 1964, 1996; Corti et al., 1996; Curró and Micgelli, 1979; Dufresne et al., 1997;

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Duke, 1998; Eisenberg et al., 1993; Garcia- Olmo, 1999). Based on these data, we can conclude that saffron contains three main pharmacologically active metabolites: 1) Saffron-colored compounds are crocins, which are unusual water-soluble carotenoids (mono and diglycosyl esters of a polyene dicarboxylic acid, named crocetin). The digentiobiosyl ester of crocetin - α-crocin is the major component of saffron. 2.) Picrocrocin is the main substance responsible for the bitter taste in saffron. 3.) Safranal is the volatile oil responsible of the characteristic saffron odor and aroma. Furthermore, saffron contains proteins, sugars, vitamins, flavonoids, amino acids, mineral matter, gums and other chemical compounds (Grisolia, 1974). Animal studies indicate that the oral $LD_{50}$ of saffron was 20.7 g/kg administered as a decoction (Himeno and Sano, 1987).

Some reports about the stability of saffron (Hosseinzadeh and Khosravan, 2002) mention that two factors (temperature and humidity) exert a strong influence on the degradation of the main pharmacologically active ingredients of saffron under different storage conditions, but the developed HPLC assay can be utilized as a quality control method for saffron (Iborra et al., 1993). On the other hand, when saffron is stored under -20°C, its pharmacological activities as a supplement remain unaltered for at least 2 years or even longer (Iborra et al., 1992). Further studies to elucidate the structure and to characterize the biologically active ingredients of saffron are now in progress in different laboratories. The scientific evidence on the cancer chemopreventive effects of saffron extract and its main ingredients are outlined here, updating previous reviews on this topic (Iborra et al., 1992). Saffron is an herb most people are unlikely to utilize, either for medicinal or culinary purposes, primarily because the material has a justified reputation for being extraordinarily expensive.

The aim of this review is to provide an investigation focused on the anticancer activity of saffron (*C. sativus* L.) and its principal ingredients. Potential use of these natural agents in cancer therapy are also discussed.

## Literature review

Saffron is collected from *C. sativus* (*Iridaceae*), which originated in the Middle Eastern region of the Eurasian continent, from Greece to Persia (Iran). Iran, the major saffron producer for about 85 percent of the global share, has been investing in research into saffron's potential medicinal applications (Kubo and Kinst-Hori, 1999).

The medicinal properties attributed to saffron are extensive. Topically, it is applied for inhibiting growth of cancer cells, improve the skin condition overall and specifically to treat acne. Internally, it is used to improve blood circulation, regulate menstruation, treat digestive disturbance, ease cough and asthmatic breathing, reduce fever and inflammation, calm. Extracts of saffron have been shown to inhibit the formation of tumors and/or to retard tumor progression in a variety of experimental animal systems. The topical application of a saffron extract has been shown to inhibit both the initiation and the promotion of cancer by a common carcinogen, DMBA, which is used to induce skin cancer for experimental purposes. The exciting news is that saffron extracts have been shown to significantly prolong-almost by three-fold-the life spans of mice undergoing experimental chemotherapy with the toxic anticancer drug, cisplatin. Saffron also partially prevented the decrease in body weight, hemoglobin levels and leukocyte counts associated with that form of chemotherapy (Nair, 1991). Another study showed that when saffron was combined with two other substances, the amino acid cysteine and the antioxidant vitamin E, it had a protective effect against the toxicity of cisplatin. Together, these three protective agents significantly reduced blood urea nitrogen, serum creatinine and blood glucose levels, as well as reduced many other harmful chemical changes in the body (el Daly, 1998).

Taken together, these studies indicate that saffron (with or without other substances, such as antioxidants or their precursors) has the potential to alleviate the toxicity of cisplatin, including the nephrotoxicity (damage to the kidneys), which is one of cisplatin's most serious side effects. This potential use of saffron has gone largely unexplored by conventional oncology since it first became known in 1991.

In other studies, Nair and colleagues showed that the oral administration of saffron extract inhibited the growth of mouse tumors that were derived from three different kinds of cancer cells (S180, DLA and EAC) and significantly increased (again by two- to three-fold) the life spans of treated tumor-bearing mice (Nair, 1997b).

Later, these same Indian authors reported that giving saffron by mouth to lab animals significantly slowed the growth of two different kinds of cancer cells (DLA and S-180). The authors suggested that the increased levels of carotenes and Vitamin A may have accounted for this anticancer effect. Interestingly, when saffron extract was encapsulated with lipids and then injected into the mice, there was an increase in the antitumor effect of this extract towards several solid tumors, including EAC tumor cells that had formerly been insensitive to orally administered saffron extract (Nair, 1992). Dr. Abdullayev and his colleagues have also found that naturally occurring saffron extract, in combination with two synthetic compounds, sodium selenite or sodium arsenite, may have a synergistic effect with saffron and may, therefore, have an important role in cancer chemoprevention (Riverón-Negrete, 2002; Gresta et al., 2008).

A computerized search of published articles was performed using the MEDLINE database from 1990 to 2004. Search terms utilized including saffron, carotenoids, chemoprevention and cancer. All articles were obtained as reprints from their original authors. Additional

sources were identified through cross-referencing. Studies in animal models and with cultured human malignant cell lines have demonstrated antitumor and cancer preventive activities of saffron and its main ingredients, possible mechanisms for these activities are discussed. More direct evidence of anticancer effectiveness of saffron as chemopreventive agent may come from trials that use actual reduction of cancer incidence as the primary endpoint. This work suggests that future research be warranted that will define the possible use of saffron as effective anticancer and chemopreventive agent in clinical trials. (Li et al., 1999; Lozano et al., 1999; Morjani et al., 1990)

## Market survey

In some countries, such as Spain, Iran, and India, people know that saffron is worth its price and make good use of it. To meet the demand, world annual production is about 265 tons per year, which is grown on about 90,000 acres of land (if efficiently cultivated, each acre produces about 6 pounds of saffron a year). (Nadkarni, 1976) It takes about 170 – 200 h of work to collect the flowers and remove the stamens for drying in order to produce just 1 pound of saffron, which is a large part of the expense for the spice.

## METHODOLOGY

Research into the effect of saffron on neoplastic cells has seen a renaissance in the last decade, and a growing body of evidence indicates that saffron and its characteristic components possess anti carcinogenic and antitumor activities *in vivo* and *in vitro* (Nair et al., 1995). Saffron extract has been shown capable of inhibiting and/or retarding tumorigenesis in a variety of experimental models *in vivo* (Nair et al., 1991b). Much of the ground-breaking research related to saffron and its anti-cancer and anti-tumor properties is being carried out by Azerbaijani scientist Dr. Fikrat Abdullayev, who heads a research team at the National Institute of Pediatrics in Mexico City. He ideally positioned to lead an international investigation into the medicinal properties of saffron.

For the past 30 years, Dr. Ralph Moss has been studying the field of cancer therapy and prevention, monitoring developments in the world of oncology, helping cancer patients and their families weigh up the benefits and drawbacks of treatments both conventional and alternative. Topical application of saffron extract (100 mg/kg body wt) inhibited two-stage initiation/promotion dimethylbenz[a]anthracene (DMBA)-induced skin carcinogenesis and oral administration of saffron extract in the same dose restricted 20-methylchloanthrene (MCA)-induced soft tissue sarcomas in mice (Nair et al., 1991a). Later, it was demonstrated that saffron extract significantly prolonged (almost 3-fold) the life spans of cisplatin-treated (2 mg/kg body wt) mice and partially prevented the decrease in body weight, hemoglobin levels and leukocyte counts (Nair et al., 1991). Another study (Nair et al., 1992) examined the protective effect of concurrent administration of cysteine (20 mg/kg body wt) together with vitamin E (2 mg/kg body wt) and saffron extract (50 mg/kg body wt) against cisplatin-induced (3 mg/kg body wt) toxicity in rats. It was shown that treatment of animals with protective (saffron together with vitamin E and cysteine) agents significantly reduces blood urea nitrogen, serum creatinine levels and blood glucose levels, as well as partially prevents many changes in the activities of different serum enzymes (Nair et al., 1994). Taken together, these studies indicated that saffron may be a promising agent for reducing cisplatin-toxic side effects, including nephrotoxicity.

Oral administration of saffron extract (200 mg/kg body wt) induced a dose-dependent inhibition of the growth in mice of ascite tumors derived from sarcoma-180 (S-180), Ehrlich ascites carcinoma (EAC), Dalton's lymphoma ascites (DLA), and significantly increased (2- to 3-fold) life spans of treated tumor-bearing mice (Narasimhan et al., 1992). Later, these authors reported that oral administration of saffron extract significantly suppressed the growth of DLA and S-180 tumor cells, but did not affect the growth of EAC tumor cells in mice (Negbi, 1999). The authors suggested that increase in the levels of ß-carotene and vitamin A in the serum of the experimental animals receiving saffron might be one explanation for this antitumor effect of saffron. Interestingly, when liposome-encapsulated saffron extract was injected i.p. into mice, the increasing of antitumor effect of this extract towards several solid cells was observed, including the EAC tumor cells, which were insensitive to orally administered extract (Noorbala, 2005). These authors suggested that enhancement in antitumor activity of saffron extract could be due to site-directed drug delivery or to carrier-mediated increased drug solubility. More recently, it was reported (Oberdieck, 1991) that crocin, a carotenoid isolated from saffron, increased the survival time and decreased tumor (colon adenocarcinoma) growth in female rats without any significant effects in male animals. The authors suggested that the selective antitumor action of crocin in female rats compared with male might be related to hormonal factors.

In another study (Oda and Tatsumi, 1993), crocetin at nontoxic doses inhibited genotoxic effect and neoplastic transformation in C3H1OT1/2 cells induced by benzo(a)pyrene (BP). Thus, studies *in vivo* showed that saffron extract and its purified constituent significantly increased the life span of animals with different types of tumor, but the mechanism of anti carcinogenic effect of saffron has not been elucidated.

A number of studies have demonstrated an antitumor effect of saffron and its constituents on different malignant cells *in vitro* .Observed differences in sensitivity to saffron and its ingredients between different cultured malignant cells (Palozza and Krinsky, 1992; Pfander and Schurtenberge, 1982; Rios, 1996; Riverón-Negrete et al., 2002; Rödel and Petrzika, 1991) could be due to the existence of distinct cell surface receptors, intracellular retention transport, differences in the drug uptake, or differences in the methods of extraction and determination of cytotoxicity. By using trypan-blue dye exclusion as a criterion of cell viability, the $IC_{50}$ of saffron extract was found to range from 7 to 30 µg/ml, dependent upon the type of tumor cells, whereas there was no significant effect on normal mouse spleen cells (Saito and Utsumi, 1996). Utilizing the method of colony formation as a measure of cell viability, it was demonstrated that the $IC_{50}$ of saffron extract ranged from 100 to 200 µg/ml upon the type of human malignant cells, but had no significant effect on normal human lung cells (Salomi et al., 1990, 1991). It was shown that the saffron extract inhibited cellular nucleic acid synthesis and had no effect on protein synthesis in tumor cells (Salomi et al., 1991). Interestingly, there was a stimulatory or supporting effect of saffron extract on nonspecific proliferation of immature and mature lymphocytes *in vitro* and colony formation of normal human lung cells (Selim et al., 2000; Smith, 1998). It was also observed that saffron increased the intracellular levels of reduced glutathione and glutathione-related enzymes and suggested a possible antioxidant activity of saffron (Straubinger et al., 1998). It was shown that saffron extract and its purified characteristic compounds crocin, safranal, picrocrocin, and ß-carotene inhibited different types of tumor cell growth (Straubinger et al., 1997). Interestingly, in two studies (Sujata et al., 1992), crocetin isolated from saffron had a cytotoxic activity on tumor cells,

**Table 1.** (Dharmananda).

| Substance | Proportion (%) |
|---|---|
| Simple sugars | 12 – 15 |
| Water | 9 – 14 |
| Proteins, amino acids, other nitrogen compounds | 11 – 13 |
| Cellulose (fiber) | 4 – 7 |
| Fats | 3 – 8 |
| Minerals (measured as acid soluble ash) | 1 – 1.5 |
| Other non-nitrogen (mainly complex sugars) | about 40 |

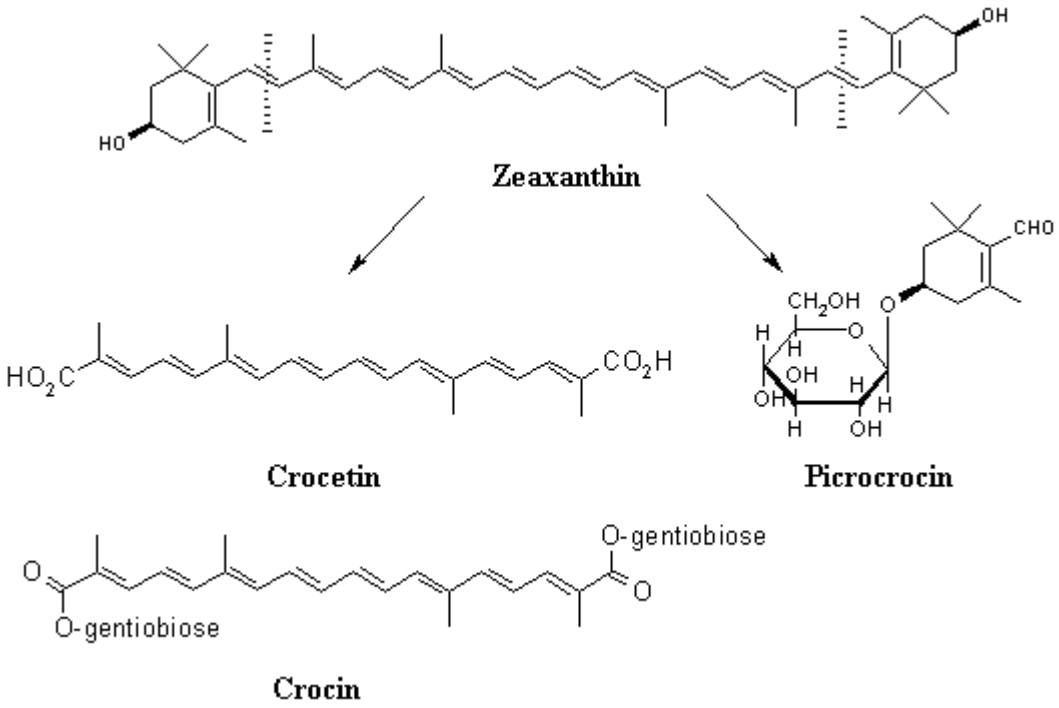


**Figure 1.** Proposed mechanisms for cancer preventive and tumoricidal effects of saffron.

but in another study, it was shown that crocetin did not show any cytotoxic effect (Suzhou, 1977). This study (Takashi, 1992) demonstrated that crocetin had no cytotoxic effect on colony formation of different tumor cells, but had a dose-dependent inhibitory effect on DNA, RNA, and protein synthesis in these human malignant cells. We also reported that treatment of tumor cells with saffron extract in combination with well-known antitumor agents such as selenium compounds caused a more effective inhibition of colony formation and nucleic acid synthesis relative to the effects of these agents alone (Tarantilis et al., 1994). It was reported that a novel glucoconjugate isolated from corms and callus of saffron possessed cytotoxic activity against different tumor cells (Tarantilis, 1994). These authors demonstrated that glucoconjucate from corms of *C. sativus* L. possessed cytotoxic activity on human tumor cells derived from fibrosarcoma, cervical epithelioid carcinoma and breast carcinoma. This compound was about eight times more cytotoxic for malignant cells than for their normal counterparts and it caused plasma membrane damage in these cells. Interestingly, that analysis of DNA fragmentation indicated that cell death was not mediated by apoptosis. Thus, extracts of saffron stigmas, corm, and callus and its ingredients possessed both anticarcinogenic and antitumor activities *in vivo* and *in vitro* (Tarantilis et al., 1994).

Only one study (Tarantilis and Polissiou, 1997) using the Ames assay had indicated that crocin and dimethyl-crocetin from saffron were nonmutagenic and nonantimutagenic. In their laboratory, it has been demonstrated that saffron extract was nontoxic, nonmutagenic and nonantimutagenic (Tarantilis et al., 1994). Thus, saffron and its constituents are suggested as alternative anticancer agents, which alone and in combination with other synthetic substances may have the potential for the prevention and the treatment of certain forms of cancer. Saffron has been analyzed extensively. It contains these plant components as pointed in Table 1.

These active compounds: Essential oil (volatile oil): 0.3 – 1.5% yellow color: crocins, derived from crocetin (about 2%) and other carotenes (about 8%). Bitter substances including picrocrocin and safranal (the main aromatic of saffron): about 4% the active constituents are degradation products of common carotenoids, mainly zeaxanthin (and to a lesser extent, lycopene and beta-carotene), as illustrated in Figure 1. Crocetin and the crocins

provide far more color than the other carotenes. Picrocrocin, derived from the terminal end of zeaxanthin, is the glycoside of safranal, which is a terpene aldehyde. Safranal is formed during the drying of the collected saffron and it provides most of the characteristic saffron fragrance. There are other volatile components (included in the essential oil fraction) that are also derived from the carotenes and have structures similar to safranal.

## RESULT AND DISCUSSION

Different hypotheses for the modes of anticarcinogenic and antitumor actions of saffron and its components have been proposed. One of the mechanisms for the antitumor or anticarcinogenic action of saffron and its components is the inhibitory effect on cellular DNA and RNA synthesis, but not on protein synthesis (Tarantilis et al., 1995). A second suggested mechanism for the antitumor action of saffron and its constituents is the inhibitory effect on free radical chain reactions, because most carotenoids are lipid-soluble and might act as membrane-associated high-efficiency free-radical scavengers, which is connected with their antioxidant properties (Tseng et al., 1995). A third proposed mechanism by which the saffron extract exerts its antitumor effect is the metabolic conversion of naturally occurring carotenoids to retinoids, but recently, it was reported that conversion carotenoids to vitamin A is not a prerequisite for anticancer activity (Verma and Bordia, 1998). A fourth suggested mechanism is that the cytotoxic effect of saffron is con=-nected with interaction of carotenoids with topoisomerase II, an enzyme involved in cellular DNA-protein interaction (Winterhalter and Straubinger, 2000).

Recently, several other mechanisms for the antitumor effect of saffron and its constituents have also been proposed. It was demonstrated that a novel glucoconjugate, isolated from corm and callus extract of saffron, caused swelling and local plasma membrane evagination and it was suggested that cytotoxicity is mediated via extracellular fluid uptake (Wuthrich et al., 1997). It was also reported that saffron contains lectins and it might also be suggested that antitumor activity of saffron is mediated via lectins. The literature also contains reports that saffron extract and/or its components inhibited activities of different cellular enzymes and it was suggested that the antitumor effect of these agents might be associated with the effect on enzyme functions. Treatment of tumor cells with saffron resulted in an increase in the level of intracellular sulphydryl compounds and this could be one explanation for the potentiation of saffron cytotoxicity. Another suggested mechanism is that cytotoxic effect of carotenoids from saffron is mediated via apoptosis (Xuabin, 1992). Interesting studies indicate that encapsulation in amorphous polymer matrices of saffron extracts or saffron carotenoid greatly improves their stabilities and enhances their antitumor effects. More recently, it was shown that $\gamma$-irradiation, necessary for microbial decontamination, did not produce significant qualitative changes of volatile essential oil constituents of saffron, but induced a slight decrease in glycosides and an increase in aglycon content in carotene constituents of saffron (Xue, 1982; Zareena et al., 2001; Zargani and Heinz, 1971; Zhang, 1994). This relative stability of saffron to irradiation should also be taken to account in the search for an explanation of the chemopreventive potential of this spice.

Although several hypotheses have been put forward, the exact mechanism(s) of anticarcinogenic and antitumor effects of saffron and its main constituents are not clear at present (Zhou et al., 1978), and it might also be suggested that antitumor activity of saffron is mediated via lectins. The literature also contains reports that saffron extract and/or its components inhibited activities of different cellular enzymes and it was suggested that the antitumor effect of these agents might be associated with the effect on enzyme functions. Treatment of tumor cells with saffron resulted in an increase in the level of intracellular sulphydryl compounds and this could be one explanation for the potentiation of saffron cytotoxicity. Another suggested mechanism is that cytotoxic effect of carotenoids from saffron is mediated via apoptosis (Xuabin, 1992). Interesting studies indicate that encapsulation in amorphous polymer matrices of saffron extracts or saffron carotenoid greatly improves their stabilities and enhances their antitumor effects. More recently, it was shown that $\gamma$-irradiation, necessary for microbial decontamination, did not produce significant qualitative changes of volatile essential oil constituents of saffron, but induced a slight decrease in glycosides and an increase in aglycon content in carotene constituents of saffron (Xue, 1982; Zareena et al., 2001; Zargani and Heinz, 1971; Zhang, 1994). This relative stability of saffron to irradiation should also be taken to account in the search for an explanation of the chemopreventive potential of this spice.

Although several hypotheses have been put forward, the exact mechanism(s) of anticarcinogenic and antitumor effects of saffron and its main constituents are not clear at present (Zhou et al., 1978).

### Conclusion

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

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

Since the relationship between saffron and cancer is an important concern, comprehensive, in-depth studies need to be conducted further along the following lines: 1.) Define the mechanism(s) involved in the therapeutic properties of saffron; 2.) Investigate the mechanism(s) involved in saffron cancer chemoprevention; 3.) Determine the biologically active components of saffron; and 4.) Perform human studies to define efficacy of saffron in cancer treatment and prevention.

The scarcity and expense in obtaining large quantities of saffron may provide impediments to human chemoprevention and cancer treatment using this agent; however, an indoor cultivation method is advantageous in achieving the highest quality of saffron and for decreasing its price. The results of each of these researches provide parts of the scaffolding to construct a logical platform for the appearing of a new scientific discipline to be called saffronology.

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