Antitumor Effect of Saffron (*Crocus sativus* L.): Overview and Perspectives

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Keywords: cancer, chemoprevention, cytotoxicity, genotoxicity, human normal and malignant cells, saffron

Abstract

From ancient times, saffron dried stigmas of *Crocus sativus* L. is widely used as a drug against different human diseases. In the beginning of 1990´s scientific literature reported for the first time the antitumor activity of saffron. During the last decade different laboratories of the world (including ours) have accumulated sufficient scientific evidence to suggest that saffron, and its main ingredients, can inhibit the process of carcinogenesis in vivo and in vitro effectively. The aim of this work has been to summarize and discuss the scientific results obtained in our laboratory, together with data collected from the literature, about the antitumor and anticarcinogenic activities of saffron and its main ingredients, and the possible molecular mechanism of these actions. Saffron and its carotenoid ingredients are chemopreventive in cultivate human malignant cells and animal models, inhibiting cell growth. This could explain how they reduce outgrowth of tumor cells in vivo. It has been shown that those malignant cells are more sensitive than normal cells to the inhibitory effect of saffron. Inhibition of intracellular nucleic acid synthesis and free radical chain reactions may contribute to explain the molecular mechanism of antitumor effect of saffron. Although, it may be too soon to celebrate, the antitumor activity of saffron is quite promising and warrants further investigations, especially in clinical trials.

INTRODUCTION

Plant based natural products have been a fertile source of cure for cancer, which is projected to become the major causes of death in this century. There are at least 250,000 species of plants out of which more than one thousand plants have been found to possess significant anticancer properties (Abdullaev, 2001). Commercial saffron is derived from the stigmas of *Crocus sativus* L. a member of the *Iridaceae* family.

The taxonomic classification of saffron is the following:
Division: Spermatophyta
Subdivision: Angiospermae
Class: Monocotyledonae
Subclass: Lilidae
Order: Liliales
Family: Iradaceae
Genus: Crocus

Characteristic ingredients of saffron (Figure1) are crocin –responsible of the color, picrocrocin –responsible of the bitter taste and safranal –responsible of the odor and aroma (Abdullaev, 1993). The use of saffron for medical benefit has played an important role in traditional medicine of different cultures on earth (Abdullaev, 1993). Anecdotal data comprise much of the popular information available about saffron in folklore medicine. In modern pharmacy saffron has reputed to be useful in treatment of numerous human diseases including cancer (Abdullaev, 2002a). The widespread use of saffron, either directly or as dietary supplement, has raised many scientific questions. One of them is, are saffron preparations safe? Animal studies indicated that oral LD_{50} of saffron, administered as a decoction, was 20.7 g/kg. Our data demonstrated that oral

administration of saffron at concentrations from 0.1 to 5 g/kg was nontoxic in mice (Abdullaev, 2002b). In the beginning of 1990´s scientific literature reported for the first time the antitumor activity of saffron (Nair et al, 1991; Abdullaev and Frenkel, 1992a,b). During the last decade, results of saffron antitumor research were published in 29 experimental articles and 8 review articles (Tables 1 and 2). It is a fact that saffron anticancer research clearly requires multinational efforts, involving scientists from Azerbaijan, Greece, Hungary, India, Japan, Mexico, Spain, USA and others. This review will focus on research findings, mainly from our laboratory, regarding the antitumor and anticarcinogenic activities of saffron and its main ingredients, possible molecular mechanism(s) of action(s) and perspectives in this area of research.

ANTITUMOR EFFECTS OF SAFFRON AND ITS INGREDIENTS ON TUMOR CELLS IN VITRO

Several of studies from our laboratory (Abdullaev and Frenkel, 1992a,b; Abdullaev, 1994; Abdullaev and González de Mejía, 1995; Abdullaev et al, 2002a,b, 2003a,b) have demonstrated the cytotoxic effect of saffron extract on different human normal and malignant cells in vitro (Table 3). Saffron extract had no effect on two normal human cell lines, but inhibited the growth of all tested human malignant cells (six) in dose-dependent manner. The most sensible to the inhibitory effect of saffron were A204 human rhabdomyosarcoma cells. It is interesting to note that saffron showed inhibitory effect of tested human malignant cells from different origin. Data presented in Table 4 indicate that saffron extract had no effect on macromolecular synthesis neither in normal or malignant human cells in vitro, but exhibited a dose-dependent inhibitory on nucleic acid synthesis in the tested malignant cells. No effect of saffron extract was observed on protein synthesis neither in normal or malignant cells. Commercial crocetin isolated from saffron had no cytotoxic effect on colony formation of normal and malignant human cells (Table 5). Our results demonstrated that crocetin inhibited synthesis of DNA, RNA and protein in dose-dependent manner only in malignant human cells. Data presented in Table 6 demonstrated inhibitory effect of different isolated carotenoid ingredients of saffron on colony formation of human HeLa cells. It was shown that trans-crocin-3, isolated from saffron, was more effective. It would be interesting to ascertain: Does saffron interact with other antitumor agents to enhance or reduce their efficacy? In other of our articles, presented in this volume (chapter 58), we have demonstrated that saffron in combination with a well known antitumor compound, sodium selenite, caused an additive inhibitory effect on four human malignant cells tested in vitro (Riverón-Negrete et al., 2004). The effect of saffron extract on viability on four human cells lines is presented in Table 7. Cell lines utilized in these experiments were SKNSH (malignant cells derived from a bone metastases of a neuroblastome), HeLa (malignant cells from an adenocarcinoma from uterine cervix), MCF-7 (malignant cells from a breast tumor), and normal fetal lung fibroblast cells. All the cells lines are of human origin, and they were obtained from ATCC (American Type Culture Collection). Saffron extract inhibited the viability of human malignant cells in dose dependent manner. IC_{50} for normal cells was about 10 times higher than for malignant cells. By using the Ames/*Salmonella* plate incorporation assay with five strains of these bacteria, both with and without S9 activation, no mutagenic activity of saffron extract up to concentration of 1500 μg/plate was detected (Table 8). The mutation index (MI) was less than 2, at all the tested concentrations of saffron extract. Previously, it was shown that saffron extract was non antimutagenic with benzo (a) pyrene (Abdullaev et al., 2003a). In Table 9 literature data on the cytotoxic effect of saffron and its ingredients on different malignant cells are summarize. Literature data about anticarcinogenic and antitumor effect of saffron in animal models has been discussed in a recent review (Abdullaev, 2002).

Different hypothesis for the antitumor mechanism of action of saffron and its ingredients have been proposed, e.g. inhibition of intracellular DNA and RNA synthesis, inhibition of free radical chain reactions, stability to irradiation, metabolic conversion of carotenoids to retinoids, increase of intracellular SH-compounds, inhibition of

genotoxicity, induction of apoptosis, inhibition of cell proliferation, inhibition of different cellular enzyme activities, and others (Abdullaev, 2002). However, the precise molecular mechanism of antitumor activity of these natural agents is not well understood at present. Thus, saffron extracts from stigmas, corm and callus and their main ingredients have shown antitumor and anticarcinogenic activities both in vitro and in vivo. These findings have great significance regarding the direction of clinical trials, in order to investigate the use of saffron and/or its main ingredients as natural agents for prevention and treatment of different human cancers.

CONCLUSIONS

Saffron and its main carotenoid constituents are suggested as alternative natural antitumor agents, which alone and in combination with other well-know antitumor substances, may have potential to prevent and/or to treat certain forms of cancer. Because the relationship between natural agents and cancer is an important concern, in-depth studies are necessary to be conducted further along in the following lines (Abdullaev, 2003b):

1. To determine the biological active ingredients of saffron, responsible for its anticancer effect.
2. To carry out epidemiological studies on effect of saffron consume in cancer prevention.
3. To investigate the molecular mechanism(s) involved in the antitumor and anticancer effects of saffron.
4. To define efficacy and safety of saffron and its main ingredients for cancer prevention and treatment, both in animal models and clinical trials.

Unfortunately, we spent many years and money focusing on cures of cancer, but focusing on prevention by natural anticancer agents instead would have made winnable the war on this terrible disease. Investigation of saffron and its constituents should follow a structured design, incorporating parallel preclinical studies of the spice source and the isolated chemical agents in terms of efficacy, toxicity, biological mechanisms, and pharmacokinetics. Pilot clinical trials on the pharmocokinetics and mechanism-based markers of efficacy of the selected intervention should precede phase I-III development in suitable populations. Chemoprevention of cancer is a challenging task in the 21st century and more knowledge regarding the relationship between natural products and cancer is needed.

ACKNOWLEDGEMENTS

This work was partially supported by funds from CONACyT (grant 28513-N and grant 40011).

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Tables

Table 1. Experimental publications on antitumor or anticancer effect of saffron

Year	Authors	Journal	Country
1991	Nair SC et al	*Cancer Lett 57: 109-114*	India
1991	Salomi MJ et al	*Nutr Cancer 16: 67-72*	India
1991	Nair SC et al	*Ethnopharmacol 31: 75-83*	India
1992	Abdullaev FI, Frenkel GD	*Biofactors 3: 201-204*	USA
1992	Abdullaev FI, Frenkel GD	*Biofactors 4: 43-45*	USA
1992	Nair SC et al	*Biofactors 4: 51-54*	India
1994	Abdullaev FI	*Toxicol Lett 70: 243-251*	USA
1994	Tarantilis PA et al	*Anticancer Res 14: 1913-1918*	Greece
1994	Nair SC et al	*Int J Pharmacog 32: 105-114*	USA
1995	Abdullaev FI, González de Mejía E	*Biofactors 5: 133-138*	Mexico
1996	Escribano J et al	*Cancer Lett 100: 23-30*	Spain
1998	Verma SK, Bordia A	*Indian J Med Sci 52: 205-207*	India
1998	El Daly ES	*J Pharm Belg 53: 87-95*	Egypt
1998	Kacumov FJ et al	*Dokl Azerb Acad Sci LIV, 1: 70-73*	Azerbaijan
1999	Kubo I, Kinst-Hori I	*J Agric Food Chem 47: 4121-4125*	USA
1999	Escribano J et al	*Biochem Biohys Acta 1426: 217-222*	Spain
1999	García-Olmo DC et al	*Nutr Cancer 35: 120-126*	Spain
1999	Escribano J et al	*Cancer Lett 144: 107-114*	Spain
1999	Escribano J et al	*J Biotechnol 73: 53-59*	Spain
2000	Fernández JA et al	*J Exp Bot 51: 731-737*	Spain
2000	Molnar J et al	*Anticancer Res 20: 861-867*	Hungary
2000	Escribano J et al	*Planta Med 66: 157-162*	Spain
2001	Premkumar K et al	*Drug Chem Toxicol 24: 421-428*	India
2001	Soeda S et al.	*Life Sci 69: 2887-2898*	Japan
2001	Zareena AV et al	*J Agric Food Chem 49: 687-691*	India
2002	Martin G et al	*Food Chem Toxicol 40: 959-964*	USA
2002	Abdullaev FI et al	*Rev Inv Clin 54: 430-436*	Mexico
2003	Premkumar K et al	*Phytother Res 17: 614-617*	India
2003	Abdullaev FI et al	*Toxicol in Vitro 17: 731-736*	Mexico

Table 2. Review articles on the biological effects of saffron

Year	Authors	Journal/Book	Country
1993	Abdullaev FI	*BioFactors 4: 83-86*	USA
1995	Nair SC et al	*Cancer Biother 10: 257-264*	USA
1996	Rios JL et al	*Phytother Res 10: 189-193*	Spain
1999	Abdullaev FI, Frenkel GD	*"Saffron, Crocus sativus L." Negbi M. (ed) Medicinal & Aromatic Plants –Industrial Profiles. Vol. 8, Harwood Acad Pub, Amsterdam, 10, 103-113*	Mexico/USA
2000	Winterhalter P, Straubinger M	*Food Rev Int 16: 39-59*	Germany
2002	Deng Y et al	*Zhongguo Zhong Yao Za Zhi 27: 565-568*	China
2002	Abdullaev FI	*Exp Biol Med 227: 20-25*	Mexico
2003	Abdullaev FI	*Recent Progress in Medicinal Plants 8 69-82*	Mexico/Azerbaijan

Table 3. Inhibitory (IC_{50}) effect of saffron extract on colony formation of different human cultured cells in vitro

Cells	IC_{50} (µg/ml)
Normal fetal lung fibroblast (WI38)	No effect
Normal lung fibroblast-like (CCD-18Lu)	No effect
SV-40 transformed WI38 (VA13)	250
Lung adenocarcinima (A549)	300
Cervix epitheloid carcinoma (HeLa)	180
Hepatocallular carcinoma ($HepG_2$)	220
Rhardomyosarcoma (A204)	95
Colon adenocarcinoma (SW480)	195

Table 4. Inhibitory (IC_{50}) effect of saffron extract on DNA, RNA and protein synthesis in different human cultured cells in vitro

Cells	IC_{50} (µg/ml)		
	DNA	RNA	Protein
Normal fetal lung fibroblast (WI38)	No effect		
Normal lung fibroblast-like (CCD-18Lu)	No effect		
SV-40 transformed WI38 (VA13)	250	80	No effect
Lung adenocarcinima (A549)	200	100	No effect
Cervix epitheloid carcinoma (HeLa)	150	160	No effect
Hepatocallular carcinoma ($HepG_2$)	220	110	No effect
Rhardomyosarcoma (A204)	105	70	No effect
Colon adenocarcinoma (SW480)	190	130	No effect

Table 5. Inhibitory (IC_{50}) effect of crocetin on colony formation and DNA, RNA, Protein synthesis in different human cultured cells in vitro

Cells	IC_{50} (µg/ml)			
	Colony formation	DNA	RNA	Protein
Normal fetal lung fibroblast (WI38)	No effect		No effect	
SV-40 transformed WI38 (VA13)	No effect	50	70	55
Lung adenocarcinima (A549)	No effect	42	57	45
Cervix epitheloid carcinoma (HeLa)	No effect	10	30	100

Table 6. Inhibitory effect (%) of different ingredients of saffron on colony HeLa cells formation.

Ingredients of saffron (200µg/ml)	% Inhibition
Crocin 1	7.0±0.9
Crocin 2	10,0±1.2
Crocin 3	25,0±2.1
Picrocrocin	27.0±2.7
Picrocrocin (acid forms)	30,0±1.9
Trans-crocin 2´	10,0±1.6
Trans-crocin 3	36.0±2.4
Trans-crocin 4	27,0±2.3
Cis-crocin 3	18,0±1.8
Crocetin	25,0±2.0

HeLa cells were exposed to indicate concentrations of saffron ingredients for 3 h. The number of cells that were able to form colonies was determined as described in Materials and Methods. Results are presented as the percentage of colony formation inhibition (colonies/dish) in relation to untreated cells 100% (HeLa cells = 70). Each value represents the mean ±S.D. of triplicate plates.

Table 7. Effect of saffron extracts on viability of human cultured cells.

IC_{50} (mg/ml)			
MCF-7	SKNH	HeLa	Normal human fibroblasts
0.78	1.66	1.92	19.99

Table 8. Non-mutagenic activity of saffron extract assayed in the *Salmonella typhimurium* TA97, TA98, TA100, TA102 and TA1538 strains plate incorporation test [a]

Saffron extract	Mutation Index (MI)[b]				
(µg/plate)	TA97	TA98	TA100	TA102	TA1538
none	-	-	-	-	-
50	0.5	0.9	0.8	0.9	0.7
100	0.6	0.8	0.9	1.0	1.0
250	0.8	0.8	1.0	1.0	0.6
500	0.8	0.8	0.9	1.0	0.6
1000	0.8	0.8	0.9	1.0	0.7
1500	0.8	0.8	0.8	1.1	0.9
BP (10 µg per plate)	9.1	8.7	8.3	7.9	-

[a] Each value represents the mean ± Standard deviation (S.D.) of triplicate plates

[b] MI: Mutation Index

The mutation index was calculated as MI= x_1/x_0 where x_1 is the number of revertant colonies at each dose of agent assayed, and x_0 is the number of revertants in the negative control.

A test compound was considered mutagenic if the number of the His^+ revertant colonies was increased at least twice over the value of the corresponding control (MI > 2), over at least three doses levels, and a reproducible dose-response curve could be demonstrated.

Table 9. Antitumor effect of saffron and its ingredients on tumor cell.

Agents	Cells	IC_{50}	Literature cited
Saffron	S-180; EAC;DLA;P388	7-30 μg/ml	Nair et al. 1991;1994;1995; Salomi et al. 1991;
Saffron	HeLa;A549;WI-38VA	100-250 μg/ml	Abdullaev et al. 1992a
Saffron	A204;HeLa;HepG2;SW480	150-200 μg/ml	Abdullaev et al. 2003a
Saffron	HeLa	2,3 mg/ml	Escribano et al. 1996
Crocetin	HL60;K562	2 μM	Morjani et al. 1990
Dimethylcrocetin	HL60;K562	0.8 μM	Morjani et al. 1990; Tarantilis et al. 1994
Crocin	HL60;K562; HeLa; HT29; DHD/K12-PROb	2 μM 3 μM 0.4 mM 1mM	Morjani et al. 1990; Tarantilis et al. 1994, Escribano et al. 1996; García-Olmo et al. 1999
B-carotene	K562	3μM	Morjani et al. 1990
Picrocrocin	HeLa	0.8 mM	Escribano. et al. 1996
Safranal	HeLa	3 mM	Escribano et al. 1996
All-trans retinoic acids	HL60	0.12 μM	Tarantilis et al. 1994
Saffron corm callus extract	HeLa	100-150 μg/ml	Escribano et al. 1999a,b,c, 2000; Fernández et al. 2000
Glycoconjugate from saffron corms	HeLa; HT-1080 MDA-MB-231 Tobacco-BY-2cells, Protoplasts	7 μg/ml 9 μg/ml 22 μg/ml 0.5 μg/ml 2 μg/ml	Escribano et al. 1999a, 2000 Fernández et al. 2000