Evaluation of the Mutagenic and Antimutagenic Activities of Saffron

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Keywords: antimutagenicity, *Crocus sativus* L., mutagenicity

Abstract

We have analyzed the mutagenic and antimutagenic activities of different concentrations of aqueous and alcoholic saffron extracts, by using classic Ames/*Salmonella typhimurium* assay

With strains TA98 or TA100 and two well-known mutagens: 2-aminoantrhracene (2-AA) and benzo(a)pyrene (B(a)P). Our results indicated that under conditions tested (50-1500 μg/ml) saffron did not display mutagenic, antimutagenic or toxic effects on bacterial strains used. Saffron extracts were unable to modify the number of mutant colonies induced by B(a)P , but in case of 2-AA, we observed a dose-dependent co-mutagenic effect of saffron. Results of experiments with isolated ingredients of saffron showed that the responsible molecule for this unusual co-mutagenic effect of saffron was safranal. The molecular basis for the observed differential effects of saffron on B(a)P and 2-AA induced mutagenesis is currently under investigation. Taken together, our results indicate that saffron extract itself is non-toxic, and non-mutagenic and can be considered as a potential cancer chemopreventive agent.

INTRODUCTION

In view of the renewed interest in chemopreventive plant agents, based on evidence from in vivo and in vitro experimental studies indicating that natural products might protect against the development of various diseases, including cancer (Abdullaev, 2001). Since ancient times, *Crocus sativus* L. which is harvested from the dried, dark red stigmas of saffron flowers, has not only been used as a spice for flavoring and coloring food and as a perfume, but also for treating several diseases. Recent data show that the saffron extract and its components possess anticarcinogenic (inhibition of chemical carcinogenesis) and antitumor (inhibition of tumor growth) in vivo and in vitro activities (Abdullaev, 2002). Characteristic compounds of saffron include crocin, safranal, picrocrocin, crocetin and β– carotene (Tarantilis et al., 1994; Escribano et al., 1996). Previously, a study using the Ames/Salmonella assay and sodium azide as a mutagen, indicated that crocin and dimethyl-crocetin, isolated from saffron, were non-mutagenic, non-antimutagenic, and non-comutagenic (Nair et al., 1995). Another study reported that the saffron extract itself was also non- mutagenic (Rockwell et al., 1979). The aim of the present study was to assess the potential mutagenic or antimutagenic effects of saffron (*C. sativus*).

MATERIALS AND METHODS

Chemicals

All other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO).

Preparation of the Saffron Extract

Stigmata of pure saffron (Mancha, Spain) were purchased from a local market and

stored in the dark at 4°C. Herbarium vouchers are held in the "Herbario ALBA" (Index Herbariorum New York), Castilla-La Mancha University, Spain, with the number 5994. The chemical analysis of the saffron extract revealed that its characteristic compounds include three main chemical components: 1. the bright yellow coloring carotenoids (the main coloring ingredients of saffron are a water-soluble α-crocin); 2. a bitter tasting picrocrocin (a glucoside of safranal); and 3. a spicy aroma, safranal (one of the main ingredients responsible for the aroma of saffron, a monoterpene aldehyde). Saffron was found to contain also sugar, alkaloids, amino acids, vitamins A, B, C, H_2O, fixed and volatile oil, wax and ash (Abdullaev, 1993). The concentrated saffron extract was prepared as previously described (Abdullaev and Frenkel, 1992). Briefly, aliquots of saffron (dried whole stigmata) were ground, and then were three times extracted with 75 % aqueous ethanol overnight with magnetic stirring while in the dark. The resulted pooled extracts were centrifuged at 30,000 g for 15 min and filtered through a Whatman filter (Gf/F, 0,7 μm) to separate the plant residue, which was discarded. Obtained extracts of saffron were lyophilized (24 h) in Fast Freeze and Lyth-Lock Freeze Dry Flask, LABCONCO Co., U.S.A. The saffron extract concentration was modified depending on the assay as reported in the text.

Mutagenicity and Antimutagenicity Assays

Dr. B.N. Ames from the University of California at Berkeley kindly provided the *Salmonella typhimurium* TA98 tester strain. The mutagenic and the antimutagenic activities of the saffron extract were determined using the plate incorporation test (Maron and Ames, 1983) and indirect-acting mutagen: benzo [a] pyrene (BP). BP (10 μg/plate) was chosen as positive control for the antimutagenicity studies, since this dose is not toxic for TA98 strain. 0.5 ml of S9 mixture (10% rat liver S9 fraction, 8 mM $MgCl_2$, 33 mM KCl, 4 mM NADP, 5 mM glucose-6-phosphate and 100 mM sodium phosphate buffer (PBS), pH 7.4 were added to the molten top agar before platting. Toxicity of the tested agents was assessed by the observation of the background bacterial growth in the minimal agar plates due to the trace of histidine in the medium. Revertant colonies were counted after a 72-h incubation period at 37ºC using a MiniCount colony counter (Biotran II, New Brunswick Scientific, Edison NJ). All determinations were done in triplicate. Because the plate-incorporation reversion test used in our studies does not directly measure possible toxicity of the tested agents, a decrease in reversion could be due to a toxic effect of the agents. To examine this possibility, the background lawn growth on the plates was observed under a microscope when the revertant colonies were counted. No obvious toxic effect of saffron extract or its ingredients at all concentrations used was noted.

Statistical Analysis

Data were analyzed using Statistical Analysis System software (SAS Institute Inc., Cary, NC 27511, U.S.A. Release 6.02). Values were considered significant when $p< 0.05$.

RESULTS AND DISCUSSION

After using the plate incorporation assay, no mutagenic activity of the saffron extract up to the concentration of 1500 m plate was detected (Table 1) when using the S. typhimurium tester strain TA98,both with and without S9 activation. The mutation index (MI)was less than 2 at all tested saffron extract concentrations. Non-mutagenic effect of saffron extract was observed in experiments using Ames/*Salmonella* assay (Table 1). No marked effect of the saffron extract on BP-induced mutagenic activity including the highest concentration used (1500 m plate)was seen (Table 2) in the Ames/*Salmonella* test system with the TA98 strain+S9 . Although all tested concentrations of the saffron extract slightly reduced BP mutagenicity, no clear dose -response effect was obtained. The saffron extract induced a comutagenic effect on 2-AA induced mutagenicity in a dose-dependent manner, although saffron itself had no any mutagenic activity (Table 1 and 2).The saffron extract at a concentration of 1500 μg/ plate more than tripled 2-AA 's mutagenic activity. Examination of the effects of four main ingredients of the saffron

extract demonstrated that safranal might be one of the components responsible for this co-mutagenic effect (Table 3).

It is known that mutations are important early steps in carcinogenesis, therefore, short-term genetic test such as the Salmonella /reversion assay has been successfully used for the detection of mutagens/carcinogens, as well as of antimutagens/anticarcinogens (Rausher et al., 1998). Our previous study showed that the oral administration of the saffron extract at concentrations from 0.1 to 5 g/kg was non-toxic in mice (Abdullaev et al., 2002). It is evident from our results that the saffron extract exhibited non-mutagenic (Table 1) and non-antimutagenic (Table 2) effects on BP mutagenicity in the Salmonella tester strain TA98. We also observed that saffron increased mutagenicity (Table 2) of 2-AA and t is co-mutagenic effect depended on the concentration of saffron. It was also demonstrated that safranal was potentially responsible for the co-mutagenic effects of saffron on 2-AA mutagenicity (Table 3).

Additional studies are required for determining the biological effect of the saffron ingredients, especially concerning the co-mutagenic effect of saffron on 2-AA mutagenicity.

Thus, our results reveal that the saffron extract is non-toxic, non-comutagenic with BP, but exhibits a co-mutagenic effect with 2-AA. In conclusion, the present study supports increasing evidence that naturally occurring saffron extract may have an important role in cancer chemoprevention.

ACKNOWLEDGMENTS

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

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Tables

Table 1. Non-mutagenicity of saffron extract, assayed in the *Salmonella typhimurium* plate incorporation test, with metabolic activation (S_9mix) and 10 µg per plate of BP or 20µg per plate of 2AA.

His^+REVERTANTS PER PLATE (mean ± SD)[a]			
TA98			
Test agents (µg/plate)	His^+ Revertants/plate (mean ± SD)[a]		
	S_9	$+S_9$	MI
None	39±9.5	45±6.7	0.8
50	33 ±7.1	41±8.3	0.9
100	28±10.3	35±5.4	0.8
200	26±12.7	37± 7.3	0.8
300	25±9.2	35±8.9	0.8
500	24± 8.1	36±7.1	0.8
1000	30±3.9	38±6.5	0.8
1500	22±5.0	34±3.8	0.7
BP (10 µg per plate)	24±6.8	391±19.5	8.7
2AA (20 µg per plate)	32±3.6	915±9.7	20.3

MI: Mutation Index.

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

Table 2. Antimutagenicity of saffron extract and sodium selenite, alone and in combination, assayed in the *Salmonella typhimurium* plate incorporation test, with metabolic activation (S_9mix) and 10 µg per plate of BP.

His^+REVERTANTS PER PLATE (mean ± SD)[a]	
TA98	
Test agents (µg/plate)	His^+ Revertants/plate (mean ± SD)[a]
None	36±2.3
Saffron extract	
500	347±30
1000	344±30.4
1500	343±32.7
BP (10 µg per plate)	391±19.5
None	45±5.6
Sodium selenite	
173	350±8.3
346	289±22.4
519	269±11.7
672	255±9.7
BP (10 µg per plate)	405±16.2
None	39±8.7
Saffron extract + sodium selenite	
1500+173	310±13,2
1500+346	242±20.1
1500+519	190±11.7
1500+672	186±10.8
BP (10 µg per plate)	315±12.9

[a]Each value represents the mean ± Standard Deviation (SD) of triplicate plates

Table 3. Comutagenicity of some ingredients of saffron, assayed in the *Salmonella typhimurium* plate incorporation test with 2-AA

His^+REVERTANTS PER PLATE (mean ± SD)[a]	
TA98	
Test agents (µg/plate)	His^+ Revertants/plate (mean ± SD)[a]
	CROCIN
None	39±12.2
100	403±37.6
200	359±32.5
400	348±54.3
AA (20 µg per plate)	440±40.8
	KAEMFEROL
None	43±10.1
100	286± 73.2
200	276±43.1
400	307±20.8
AA (20 µg per plate)	319±7.8
	PICROCROCIN
None	27±3.6
100	85± 13.1
200	103±14.4
400	84±10.1
AA (20 µg per plate)	103±10.1.8
	SAFRANAL
None	27±4.46
100	560±10.0
200	685±31.9
400	766±46.5
AA (20 µg per plate)	304±17.5

[a]Each value represents the mean ± Standard Deviation (SD) of triplicate plates