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Protective Effects of Saffron (*Crocus sativus* Linn.) on Genotoxins-induced Oxidative Stress in Swiss Albino Mice

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The modifying effects of the aqueous extract of saffron (dried stigmas of *Crocus sativus* Linn.) on cisplatin (CIS), cyclophosphamide (CPH), mitomycin-C (MMC) and urethane (URE) induced alterations in lipid peroxidation and antioxidant status were investigated in Swiss albino mice. Three doses of saffron (20, 40 and 80 mg/kg body weight) were orally administered to mice for 5 consecutive days prior to administration of genotoxins. A significant reduction in the extent of lipid peroxidation with a concomitant increase in the liver enzymatic (SOD, CAT, GST, GPx) and non-enzymatic antioxidants (reduced glutathione) were observed in saffron pretreated animals compared with the genotoxins alone treated animals. However, the modulatory effects were not always dose dependent. Our data suggest that saffron may exert its chemopreventive effects by modulation of lipid peroxidation, antioxidants and detoxification systems. Copyright © 2003 John Wiley & Sons, Ltd.

Keywords: saffron; lipid peroxidation; antioxidant status.

INTRODUCTION

It is well established that a large number of dietary agents are capable of inhibiting genotoxicity and carcinogenicity (Ames, 1983; Ferguson, 1994). As a result, the intake of food and beverages with chemopreventive constituents has been regarded as an effective strategy for strengthening our defence against the deleterious effects of genotoxins and carcinogens in the environment (Chatterjee *et al.*, 1995; Morse and Stoner, 1993; Ramel *et al.*, 1986; Rogers *et al.*, 1993).

Saffron (dried stigmas of *Crocus sativus* Linn.) is a highly valued spice, commonly used for flavouring and colouring food. Since time immemorial, it has been consumed in different parts of the world as a folk remedy for various ailments (Nair *et al.*, 1995; Na Li *et al.*, 1999; Premkumar *et al.*, 2001). The value of saffron as an antispasmodic, diaphoretic, carminative, emmenagogic and sedative is well recognized. It has properties such as an abortifacient and a uterine stimulant. However, there is no toxicity when taken in the recommended dosage (Nadkarni, 1976). During the past few years the antitumoural properties of crude saffron stigma extracts, both *in vitro* and *in vivo*, have been demonstrated (Abdullaev, 1993; Abdullaev and Frenkel, 1992; Salomi *et al.*, 1991). Previously, we reported inhibition of cisplatin, cyclophosphamide, mitomycin-C and urethane induced genotoxicity in male Swiss albino mice by the oral administration of saffron extract (Premkumar *et al.*, 2001). It is well known that antimutagenic agents are almost universal antioxidants (De Flora, 1998).

The present study was designed to investigate the modifying effects of saffron extract on lipid peroxidation and antioxidant status in Swiss albino mice treated with chemical genotoxins.

MATERIALS AND METHODS

Saffron was purchased from Indian Medical Practitioners Cooperative Pharmacy and Stores (IMPCOPS), Chennai, India. One gram of saffron was soaked in 100 mL distilled water. After 2 h it was homogenized in the same distilled water, stirred for 1 h and filtered. The residue was re-extracted with fresh distilled water. This aqueous extract was lyophilized and stored at 4 °C until further use. The genotoxins, cyclophosphamide (CPH) and mitomycin-C (MMC) were purchased from Sigma Chemical Company (USA). Urethane (URE) and cisplatin (CIS) were obtained from Fluka (Switzerland) and Tamil Nadu Dhada Pharmaceuticals Ltd (Chennai, India), respectively. All the other chemicals used were of the highest purity and analytical grade.

Male mice of Swiss albino strain aged 10–12 weeks old were obtained from the National Institute of Nutrition, Hyderabad, India. They were maintained under standard environmental conditions and fed with pelleted feed (Hindustan Lever Limited, Mumbai, India) *ad libitum* and had free access to tap water.

A total of 120 mice were divided into five major groups (groups I–V). Each group was further subdivided

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into four groups: a, b, c and d, of six animals each. Animals in subgroup a, b, c and d, were administered by gavage (10 mL/kg body weight) with 0, 20, 40 and 80 mg/kg body weight of saffron extract, respectively, for 5 consecutive days. Duration of pretreatment and the test doses of saffron were decided on the basis of findings from preliminary studies. Special care was taken to ensure that pretreatment with the three doses of saffron did not lead to suppression of cell proliferation. Two hours after the administration of final dose of saffron extract the genotoxins, namely. CIS, CPH, MMC and URE were injected intraperitoneally (10 mL/kg) to the experimental groups II, III, IV and V, respectively (except group I). Control animals (group Ia) received the same volume of distilled water.

At the termination of this study, i.e. 24 h after treatment, the animals of all the groups were killed by cervical decapitation. Livers were immediately excised from the animals and weighed. A 10% homogenate was prepared in 0.1 M, Tris-HCl buffer (pH 7.4) using a Potter-Elvehjem homogenizer with a teflon pestle.

Standard procedures were used to estimate lipid peroxides (LPO) (Ohkawa *et al.*, 1979), reduced glutathione (GSH) (Moron *et al.*, 1979) and the activities of superoxide dismutase (SOD) (Marklund and Marklund, 1974), catalase (CAT) (Sinha, 1972), glutathione peroxidase (GPx) (Rotruck *et al.*, 1973) and glutathione S-transferase (GST) (Habig *et al.*, 1974). The protein content was determined by the method of Lowry *et al.* (1951).

Statistical significance was determined using Student's *t*-test. The results are expressed as mean ± SD for six animals.

RESULTS

Table 1 indicates the extent of lipid peroxidation and antioxidant status in the livers of control and experimental animals. When compared with the control (group Ia), there was no significant alteration in the LPO and antioxidants after pretreatment with 20, 40, and 80 mg/kg saffron. In the genotoxins alone treated animals (groups IIa, IIIa, IVa and Va) the extent of lipid peroxidation levels were significantly higher and antioxidants were markedly lower compared with that of the control (group Ia). In saffron pretreated animals, the extent of lipid peroxidation levels were significantly decreased, whereas GSH concentration and the activities of SOD, CAT, GPx and GST were significantly higher when compared with genotoxin alone treated animals.

DISCUSSION

The results in the present study demonstrate that the aqueous extract of saffron effectively suppressed

Table 1. Levels of lipid peroxidation and antioxidant status (enzymatic and non-enzymatic) in the liver of control and experimental animals (mean ± SD for six animals)

Group (genotoxin)	Sub group	Saffron (mg/kg bw)	LPO	SOD	CAT	GST	GPx	GSH
	a	0	1.81 ± 0.22	5.75 ± 0.51	465 ± 36.08	1.04 ± 0.09	46.57 ± 5.21	1.76 ± 0.18
Group I	b	20	1.82 ± 0.19^{yNS}	5.99 ± 0.55^{yNS}	472 ± 50.05^{yNS}	1.07 ± 0.11^{yNS}	45.92 ± 4.44^{yNS}	1.86 ± 0.21^{yNS}
(Control)	c	40	1.73 ± 0.16^{yNS}	6.36 ± 0.58^{yNS}	484 ± 43.61^{yNS}	1.14 ± 0.12^{yNS}	49.19 ± 4.74^{yNS}	1.95 ± 0.18^{yNS}
	d	80	1.93 ± 0.20^{yNS}	5.88 ± 0.56^{yNS}	477 ± 43.52^{yNS}	1.08 ± 0.11^{yNS}	49.70 ± 5.04^{yNS}	1.88 ± 0.18^{yNS}
	a	0	2.82 ± 0.25^{xc}	4.08 ± 0.33^{xc}	266 ± 32.54^{xc}	0.71 ± 0.07^{xc}	25.60 ± 3.20^{xc}	0.69 ± 0.07^{xc}
Group II	b	20	2.32 ± 0.21^{yb}	4.66 ± 0.54^{ya}	334 ± 31.27^{yb}	0.92 ± 0.10^{yb}	30.17 ± 3.45^{ya}	0.93 ± 0.11^{yb}
(CIS)c	40		2.11 ± 0.20^{yc}	5.41 ± 0.66^{yb}	443 ± 41.74^{yc}	1.04 ± 0.10^{yc}	43.66 ± 4.36^{yc}	1.49 ± 0.14^{yc}
5 mg/kg	d	80	2.22 ± 0.23^{yb}	5.11 ± 0.46^{yb}	403 ± 37.55^{yc}	0.99 ± 0.09^{yc}	39.70 ± 4.96^{yc}	1.25 ± 0.12^{yc}
	a	0	2.51 ± 0.32^{xb}	4.03 ± 0.39^{xc}	272 ± 29.16^{xc}	0.73 ± 0.08^{xc}	26.83 ± 2.64^{xc}	0.79 ± 0.09^{xc}
Group III	b	20	2.26 ± 0.27^{yNS}	4.79 ± 0.51^{ya}	313 ± 40.18^{yNS}	0.86 ± 0.11^{ya}	31.37 ± 3.47^{ya}	0.98 ± 0.09^{yb}
(CPH)c	40		2.02 ± 0.23^{ya}	5.17 ± 0.62^{yb}	460 ± 55.91^{yc}	1.02 ± 0.07^{yc}	41.83 ± 5.07^{yc}	1.54 ± 0.17^{yc}
40 mg/kg	d	80	2.09 ± 0.24^{ya}	5.04 ± 0.57^{yb}	372 ± 47.36^{yb}	0.94 ± 0.08^{yb}	36.94 ± 4.32^{yc}	1.11 ± 0.14^{yc}
	a	0	2.43 ± 0.26^{xb}	4.13 ± 0.42^{xc}	253 ± 28.72^{xc}	0.77 ± 0.08^{xc}	25.68 ± 2.60^{xc}	0.97 ± 0.09^{xc}
Group IV	b	20	2.08 ± 0.19^{ya}	4.82 ± 0.58^{ya}	357 ± 37.64^{yc}	0.91 ± 0.09^{ya}	29.73 ± 2.71^{ya}	1.13 ± 0.12^{ya}
(MMC)	c	40	1.94 ± 0.18^{yc}	5.20 ± 0.57^{yb}	434 ± 45.70^{yc}	1.02 ± 0.11^{yb}	38.00 ± 4.77^{yc}	1.43 ± 0.13^{yc}
1 mg/kg	d	80	2.02 ± 0.20^{ya}	5.08 ± 0.53^{yb}	386 ± 37.16^{yc}	0.98 ± 0.12^{yb}	34.97 ± 3.59^{yc}	1.17 ± 0.13^{ya}
	a	0	2.92 ± 0.28^{xc}	3.90 ± 0.46^{xc}	288 ± 33.47^{xc}	0.72 ± 0.08^{xc}	24.24 ± 2.37^{xc}	0.76 ± 0.09^{xc}
Group V	b	20	2.48 ± 0.31^{ya}	4.55 ± 0.54^{ya}	314 ± 35.08^{yNS}	0.86 ± 0.10^{ya}	31.76 ± 3.25^{yb}	0.88 ± 0.11^{yNS}
(URE)c	40		2.21 ± 0.24^{yc}	5.33 ± 0.63^{yb}	440 ± 48.03^{yc}	0.98 ± 0.12^{yb}	40.38 ± 4.78^{yc}	1.34 ± 0.16^{yc}
750 mg/kg	d	80	2.43 ± 0.26^{yb}	5.02 ± 0.52^{yb}	401 ± 49.96^{yc}	1.03 ± 0.11^{yc}	36.94 ± 3.48^{yc}	1.14 ± 0.14^{yc}

The symbols represent statistical significance (Student's *t*-test).
[a] $p < 0.05$
[b] $p < 0.01$
[c] $p < 0.001$
[NS] Not significant
[x] Group Ia compared with group Ia, IIIa, IVa and Va;
[y] Subgroup a compared with subgroup b, c and d, in respective groups. Units are expressed as: LPO: nmol of MDA formed/mg protein; GSH: μg/mg protein; GST: nmol of CDNB conjugated/min/mg protein; GPx: μmol of reduced GSH oxidized/min/mg protein; SOD: 50% inhibition of auto-oxidation of pyrogallol/min/mg protein; CAT: μmol of H_2O_2 consumed/min/mg protein.

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lipid peroxidation induced by cisplatin (CIS), cyclophosphamide (CPH), mitomycin-C (MMC) and urethane (URE) with concomitant changes in antioxidants and detoxification systems. However, the observed modifying effects were not always dose dependent. Against the genotoxins tested, the maximum protection was observed after pretreatment with a dose of 40 mg/kg aqueous extract of saffron. A further increase in dose did not enhance this protective effect.

In this study the main aim was to assess the modulatory effects associated with the use of saffron for flavouring and colouring food. Hence pretreatment was carried out with the aqueous extract. However, the doses chosen do not represent the doses used in food and medicine. The observed protective effects can be attributed to the water soluble chemical constituents of saffron. This would include mainly constituents such as crocin and picrocin (Escribano *et al.*, 1996). In rats, crocin dyes are known to exert protective effects against acute hepatic damage induced by aflatoxin B_1 and dimethylnitrosamine (Lin and Wang, 1986).

The involvement of free radical induced lipid peroxidation and the role of antioxidants in carcinogenesis are well established (Collins *et al.*, 1994). Cellular enzymatic (SOD, CAT, GPx, GST) and non-enzymatic antioxidants (GSH) act as primary line of defence to cope with the deleterious effects of these radical species (Cerutti *et al.*, 1994). There was an increased extent of lipid peroxidation with a concomitant decrease in both enzymatic and non-enzymatic antioxidant defence in CIS, CPH, MMC and URE treated animals. The observed increase in lipid peroxidation rate in genotoxin treated mice livers could be due to higher oxidative stress and poor antioxidant defence. It is known that higher levels of antioxidant enzymes are associated with decreased susceptibility to cell damage (Werts and Gould, 1986). Positive modulation of these parameters by saffron extract could be either by preventing the leakage of lipid peroxides from the tissue or by direct scavenging of free radical generation.

Intracellular lipid peroxidation is regulated by CAT, SOD, GPx and GST. Decreased levels of CAT, SOD, GST and GPx in genotoxin treated animals may be due to saturation of all these enzymes with a higher concentration of reactive free radicals or due to direct inhibition of these enzymes by free radicals. The oral administration of saffron extract significantly activated the liver antioxidants SOD and CAT.

Reports in the literature indicate depleted levels of GSH on exposure to toxic compounds (Wattenberg, 1978). Depleted levels of GSH in livers of genotoxin treated mice were observed. The excessive utilization of GSH in CIS, CPH, MMC and URE treated mice may account for depleted levels of this nucleophilic trapping agent in the livers of genotoxin treated mice. Treatment of mice with saffron extract tried to revert it, however, normal levels could not be attained. Trapping of the reactive electrophilic forms of chemical carcinogens/mutagens by GSH has been linked with chemopreventive activity (Wattenberg, 1978) and thus our findings are in confirmation with the previous reports. The depleted levels of GSH may also be held responsible for enhanced levels of lipid peroxidation in the livers of genotoxin treated mice. Saffron pretreatment showed a significant fall in lipid peroxidation levels in the livers of mice.

The phytochemicals, which are inducers of enzymes that detoxify xenobiotics and reactive carcinogen metabolites, have cancer preventing properties (Kensler *et al.*, 1993). Saffron extract administration enhanced the activity of phase II enzyme, GST, in genotoxin treated group suggestive of quick elimination of genotoxic metabolites or free radicals generated by the genotoxins.

From the results of the present study it can be inferred that oral pretreatment with the saffron aqueous extract for 5 consecutive days prior to the administration of genotoxins is associated with an increase in the levels of GSH concentration as well as the activities of GST, GPX, CAT and SOD compared with the genotoxin alone treated mice and thus exerted the scavenging and detoxifying effects on the reactive species generated by the genotoxins under investigation. These observations substantiate the antioxidant property of saffron reported by Verma and Bordia (1998). From our previous experiments it was demonstrated that the aqueous extract of saffron can effectively inhibit the *in vivo* genotoxicity of CIS, CPH, MMC and URE (Premkumar *et al.*, 2001). The present observations together with our previous results suggest that saffron aqueous extract has potential antimutagenic and antioxidant properties in experimental animals. In general, the beneficial effects of plant products such as saffron may be attributable to one or more phytochemicals present in it. Although, quantitation and characterization of individual compounds was not carried out, this present study lends support to the chemopreventive nature of saffron extract based on specific biochemical markers.

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