RESEARCH COMMUNICATION

Saffron Can Prevent Chemically Induced Skin Carcinogenesis in Swiss Albino Mice

Ila Das[1], RN Chakrabarty[2] Sukta Das[1]

Abstract

One of the most promising strategies for cancer prevention today is chemoprevention using readily available natural substances from vegetables, fruits, herbs and spices . Among the spices, saffron (Crocus sativus, L) a member of the large family Iridaceae, has drawn attention because apart from its use as a flavouring agent, pharmacological studies have demonstrated many health promoting properties including radical scavenging, anti- mutagenic and immuno-modulating effects. In the present study the effects of an aqueous infusion of saffron on two stage skin papillogenesis / carcinogenesis in mice initiated by 7-12 dimethyl benz[a] anthracin (DMBA) and promoted with croton oil were investigated. Significant reduction in papilloma formation was found with saffron application in the pre-initation and post-initation periods, and particular when the agent was given both pre- and post-initation. The inhibition appeared to be at least partly due on modulatory effects of saffron on some phase II detoxifying enzymes like glutathione-S-transferase (GST) and glutahinoe peroxidase (GPx), as well as catalase (CAT) and superoxide dismutase (SOD).

Key Words: Saffron - skin carcinogenesis - detoxification enzymes - chemoprevention

Asian Pacific J Cancer Prev, **5**, 70-76

Introduction

Saffron, the dry stigma of the plant Crocus sativus L popularly used as a spice and food colorant, has been used in traditional medicines for treatment of many diseases including tumors. The chemical components of saffron include the coloured carotenoids, crocin and crocetin, and the monoterpene aldehydes, picrocrocin and saffranol (Abdullaev 1993, Tarantilis et al, 1995; Escribano et al, 1996, Lozano et al, 2000). In addition saffron contains proteins, sugars, vitamins, flavonoids, amino-acid, minerals and gums (Abdullaev 1993, Rios et al 1996). A number of reports have indicated anti-tumor properties of saffron extract in vivo (Salomi et al 1991, Abdullaev et al 1992, Abdullaev et al 2002). Anti-promoting and non-mutagenic activity of saffron extract has also been reported (Salomi et al 1991). It has also been shown that crocins can protect from the adverse effects of hepatocarcinogenic compounds (Lin and Wang 1986) and that crocetin, the deglycosylated crocin derivative, can inhibit intracellular nucleic acid synthesis (Abdullaev 1994). In vitro proliferation of promyelocytic leukemia cells is inhibited by crocins and crocin derivatives (Tarantitis et al, 1994). Another study (el Daly 1998) demonstrated protective effects of saffron extract together with cysteine and vitamin E against cisplatin induced toxicity in rats. However, to our knowledge there has been no systematic cytotoxicity analysis of saffron compounds, crocin, crocetin, picrocrocin, and safranol performed separately. In the present study we the examined the anti carcinogenic effects of aqueous infusion of saffron, administered orally using two stage skin carcinogenesis model in mice. Since phase II detoxification enzymes play a major role in the onset of carcinogenesis by helping eliminating the damaging agents, modulatory effects of saffron treatment on a number of enzyme species in the same model were also ascertained.

Materials and Methods

Preparation of Test Material

Commercially available saffron was purchased from the Govt. of Kashmir Emporium, Kolkata and stored at $4^{o}C$ until use. An infusion was prepared by soaking 0.1 gm saffron in 12.5ml of lukewarm distilled water for 10 minutes followed by straining. The infusion was administered orally at a dose of 200 mg/kg body weight /mouse.

[1]*Department of Cancer Chemoprevention,* [2]*Department of Cancer Detection and Screening, Chittaranjan National Cancer Institute, 37, S.P.Mukherjee Road, Kolkata-700026, India. Fax: +91-33-2475-7606 Email: suk_tadas@yahoo.com*

Selection of the Dose for Treatment

Saffron infusion at different doses (50 - 500mg/kg body weight by oral gavage were administered to normal mice. Based on body weight, skin texture, food intake and general behavior observed for 7 days, the dose selected was 200 mg/kg body weight/day/mouse (maximum dose not producing change in any of these parameters).

Chemicals

DMBA (7-12 dimethylbenz [a] anthracene), croton oil, CDNB (1-chloro-2-4-dinitrobenzene, GSH (glutathione), GR (glutathione reductase), NADPH (nicotinamide adenine dinucleotide phosphate reduced form), Pyrogallol, TBA (thiobarbituric acid) and DTPA (diethylene triamine penta acetic acid) and pyridine, were purchased from Sigma Chemicals Co, St. Louis, MO, U.S.A. Sodium dodecyl sulphate (SDS) was purchased from Gibco BRL, U.S.A. and hydrogen peroxide solution (H_2O_2) (30%), acetic acid (CH_3COOH), n-butanol and potassium chloride (KCL) from Merck (India).

Experimental Animals

Female Swiss albino mice aged 5-6 weeks, and weighing 20-22 gm at the commencement, were housed in plastic cages with stainless steel wire lids (6 mice per cage). The housing facility was maintained at $30^{o}C$. The animals received standard food pellets (Lipton India Ltd.) and water ad-libitum. To induce carcinogenesis the mice received three topical applications of 100 n mol DMBA in 100µl acetone at an interval of 2 days on the shaved skin (initiator) followed by 100µl 1 gm% croton oil in acetone (promoter) after a week at the same site twice weekly for 8 weeks.

Treatment Groups

The normal control group (NC) received only distilled water every day during the treatment period, while the carcinogen control group (CC) was given three topical applications of DMBA and then croton oil followed by distilled water. The animals of Group–A (pre treatment) were administered saffron infusion orally once daily for 2 weeks prior to the application of DMBA-croton oil. Group-B (pre+post treatment group) was administration the saffron infusion starting 2 weeks prior to the application of DMBA-croton oil and continued until the sacrifice. Group-C (post treatment) was administered saffron infusions starting after 2 weeks of application of DMBA/ croton oil.

Morphological Observation of Papilloma Development

Body weights and papilloma counts in mice of groups NC, CC, A, B, and C were recorded at an interval of 7 days till sacrifice. The first set of mice (Set-I) were sacrificed after 2 weeks and the second set (Set-II) after 12 weeks. Each set consisted of 12 mice. The affected skin was collected for histology.

Preparation of Skin for Histology

Affected skin and skin with papilloma were fixed in 10% buffered formalin and dehydrated with graded alcohols starting from 50% ethyl alcohol to absolute alcohol. Then the tissues were embedded in paraffin after clearing in xylene. Serial microtome sections (4µ) were stained with haematoxylin and eosin *(*Kehar and Wahi, 1967*)*.

Methods for Biochemical Assays

Liver tissue was sampled for biochemical assays after 2 weeks and 12 weeks from all groups for the estimation of lipid peroxidation (LPO) and to measure the activity of phase II detoxification and other enzymes - glutathione-S-transferase (GST), glutathione peroxidas (GPx), catalase (CAT) and superoxide dismutase (SOD).

LPO was estimated in the liver microsomal fraction by the method of Okawa et al. (1979). The levels of lipid peroxides formed were measured using thiobarbituric acid reactive substance (TBARS) formed /mg protein by an extinction co-efficient of $1.56x10^5$ M^{-1} cm. $^{-1}$

GST activity was assayed by the method of Habig et al (1974) following the increase in absorbance at 340nm using 1-chloro-2-4-di-nitrobenzene (CDNB) as the substrate. Specific activity of the enzyme was expressed as formation of 1-chloro-2-4-dinitrobenzene-glutathione (CDNB)-GSH conjugate per minute per mg of protein.

GPx activity was measured by the method of Paglia et al. (1967) in the post mitochondrial fraction.The reaction mixture contained NADPH and glutathione reductase.The decrease in absorbance following addition of H_2O_2 was recorded at 340 nm. Enzyme activity was expressed as nmoles of NADPH utilized /minute /mg protein using molar extinction co-efficient of NADPH at 340 nm of 6200 m^{-1} cm^{-1}.

CAT activity in liver was assayed by the method of Luck (1963). The enzyme activity was determined spectrophotometrically at 250 nm and results were expressed as units/mg protein where the unit was the amount of enzyme that liberates half the peroxide oxygen from H_20_2 in 100 seconds at 25^0 C.

SOD activity was assayed by the method of Marklund et al. (1974). Partial extraction and purification of SOD was performed as described by McCord et al. (1969). SOD was determined by quantification of pyrogallol auto oxidation inhibition and the results expressed as units/mg protein. One unit of enzyme activity was defined as the amount of enzyme necessary for inhibiting the reaction by 50%. Auto oxidation of pyrogallol in Tris-HCL buffer (50mM,pH-7.5) was measured by increase in absorbance at 420 nm.

Protein was measured by the method of Lowry et al. (1951) using bovine serum albumin (BSA) as a standard.

Statistical Analysis

Data from different experimental groups were analysed and expressed as mean $\pm$SD. The difference in data from different groups were statistically analysed by Student's t test. A value of $p < 0.05$ was considered significant for all experiments.

Results

Effects of Aqueous Infusion of Saffron on Body Weight

Gradual increase in body weights was noted in all animals upto 8 weeks except in those from the CC group. At 12 weeks a sharp difference in body weight was noted between the NC and CC groups. Near normal values were noted in the treatment groups A and B (Figure-1).

Effects of Aqueous Infusion of Saffron on Development of Papillomas

Papillomas started to appear on the region of skin receiving topical application of DMBA and croton oil from the 5th to 6th weeks. Percentages of mice with papilloma continued to increase until 12th week in the CC group. Observation made after 12 weeks of treatment revealed that incidence of papilloma in the carcinogen control group was 90%. Reduction in incidence of papilloma was noted in all treatment groups. (Figure-2).

In Set-I animals which were sacrificed after two weeks of DMBA application no papillomas were visible. In Set II animals appearance of papillomas was delayed from the 5th week to the 7th week in Gr A and Gr C and to 9th week in Gr B. Treatment with saffron delayed the onset of papillomas as well as the reduced the incidence as compared to group CC. The most potent effect of treatment was noted in Gr B (pre ± post treatment group).

Effect of Aqueous Infusion of Saffron on LPO

Lipid peroxidation in liver, expressed as TBARS produced, was found to be reduced from that seen in the CC group following treatment. It can be noted from Fig.-3 and Fig.-4 that the best effect of saffron in preventing carcinogen-induced lipid peroxidation was produced in Gr B where saffron treatment started before application of DMBA-croton oil and was continued for up to week 12. After treatment for 2 weeks, a significant inhibition in lipid peroxidation was noted as revealed by decrease in LPO by 1.42 fold ($P<0.001$), 1.15 fold ($P<0.001$) and1.11 fold ($P<.01$) in Gr B, Gr A and Gr C respectively. A similar trend was also noted after 12 weeks of treament. Considering all treatment groups the most significant effect was found in Group B.

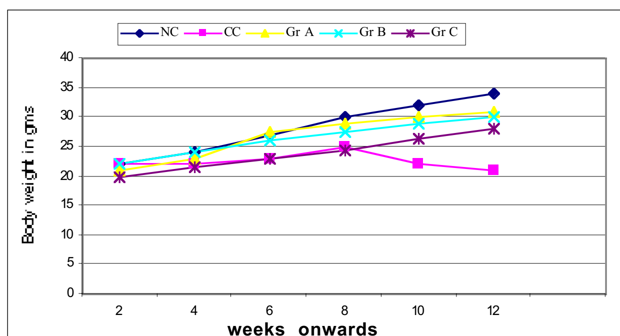

Figure 1. Effects of Saffron Infusion on Body Weights

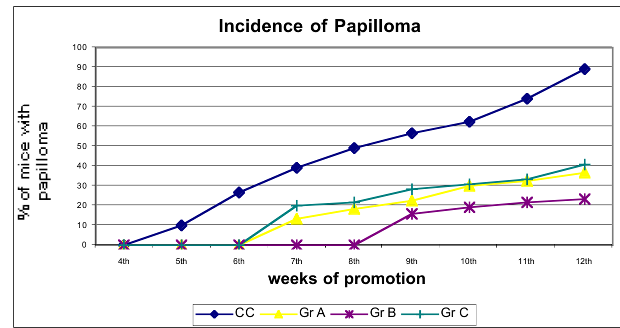

Figure 2. Effect of Aqueous Infusion of Saffron on Incidence of Skin Papillomas at Different Time Points

Effects of aqueous infusion of saffron on GST

Activity of GST in mouse liver was found to be lower in CC than NC.The enzyme activity significantly increased in Groups A and B by 1.16 fold ($P<0.01$) and 1.51 fold ($P<0.001$), respectively after 2 weeks. After 12 weeks the figures were 1.30 fold ($P<0.05$) and 1.52 fold ($P<0.001$). The results of treatment in Group C were not significant in either case (Figs 5 & 6).

Effect of aqueous infusion of saffron on GPx activity

Like GST, GPx was also found to be lower in the CC group in comparison to the NC group. Fig 7 & Fig 8 show significant increase in the activity of this enzyme in Groups

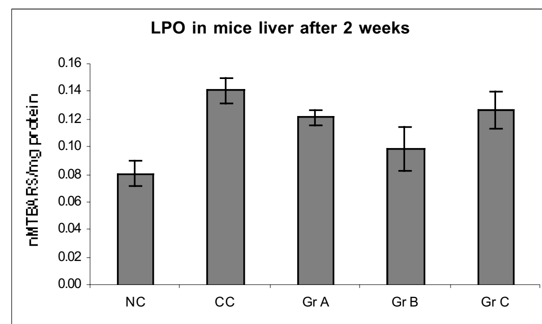

Figure 3. Effects of Infusion of Aqueous Saffron on LPO in Mouse Liver after Treatment for 2 Weeks. Data are mean ±SD

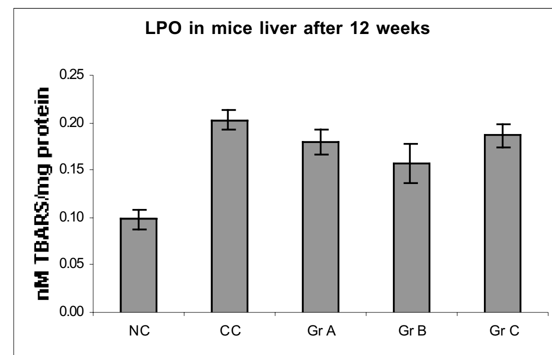

Figure 4. Effects of Infusion of Aqueous Saffron on LPO in Mouse Liver after Treatment for 12 Weeks. Data are mean ±SD

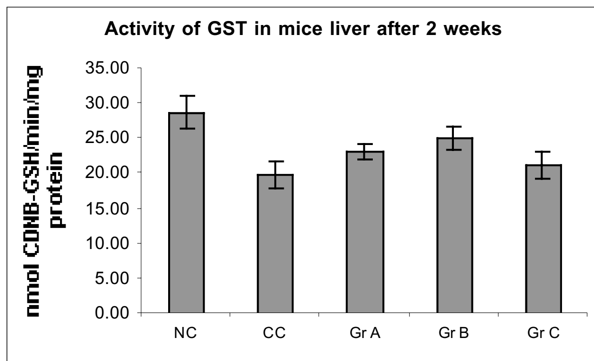

Figure 5. Effect of Aqueous Infusion of Saffron on the Activity of GST in Mouse Liver. Data are mean ±SD

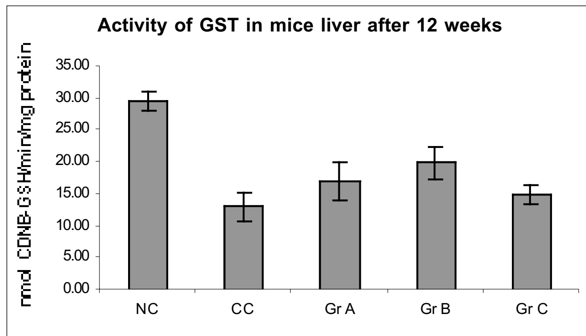

Figure 6. Effect of Aqueous Infusion of Saffron on the Activity of GST in Mouse Liver. Data are mean ±SD

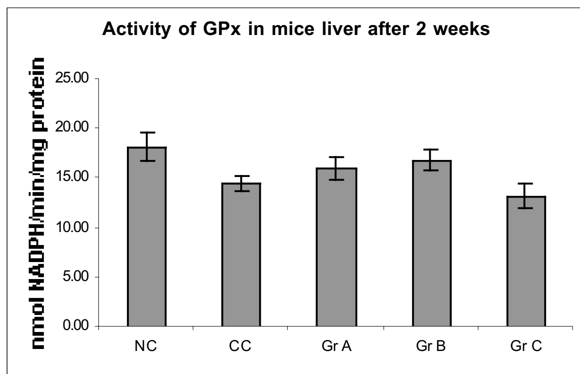

Figure 7. Effect of Aqueous Infusion of Saffron in Mouse Liver GPx Activity. Data are mean ±SD

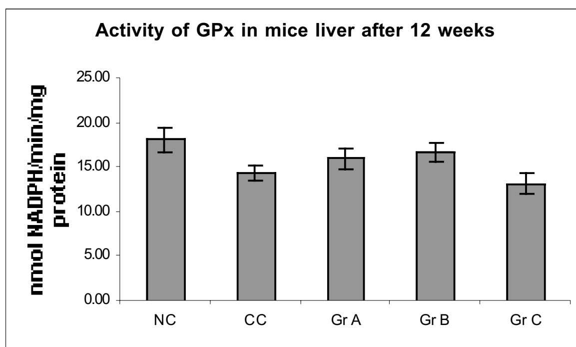

Figure 8. Effect of Aqueous Infusion of Saffron on GPx Activity in Mouse Liver. Data are mean ±SD

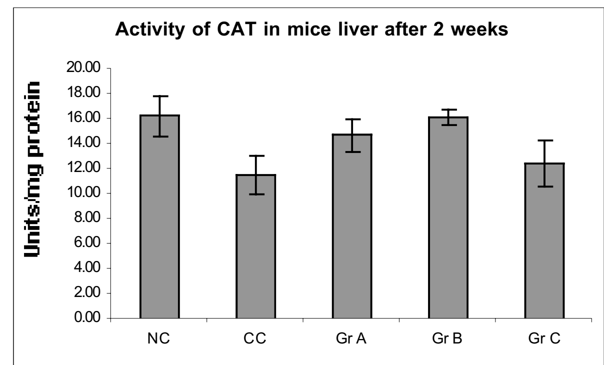

Figure 9. Effect of Aqueous Infusion of Saffron on the Liver Activity of Catalase. Data are mean ±SD

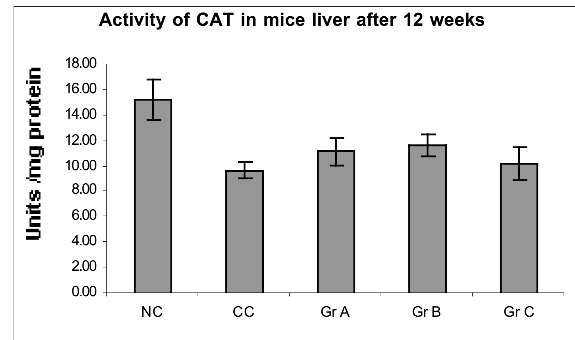

Figure 10. Effect of Aqueous Infusion of Saffron on the Liver Activity of Catalase. Data are mean ±SD

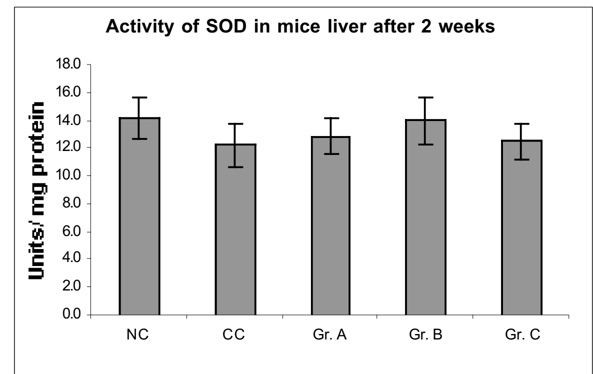

Figure 11. Effect of Aqueous Infusion of Saffron on the Activity of SOD in Mouse Liver. Data are mean ±SD

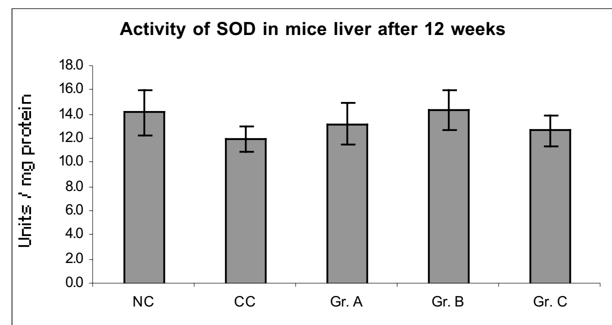

Figure12. Effect of Aqueous Infusion of Saffron on the Activity of SOD in Mouse Liver. Data are mean ±SD

A and B by 1.10 fold (P<0.1) and 1.16 fold (P<0.001) after 2 weeks and 1.16 fold (P<0.001) and 1.30 fold (0.001) after 12 weeks. The 1.11 fold increase noted in Group C was, however, insignificant.

Effect of Aqueous Infusion of Saffron on CAT

Activity of the enzyme catalase was also noted to be increased in Group A by 1.26 fold (P< 0.01) and in Group B by 1.40 (P<0.001) after treatment with saffron for 2 weeks in comparison with the carcinogen control group. After 12 weeks the enhancement was 1.15 fold (P<0.01) and 1.20 fold (P<0.001) (Figs 9 &10).

Effect of Aqueous Infusion of Saffron on SOD

Enhancement of SOD activity was significant in Group A by 1.13 fold (P<0.05) and in Group B by 1.16 fold (P<0.01) following treatment for 2 weeks but only significant in Group B observed after 12 weeks.

Discussion

A wide variety of naturally occurring substances including spices (Unnikrisnnan. et al. 1990) have been shown to inhibit chemical carcinogenesis in animal models (Williams 1984, Wattenberg 1985, Boone et al. 1999). Saffron, harvested from the dried dark red stigma of Crocus sativus.L flowers is used as a spice to add flavour to exotic food preparations and also finds mention in Indian system of medicine. Analysis of the chemical composition of saffron revealed more than 150 volatile and several non-volatile compounds of which approximately 40-50 constituents have been already identified. (Zargan et al. 1971, Sujata et al. 1992, Tarantilis et al. 1997, Lozano et al. 1999). It has been reported that Saffron can inhibit formation of new tumours as well as reduce or shrink existing tumors. (Nair et al. 1991, 1992, 1994).

In the present report we have demonstrated a chemopreventive effect of saffron on DMBA-induced skin carcinogenesis in female Swiss albino mice. Oral administration of aqueous infusion of saffron was found to produce a remarkable reduction in the incidence of mouse skin papilloma which is a pre-neoplastic benign growth. Topical application of saffron extract was also reported to inhibit skin carcinogenesis and oral administration of saffron extract in the same dose reduced 20-methylchloanthrene (MCA) induced soft tissue sarcoma in mice (Salomi.,et al.,1990; 1991). The anti-tumour and anti-carcinogenic effect of saffron may be attributed to presence of a number of biologically active carotenoids like crocin, crocetin and picrocrocin in saffron (Abdullaev 2002). Free radicals and lipid peroxidation are known to cause initiation and promotion of carcinogenesis (Bauer et al. 1980). Lipid peroxidation is increased during the carcinogenic process (Hamburger et al., 1972) and malondialdehyde (MDA) a product of lipid peroxidation was observed to be mutagenic and carcinogenic (Yay et al., 1979. Apaja, 1980, Basu et al., 1983). It is therefore implied that agents that can reduce generation of free radicals in vivo may be considered to have the potential for chemoprevention. The present study showns that saffron treatment could significantly reduce lipid peroxidation in mice exposed to DMBA and croton oil.

GST is thought to play a physiological role in initiating the detoxication of many alkylating agents (Wood, 1970) and environmental chemicals including mutagens and carcinogens (Kramer et al., 1988). Their main function is the conjugation of GSH to a variety of electrophilic compounds. GST reduced the covalent binding of epoxides of carcinogens with DNA and other macromolecules and this reduction in DNA binding was found to be effective in decreasing carcinogenesis caused by the carcinogens. GPx also plays important roles in cellular defence as well as maintenance of cellular membranes from oxidative damage of free radicals by eliminating H_2O_2 (Sunde et al. 1980).

CAT is a major peroxisomal enzyme. Peroxisomes is involved in a number of important cellular metabolic processes as well as detoxication of H_2O_2 (Singh et al.,1997; Moser et al. 1996). More than 90% of oxygen consumed by the mitocondria is converted to water (H_2O) and the rest to super oxide radical (O_2) whereas the oxygen consumed by peroxisomes is converted to H_2O_2 and only a little amount is converted to O_2 (Singh et al., 1996) These reactive oxygen species (ROS) are the normal by-products of cellular metabolism and are checked by cellular defences provided by antioxidant enzymes (Singh et al., 1996). These antioxidant enzymes provide protection to cell against ROS i.e. O_2 and H_2O_2 by detoxifying them at the site where they are produced. So increased activity of GST, GPx and CAT in liver tissue from the mice treated with saffron and decreased activity of these enzymes in the liver tissue from the mice treated with DMBA/croton oil suggested that saffron could influence host detoxificatin processes. SOD detected in a large number of tissues and organs, is also thought to protect cells from damage by superoxide radicals (Ilan et al. 1981). Superoxide radical O_2^{--} is the product of biological oxygen reduction as a result of electron transfer oxidase system. SOD catalyzes the one electron reduction of oxygen to superoxide radical. This O_2 is harmful to the respiring cells. SOD minimises the toxicity resulting from the mechanism against oxygen formation of free radicals (Ilan et al. 1981). Increased activity of SOD in the tissue of mice liver treated with saffron suggests certain role for reactive free oxygen radical in two stage carcinogenesis (Werts et al. 1986, Nishuira et al. 1992). Interestingly saffron was found to activate this enzyme also, which was depressed following carcinogen application, to almost near normal values or exceeded the normal values. The group receiving treatment that was started before initiation with DMBA and continued throughout the experimental period produced the best effect.

The increased activities of the Phase II detoxication enzymes noted in this study was accompanied by lowered lipid peroxidation in the liver and reduced incidence of papillomas. The experimental observation therefore suggest

a protective role of saffron against carcinogenic exposure by their action on the physiological detoxification processes. Further investigation using individual chemical compounds present in saffron singly or in combination in a number of in vivo carcinogenesis model would be required to establish the anti-carcinogenic and chemopreventive role of saffron.

Acknowledgement

The authors wish to thank the Director, CNCI, for support and encouragement. Thanks are also due to all staff members of the Dept. of Cancer Chemoprevention, C.N.C.I. for their help with the experiments.

Reference

Abdullaev FI (1993). Biological effect of saffron. *Biofactors*, **4**, 83-6.

Abdullaev FI (1994). Inhibitory effect of crocetin on intracellular nucleic acid and protein synthesis in malignant cells. *Toxicol Lett,* **70**, 243-51.

Abdullaev FI (2002). Cancer chemopreventive and tumoricidal properties of saffron (*Crocus sativus* ,L*). Exptl Biol Med,* **227,** 20-5.

Abdullaev FI and Frenkel GD (1992). The effect of saffron on intracellular DNA, RNA and protein synthesis in malignant and non-malignant human cells. *Biofactors*, **4**, 43-5.

Abdullaev FI and Frenkel GD (1992). Effect of saffron on cell colony formation and cellular nucleic acid and protein synthesis. *Biofactors*, **3,** 201-4.

Apaja M (1980). Evaluation of toxicity and carcinogenity of malonaldehyde. An experimental study in swiss mice. *Thesis Acta Univ Ouluensis*, Ser D 55.

Basu AK and Marnett JL (1983). Uniquivocal demonstration that malonaldehyde is a mutagen.*Carcinogenesis*, **4**, 331-3.

Bauer G and Wendel A (1980). The activity of the peroxide-metabolising system in human colon carcinoma. *Cancer Res Clin Oncol,* **97,** 267-73.

Boone CW, Kelloff GJ, Malone WE (1990). Identification of cancer chemotherapy agents and their evaluation in animal models and human clinical trials: A review. *Cancer Res*, **50,** 2-9.

Boyland E and Chasseaud L F (1969). *Adv. Enzymol* **32,** 172-219.

Curro C and Miegelli G (1979). Determinazione specttrofotometrica del potere colorante, *Americane ed odoroso dello zafferano. Boll Chem Farm*, **118,** 553–62.

el Daly ES (1998). Protective effect of cystein and vitamin A, *Crocus sativus* and *Nigela sativa* extracts on cysplatin induced toxicity in rats. *J Pharm Belg,* **53,** 93-5.

Escribano J, Alonso GL, Coca-Prados M, Fernandez JA (1996). Crocin, safranal and picrocrocin from saffron (Crocus sativus L) inhibit the growth of human cancer cells in vitro. *Cancer Lett.* **100,** 23-30.

Habig WH, Pabst MJ, Jacoby WB (1974). Glutathione-S-Transferase :The first enzymatic step in mercapturic acid formation. *J Biol Chem*, **249**, 7130-9.

Ilan Y and Rabani J (1981). Superoxide dismutase activity of an iron porphyrin. Inorg. *Nuclear Chem Lett,* **17,** 93-6.

Kehar U and Wahi PN (1967). Cytologic and histologic behavior patterns of the premalignant lesions of the cervix in experimentally induced cervical dysplasia, *Acta Cytologica*, **11**, 1-15.

Kraemer KH, Di Giovanna J, Moshell N (1988). Prevention of skin cancer in xeroderma pigmentosum. *N Engl J Med***, 318,** 1633-7.

Lin JK and Wang CJ (1986). Protection of crocin dyes on the acute hepatic damage induced by aflatoxin B1 and dimethylnitrosamine in rats. *Carcinogenesis*, **7,** 595-9.

Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ (1951). Protein measurment with the folin phenol reagent. *J Biol Chem*, **193**, 265-76.

Lozano P, Castellar MJ, Simancas MJ, Iborra JL (1990). Quantitative HPLC methods to analyze commercial saffron (*Crocus sativus*. L) products. *J Chromatogr*, **830**, 477-83.

Lozano P, Delgado D, Rubio M, Iborra JL (2000). A non destructive method to determine the safranol content of saffron (Crocus sativus. L) by supercritical carbon-dioxide extraction combined with HPLC and gas chromatography. *J Biochem Biophys Methods*, **43,** 367-78.

Luck H (1963). A spectrophotometric method for the estimation of catalase In Bergmeyer,H.V.(ed*) Methods of enzymatic analysis*, *Acad. Press, New York*, pp. 886-8.

Marklund S and Marklund G (1974). Involvement of the superoxide anion radical in the auto oxidation of pyrogallol and a convenient assay for superoxide dismutase. *Europ J Biochem,* **47,** 469-74.

McCord JM and Fridovich I (1969). Superoxide dismutase : An enzymatic function for erythrocuprein. *J Biol Chem,* **244,** 6049-55.

Moser HW and Moser AB (1996). *Ann N Y Acad Sci*, **804**, 427-41.

Nair SC, Pannikar B, Pannikar KR (1991). Antitumour activity of saffron (*Crocus sativus*). *Cancer Lett,* **57,** 109-14.

Nair SC, Kurumboor SK, Hasegawa JH (1995). Saffron : Chemoprevention in biology and medicine: a review. *Cancer Biother,* **10,** 257-64.

Nair SC, Salomi MJ, Varghese CD, Pannikar B, Pannikar KR (1992). Effect of saffron on thymocyte proliferation, intracellular gluthathione levels and its antitumor activity. *BioFactors* **4,** 51-4.

Nair SC, Varghese CD, Pannikar KR, Kurumboor SK, Parathod RK(1994). Effects of saffron on vitamin A levels and its antitumor activity on the growth of solid tumors in mice. *Int J Pharmacol,* **32,** 105-14.

Newmark HL (1987). Plant phenolics as inhibitors of mutational and precarcinogenic events . *Can J Physiol Pharmacol*, **65**, 461-6.

Negbi M Ed (1999) Saffron (*Crocus sativus.*) cultivation. : Present and future prospects. *Harwood Academic Publisher, Amsterdam*, pp 1-19.

Nishuira T, Suzuki K, Kawaguchi T, et al (1992). Elevated serum manganese superoxide dismutase in acute leukemias. *Cancer Lett*, **62**, 211-5.

Ohishi N and Yagi K (1979). Okahawa H, Ohishi N and Yagi K (1979). Assay for lipid peroxides in animal tissue by thiobarbituric acid reaction. *Annal Biochem*, **95**, 351-8.

Paglia DE and Valentine WN (1967). Studies on the quantitative and qualitative characterization of erythrocyte glutathione peroxidase. *J Lab Clin Med*, **70**, 158-69.

Rios JL, Recio MC, Giner RM, Mane S (1996). An update review of saffron and its active compounds. *Phytother Res,* **10,** 189-93.

Salomi MJ, Nair SC, Panikkar KR (1990). Inhibitory effects of *Nigella sativa* and *Crocu sativus on* chemical carcinogenesis in mice and its non-mutagenic activety. *Proc Sci Congress*, **3**,

125.
Salomi MJ, Nair SC, Panikkar KR (1991). Inhibitory effects of *Nigela sativa* and saffron (*Crocus sativus*) on chemical carcinogenesis in mice. *Nutr Cancer,* **16,** 67-72.
Salomi MJ, Nair SC, Panikkar PR (1991). Cytotoxicity and non-mutagenicity of *Nigela sativa* and saffron *(Crocus sativus*] in vitro*. Proc Ker Congr,* **5**, 244.
Shamberger RJ (1972). Increase of peroxidation in carcinogenesis. *J Natl Cancer Inst,* **48**, 1491-7
Singh I (1996). *Ann N Y Acad Sci*, **804**, 612-27.
Singh I (1997) *Mol Cell Biochem*, **167**, 1-29.
Sparnins VL, Venegas FL, Wattenberg LW (1982). Glutathione S-Transferase*. Cancer Inst*, **68**, 493-6.
Sujata V, Ravishankar GA, Venkataraman LV (1992). Methods for the analysis of the saffron metabolites crocin, crocetind, picrocrocin and safranal for the determination of the quality of spice using thin-layer chromatography, HPLC and GC. *J Chromatography,* **624,** 497-502.
Sunde RA and Hoekstra WG (1980). Structure, synthesis, and function of Glutathione peroeidase. *Nutr Rev,* **8**, 265-73.
Taniguchi N (1992). Elevated serum manganese superoxide dismutase in acute leukemias. *Cancer Lett***, 62,** 211-5.
Tarantilis PA, Polissiou M, Manfait (1994). Separation of picrocrocin, Cis-trans crocins and safranal of saffron using HPLC with photodiode array detection. *J Chromatography*, **664**, 55-61.
Tarantilis PA, Tsoupras G, Polissiou M (1995). Determination of saffron (*Crocus sativus*. L) components in crude plant extract using HPLC-U V Visible photodiode-array detection-mass spectrometry. *J Chromatography,* **699,** 107-18.
Tarantilis PA and Polissiou M (1997). Isolation and identification of the aroma constitutes of saffron (Cricus sativus L*). J Agric Food Chem***, 45,** 459-62.
Unnikrishnan MC and Kuttan R (1990). Tumor reducing and anticarcinogenic activity of selected spices. *Cancer Lett*, **51,** 85.
Wattenberg LW (1985). Chemoprevention of cancer. *Cancer Res*, **45,** 1-8.
Werts ED and Gould MN (1986). Relationships between superoxide dismutase and susceptibility to chemically induced cancer in the rat mammary gland*. Carcinogenesis*, **7,** 1197-201.
Williams GW (1984). Modulation of chemical carcinogenesis by xenobiotics. *Fund Appl Toxicol,* **4**, 325-44.
Wood JL (1970). In Metabolic Conjugation and Metabolic hydrolysis (Fishman,W.H.ed.,) *Academic Press New York* , **2,** pp.261-299.
Yay TM (1979). Mutagenecity and cytotoxicity of malonaldehyde in mammalian cells. *Mech Ageing Dev*, **11,** 137-44.
Zargani NS and Heinz DE (1971). The volatile constituents of saffron*. Lebensm Wiss Technol,* **4,** 43-5.