Original Article

Inhibitory effects of aqueous crude extract of Saffron (*Crocus sativus* L.) on chemical-induced genotoxicity in mice

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Saffron (dried stigmas of *Crocus sativus* L.), was evaluated in the mouse bone marrow micronucleus test for its possible protective effects against chromosomal damage induced by cisplatin (CIS), mitomycin-C (MMC) and urethane (URE). Three doses of saffron (25, 50 and 100 mg/kg body weight) were orally administered to mice for five consecutive days prior to administration of genotoxins under investigation. From the results obtained, it was evident that the administration of 50 and 100 mg saffron/kg body weight could significantly inhibit the *in vivo* genotoxicity of these genotoxins. However, all the three doses of saffron were effective in exerting a protective effect against urethane.

Key words: saffron, inhibitory effects, micronucleus test, cisplatin, mitomycin-C, urethane

Introduction

At present, there is substantial evidence to show that chemical mutagenesis and carcinogenesis can be inhibited by a large number of naturally occurring compounds of plant origin.[1-4] It has been suggested that the common use of antimutagens and anticarcinogens in every day life will be the most effective approach for preventing human cancer and slowing genetic diseases.[5-8] In order to explore this possibility, there is a need to identify the commonly consumed dietary agents which can, under *in vivo* conditions, exert protective effects against a wide spectrum of environmental mutagens/carcinogens. Saffron (dried stigmas of *Crocus sativus* L.) is a highly valued spice, commonly used for flavouring and colouring food. Since time immemorial, it has been used in folk medicine for various ailments such as an aphrodisiac, antispasmodic and expectorant.[9,10] Chemical studies on *C. sativus* have shown the presence of constituents such as crocin, crocetin, safranal and picrocrocin.[11,12] The inhibitory effects of saffron on chemical carcinogenesis in mice using a two-stage assay system and the effect of crocetin on skin papillomas and Rous sarcoma have also been described.[13,14] Recently Konoshima *et al*.,[15] found that crocin and crocetin derivatives inhibit skin tumour promotion in mice. The present investigation was undertaken to evaluate in an *in vivo* mammalian test system the effect of orally administered saffron on the genotoxicity of cisplatin (CIS), mitomycin-C (MMC) and urethane (URE) - well known genotoxins with different mechanisms of action.

The short-term *in vivo* mouse bone marrow micronucleus test, which provides information on *in vivo* chromosome breakage, spindle dysfunction and mitotic non-disjunction of whole chromosomes, was used.[16,17] We used aqueous crude extract of saffron instead of its lipophilic principle, because saffron is consumed as a whole spice in a variety of food preparations in India.

Subjects and Methods

Animals

All the experiments were carried out with 10-12 weeks old male Swiss albino mice weighing 25-30 g. These animals were obtained from National Institute of Nutrition (NIN) Hyderabad, India and maintained in the University Animal House on the standard mouse diet (pellets from Hindustan Lever Limited, Mumbai, India) and water *ad lib*. The animals used in the present study were maintained in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), India and approved by the Institute's ethical committee.

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Chemicals

Mitomycin-C (MMC) was 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. Saffron (dried stigmas of *Crocus sativus* L.) was purchased from Indian Medical Practitioners Co-operative Pharmacy and Stores (IMPCOPS), Chennai, India.

Preparation of saffron extract

An aqueous extract of saffron was prepared using dried stigmas of *Crocus sativus* L, which were soaked in double distilled water for one hour and homogenized. The homogenate was centrifuged at 2000 rpm for 10 min to remove the particles and the supernatant was used for the experiment. The doses were calculated on the basis of the weight of dried stigmas (mg) used to prepare 1 ml extract.

Treatment schedule

Three test doses (25, 50 and 100 mg/kg body weight) of freshly prepared saffron extract were administered by gavage (10 ml/kg body weight) to the experimental animals for 5 consecutive days. Duration of pretreatment and test doses of saffron were decided on the basis of findings from preli-minary studies. The control animals received the same volume of distilled water. The genotoxins CIS, MMC and URE were dissolved in saline and injected intraperitoneally (10 ml/kg) 2h after the final pretreatment with saffron. Each pretreatment group consisted of six mice.

Micronucleus test

Genotoxic effects were evaluated in the mouse bone marrow micronucleus test, which was carried out according to Schmid.[17] The bone marrow cells from both femurs were flushed in the form of a fine suspension into a centrifuge tube containing human AB serum. This cell suspension was centrifuged at 2000 rpm for 10 min, and the pellet was resuspended in a drop of serum before being used for preparing slides. Air-dried slides were stained with May Grunwald and Giemsa as described by Schmid.[17] For each experimental point, six mice were used and 3000 polychromatic erythrocytes (PCEs) were scored per animal per slide to determine the frequency of micronucleated polychromatic erythrocytes (Mn PCEs). All the slides were scored by the same observer

Statistical analysis

Student's t-test was used for comparing the effects of pretreatment with saffron on genotoxicity.

Results

The data presented in Table 1 shows the influence of pretreatment with aqueous crude extract of saffron on the frequencies of Mn PCEs in bone marrow cells of mice induced by CIS, MMC and URE. A significant reduction was observed in the incidence of Mn PCEs induced by the genotoxins CIS and MMC, following the administration of 50 and 100 mg saffron extract/kg body weight. However, all the three doses of saffron tested were effective in exerting a protective effect against urethane.

Discussion

This investigation was carried out with the objective of evaluating the possible role of aqueous crude extract of saffron in modulating the *in vivo* genotoxicity of environmental mutagens and carcinogens. The results show that the oral administration of saffron can cause a significant reduction in the incidence of Mn PCEs in mouse bone marrow induced by CIS, MMC and URE, which are all known genotoxins with different mechanisms of action.[18-20] Since the micronuclei in young erythrocytes arise mainly from chromosomal fragments that are not incorporated into the daughter nuclei at the time of cell division in the erythropoietic blast cells,[16] the observed decrease in the incidence of Mn PCEs can be considered to indicate an inhibitory effect of saffron on the *in vivo* chromosomal damage induced by cisplatin, mitomycin-C and urethane. Saffron had inhibitory effects on the genotoxicity of CIS, MMC and URE even though their mechanisms of action are different. It was shown that saffron extracts contain characteristic compounds such as carotenoids, crocin and crocetin, monoterpene aldehydes, picrocrocin and safranal.[21] The majority of crocetin glycosides were able to inhibit the growth of human cancer cells in vitro.[12] The antimutagenic effect of *C. sativus* can be attributed to the total activity of the crude extract. Several explanations have been offered for antimutagenic activity of herbs and spices, one of which relates to the large number of potent antioxidants present in plant products. In our study, orally administered saffron extract has exerted anticlastogenic effects against the intraperitoneally injected directly acting agents CIS and MMC, and indirectly acting URE. Inhibition of URE may be mainly due to inhibition of their activation mediated by P-450 enzymes. The extract was also found to modulate antioxidants and topoisomerase II,[22-24] indicating that other than the inhibition of the activation of carcinogens, the extract has multifocal activity. In conclusion, the findings from the present

Table 1. Effect of saffron (Crocus sativus L.) on the frequencies of Mn PCEs induced by chemical-genotoxins

Group	Treatment	Mn PCEs/3000 PCEs Mean ± SEM[#]
1	Control	5.67 ± 2.34
2	Cisplatin (5 mg/kg)	27.5 ± 6.28 [a***]
3	Saffron (25 mg/kg) + CIS	28.8 ± 6.85
4	Saffron (50 mg/kg) + CIS	19.5 ± 6.92 [b*]
5	Saffron (100 mg/kg) + CIS	16.8 ± 7.33 [b**]
6	Mitomycin –C (1 mg/kg)	52.5 ± 7.84 [a***]
7	Saffron (25 mg/kg) + MMC	50.7 ± 6.97
8	Saffron (50 mg/kg) + MMC	43.3 ± 5.88 [c**]
9	Saffron (100 mg/kg) + MMC	38.5 ± 5.57 [c**]
10	Urethane (750 mg/kg)	67.3 ± 11.34 [a***]
11	Saffron (25 mg/kg) + URE	34.0 ± 9.69 [d**]
12	Saffron (50 mg/kg) + URE	38.0 ± 8.55 [d**]
13	Saffron (100 mg/kg) + URE	22.2 ± 7.70 [d**]

[#]Transformed values are presented as mean ± SEM for groups of 6mice. Statistical significance: **P*<0.05; ***P*<0.01; ****P*<0.001. Student's *t*- test. Comparisons were made: [a] group 1 vs. 2, 6 and 10; [b] group 2 vs. 4 & 5; [c] group 6 vs. 8 & 9; [d] group 10 vs. 11, 12 & 13.

study suggest that pre-treatment with the naturally occurring flavouring and colouring agent, saffron can lead to moderate protective effects against *in vivo* geno-toxicity.

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