Original Article

# Interactive effects of saffron with garlic and curcumin against cyclophosphamide induced genotoxicity in mice

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Saffron is a well-known spice and food colorant commonly consumed in different parts of the world. Recently, much attention has been focused on the biological and medicinal properties of saffron. In the present study the interactive effects of saffron with two commonly consumed dietary agents, garlic and curcumin was evaluated for anti-genotoxic effects against cyclophosphamide (CPH) in the mouse bone marrow micronucleus test. Experimental animals were orally pretreated with saffron (100 mg/kg body weight), garlic (250 mg/kg body weight) and curcumin (10 mg/kg body weight), either alone or in combination for five consecutive days, 2h prior to the administration of CPH. Maximum reduction in the frequencies of micronucleated polychromatic erythrocytes (Mn PCEs) induced by CPH was observed when all the three test compounds were administered together. Furthermore, the protective effects were more pronounced in the garlic-administered groups compared to curcumin and/or saffron administered groups.

**Key Words: saffron, garlic, curcumin, antigenotoxic effects, micronucleus test, cyclophosphamide.**

## Introduction

Saffron obtained from dried stigmas of *Crocus sativus* L. (Iridaceae), is a spice commonly used for flavouring and colouring food. Over the decades, it has been used in folk medicine for various ailments.[1-3] Extracts of saffron has been shown to be capable of inhibiting and/or retarding tumorigenesis in a variety of experimental models *in vivo*.[4-5] Antitumor effect of saffron and its constituents have also been demonstrated on different malignant cells *in vitro*.[6-8] Recently we reported the antimutagenic and antioxidant effects of saffron.[9-11]

Spices are dietary constituents consumed daily by most of the world population to enhance the flavour or taste of human food. Many of them have been identified as potential chemopreventive agents. Among the species, garlic and turmeric are widely used. Garlic, cloves of *Allium sativum,* and Curcumin, a yellow pigment obtained from rhizomes of *Curcuma longa,* a major component of turmeric are commonly used spices, which have been shown to possess many medicinal properties including immunomodulatory, hepatoprotective, anti-oxidant, antimutagenic and anticarcinogenic effects.[12-15]

Depending on the composition of the food, humans are likely to ingest different combinations and varying quantities of these chemopreventive agents. In order to simulate some of these conditions in the present investigation, the possible antigenotoxic effects resulting from the interaction of saffron with two commonly consumed spices - garlic and curcumin - was evaluated using short-term bone marrow micronucleus test.

## Materials and Methods

### *Animals*

All the experiments were carried out with 10-12 weeks old male Swiss albino mice weighing 25-30g. These animals were obtained from the 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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**Table 1.** Effects of saffron (S), garlic (G) and curcumin (C) on micronuclei induced by CPH

| Pretreatment groups (mg/kg body weight) | Mn PCEs/3000 PCEs Mean ± SEM |
|---|---|
| Control | $4.8 \pm 0.4^{a}$ |
| CPH (40) | $67.5 \pm 2.7^{b}$* |
| S (100) | $43.2 \pm 1.9^{c}$* |
| G (250) | $34.8 \pm 1.7^{d}$* |
| C (10) | $36.7 \pm 1.6^{d}$* |
| S + G | $30.0 \pm 1.2^{e}$* |
| S + C | $34.5 \pm 1.5^{d}$* |
| G + C | $28.2 \pm 1.4^{f}$* |
| S + G + C | $21.0 \pm 1.8^{g}$* |

Values are presented as mean ± SEM from 6 mice in each group. Values not sharing a common superscript letter differ significantly at *$P$< 0.05 (Student's $t$- test).

***Chemicals and test materials***

Cyclophosphamide (CPH) and curcumin were purchased from Sigma Chemical Company (St. Louis, MO, USA). All the other chemicals used were of the highest purity and analytical grade. Saffron (dried stigmas of Crocus sativus L.) and fresh garlic were procured from Indian Medical Practitioners Co-operative Pharmacy and Stores (IMPCOPS), Chennai, India and local vegetable market respectively.

***Preparation of test materials***

Aqueous extracts of saffron and garlic were prepared using dried stigmas of *Crocus sativus* L. and freshly peeled cloves of garlic respectively, 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. Curcumin was dissolved in peanut oil before being used. The doses were calculated based on weight (in mg) of spices used for preparing 10 mL extract (which is the volume administered per kg body weight).

***Treatment schedule***

The experimental animals were administered orally with the dietary test compounds viz. saffron (100 mg/kg body weight), garlic (250 mg/kg body weight) and curcumin (10 mg/kg body weight) for five consecutive days either alone or in combination (Table 1). The genotoxin, cyclophosphamide (40 mg/kg bw) was dissolved in saline and injected intraperitoneally (10 ml/kg) 2h after the final pre-treatment with dietary test compounds. The animals were sacrificed 24h after injecting the genotoxin. Control animals received same volume of distilled water. 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.[16] 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-Grünwald and Giemsa as described by Schmid.[16] For each experimental point, six mice were used and 3000 polychromatic erythrocytes (PCEs) were scored per animal 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 different pretreatments on genotoxicity.

**Results**

The data presented in the table shows the effect of pretreatment of saffron, garlic and curcumin either individually or in combination in modulating the CPH induced

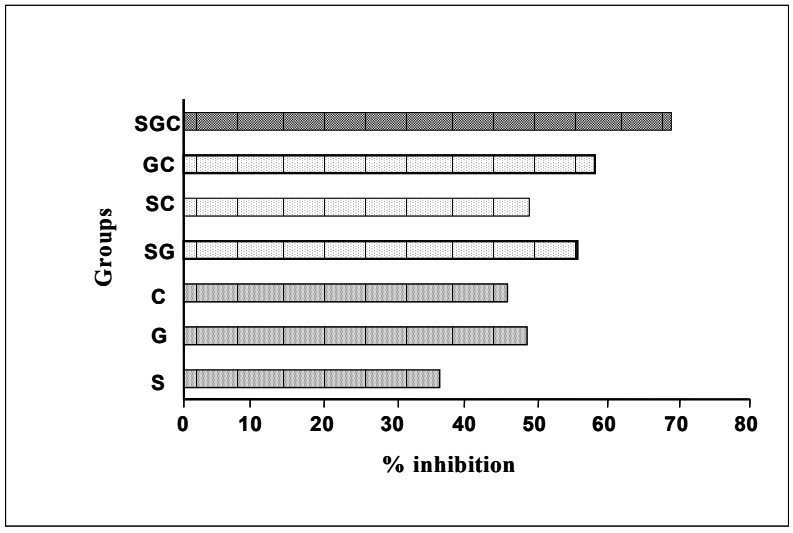


**Figure 1**. Inhibitory effects of safron, garlic and curcumin on CPH induced genotoxicity

genotoxicity. A significant protection against the CPH induced genotoxicity was observed when the test compounds were administered either alone or in a combination of two. However, the maximum reduction (69%) in the frequencies of Mn PCEs induced by CPH was observed when all the three test compounds were administered together. The protective effects were more pronounced in the garlic-administered groups compared to curcumin and/or saffron administered groups.

## Discussion

The present study was carried out with the objective of assessing the possible outcome of *in vivo* interaction of saffron with garlic and curcumin from the standpoint of anti-genotoxic potential. The results of this study suggests that the administration of saffron together with garlic and curcumin can lead to an increase in the *in vivo* anti-genotoxic effects, compared with what is observed when these agents are given separately. These observations indicate the possible interaction of saffron with garlic and curcumin.

The dietary agents included in this study represent a sample of those that are commonly ingested together by a large section of the human population. Studies on the anti-genotoxicty of these test compounds in mice have shown that the doses required to obtain a significant effect are 100, 250 and 10 mg/kg body weight for saffron, garlic and curcumin respectively.[11,17,18] Hence, in this study a similar dose was used. Several explanations have been offered for antimutagenic activity of herbs and spices, one of which relates to the large number of potent anti-oxidants present in plant products. The observed protective effect against CPH induced genotoxicity may be due to one or more of the following: antioxidant action, trapping of free radicals, formation of complex with mutagen, modulation of mutagen metabolism or by adsorbing the xenobiotic. This is feasible because many naturally occurring compounds are known to exhibit discrete mechanisms of protection[19,20].

In the present study, saffron pretreatment showed less protective effect compared to garlic and curcumin. However, the intake of saffron together with garlic and curcumin has resulted in a significant enhancement in the anitgenotoxic effects. In conclusion, the findings from the present investigation highlight the importance of interaction studies involving commonly consumed dietary agents that are individually antigenotoxic.

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