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 the inhibitory activity of saffron against the genotoxicity and carcinogenicity 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...
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) (Okawa 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)

<table>
<thead>
<tr>
<th>Group (genotoxin)</th>
<th>Subgroup</th>
<th>Saffron (mg/kg bw)</th>
<th>LPO</th>
<th>SOD</th>
<th>CAT</th>
<th>GST</th>
<th>GPx</th>
<th>GSH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I (Control)</td>
<td>a</td>
<td>0</td>
<td>1.81 ± 0.22</td>
<td>5.75 ± 0.51</td>
<td>465 ± 36.08</td>
<td>1.04 ± 0.09</td>
<td>46.57 ± 5.21</td>
<td>1.76 ± 0.18</td>
</tr>
<tr>
<td>b</td>
<td>20</td>
<td>1.82 ± 0.19</td>
<td>5.99 ± 0.55</td>
<td>472 ± 50.05</td>
<td>1.07 ± 0.11</td>
<td>45.92 ± 4.44</td>
<td>1.86 ± 0.21</td>
<td>53.78 ± 6.46</td>
</tr>
<tr>
<td>c</td>
<td>40</td>
<td>1.73 ± 0.16</td>
<td>6.36 ± 0.58</td>
<td>484 ± 43.61</td>
<td>1.14 ± 0.12</td>
<td>49.19 ± 4.74</td>
<td>1.95 ± 0.18</td>
<td>55.32 ± 7.22</td>
</tr>
<tr>
<td>d</td>
<td>80</td>
<td>1.93 ± 0.20</td>
<td>5.88 ± 0.56</td>
<td>477 ± 43.52</td>
<td>1.08 ± 0.11</td>
<td>49.70 ± 5.04</td>
<td>1.88 ± 0.18</td>
<td>54.32 ± 6.78</td>
</tr>
<tr>
<td>Group II (CIS)c</td>
<td>a</td>
<td>0</td>
<td>2.62 ± 0.25</td>
<td>4.08 ± 0.33</td>
<td>266 ± 32.54</td>
<td>0.71 ± 0.07</td>
<td>25.60 ± 3.20</td>
<td>0.69 ± 0.07</td>
</tr>
<tr>
<td>b</td>
<td>20</td>
<td>2.32 ± 0.21</td>
<td>4.66 ± 0.54</td>
<td>334 ± 31.27</td>
<td>0.92 ± 0.10</td>
<td>30.17 ± 3.45</td>
<td>0.93 ± 0.11</td>
<td>35.42 ± 3.72</td>
</tr>
<tr>
<td>c</td>
<td>40</td>
<td>2.11 ± 0.20</td>
<td>5.41 ± 0.66</td>
<td>443 ± 41.74</td>
<td>1.04 ± 0.10</td>
<td>43.66 ± 4.36</td>
<td>1.49 ± 0.14</td>
<td>39.70 ± 4.96</td>
</tr>
<tr>
<td>5 mg/kg</td>
<td>a</td>
<td>0</td>
<td>2.51 ± 0.32</td>
<td>4.03 ± 0.39</td>
<td>272 ± 29.16</td>
<td>0.73 ± 0.08</td>
<td>26.83 ± 2.64</td>
<td>0.79 ± 0.09</td>
</tr>
<tr>
<td>b</td>
<td>20</td>
<td>2.26 ± 0.27</td>
<td>4.79 ± 0.51</td>
<td>313 ± 40.18</td>
<td>0.86 ± 0.11</td>
<td>31.37 ± 3.47</td>
<td>0.98 ± 0.09</td>
<td>34.70 ± 3.74</td>
</tr>
<tr>
<td>Group III (MCC)c</td>
<td>a</td>
<td>0</td>
<td>2.02 ± 0.23</td>
<td>5.17 ± 0.62</td>
<td>460 ± 55.91</td>
<td>1.02 ± 0.07</td>
<td>41.83 ± 5.07</td>
<td>1.54 ± 0.17</td>
</tr>
<tr>
<td>b</td>
<td>20</td>
<td>2.09 ± 0.24</td>
<td>5.04 ± 0.57</td>
<td>372 ± 47.36</td>
<td>0.94 ± 0.08</td>
<td>36.94 ± 4.32</td>
<td>1.11 ± 0.14</td>
<td>39.70 ± 4.96</td>
</tr>
<tr>
<td>1 mg/kg</td>
<td>a</td>
<td>0</td>
<td>2.43 ± 0.26</td>
<td>4.13 ± 0.42</td>
<td>253 ± 28.72</td>
<td>0.77 ± 0.08</td>
<td>25.68 ± 2.60</td>
<td>0.97 ± 0.09</td>
</tr>
<tr>
<td>b</td>
<td>20</td>
<td>2.08 ± 0.19</td>
<td>4.82 ± 0.58</td>
<td>357 ± 37.64</td>
<td>0.91 ± 0.09</td>
<td>29.73 ± 2.71</td>
<td>1.13 ± 0.12</td>
<td>31.87 ± 3.65</td>
</tr>
<tr>
<td>Group IV (URE)c</td>
<td>a</td>
<td>0</td>
<td>1.94 ± 0.18</td>
<td>5.20 ± 0.57</td>
<td>434 ± 46.70</td>
<td>1.02 ± 0.11</td>
<td>38.00 ± 4.77</td>
<td>1.43 ± 0.13</td>
</tr>
<tr>
<td>b</td>
<td>20</td>
<td>2.02 ± 0.20</td>
<td>5.08 ± 0.53</td>
<td>368 ± 37.16</td>
<td>0.98 ± 0.12</td>
<td>34.97 ± 3.59</td>
<td>1.17 ± 0.13</td>
<td>37.64 ± 3.79</td>
</tr>
<tr>
<td>750 mg/kg</td>
<td>a</td>
<td>0</td>
<td>2.92 ± 0.28</td>
<td>3.90 ± 0.46</td>
<td>288 ± 33.47</td>
<td>0.72 ± 0.08</td>
<td>24.24 ± 2.37</td>
<td>0.76 ± 0.09</td>
</tr>
<tr>
<td>b</td>
<td>20</td>
<td>2.48 ± 0.31</td>
<td>4.55 ± 0.54</td>
<td>314 ± 35.08</td>
<td>0.86 ± 0.10</td>
<td>31.76 ± 3.25</td>
<td>0.88 ± 0.11</td>
<td>34.70 ± 3.74</td>
</tr>
</tbody>
</table>

The symbols represent statistical significance (Student’s t-test).

* p < 0.05
* p < 0.01
* p < 0.001

NS Not significant
* Group la compared with group Ia, IIIa, IVa and Va;
* 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 H2O2 consumed/min/mg protein.

lipid peroxidation induced by cisplatin (CIS), cyclo-
phosphamide (CPH), mitomycin-C (MMC) and urethane (URE) with concomitant changes in anti-
oxidants and detoxification systems. However, the
observed modifying effects were not always dose
dependent. Against the genotoxins tested, the maxi-
mum 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 mod-
ulatory 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 B1
and dimethylnitrosamine (Lin and Wang, 1986).

The involvement of free radical induced lipid pero-
oxidation 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 pre-
venting 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 chem-
ical carcinogens/mutagens by GSH has been linked
with chemopreventive activity (Wattenberg, 1978) and
thus our findings are in confirmation with the pre-
vious 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 infer-
red that oral pretreatment with the saffron aqueous
extract for 5 consecutive days prior to the administra-
tion 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 scav-
enging 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 anti-
oxidant 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 characteriza-
tion 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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