Evaluation of Radioprotective Action of a Mutant (E-25) Form of *Chlorella vulgaris* in Mice

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The possible role of orally fed *Chlorella vulgaris* (E-25) in modulating the gamma-ray induced chromosomal damage in whole-body irradiated mice was evaluated using a micronucleus test. Different doses of E-25 were administered either chronically (once, twice or thrice a day for 28 days) or as single acute doses before/after irradiation. A significant radioprotective effect was observed in both acute and chronic pretreatments, but only at doses above 400 mg/kg body weight. However, in mice that received E-25 (500 mg/kg) three times a day for 28 days, there was no protective effect, and a significant loss in their body weight was observed. Interestingly, E-25 afforded significant radioprotection even when it was administered within 0.4 hr after irradiation.

INTRODUCTION

There is growing awareness in the recent years that some of the commonly consumed food products and beverages contain potential modulators of the effects of environmental mutagens and carcinogens¹,²). Among the diets found to be “protective” in various mammalian systems are the green leafy vegetables, fresh fruits and components of coffee and tea³–⁵). More specifically, dietary vitamins, their precursors, trace minerals, chlorophylls, proteins and phenols of plant origin have been shown to reduce the incidence of radiation and chemical carcinogen-induced chromosome breakage in a number of test systems. In the present study, we have tested the radioprotective potential of *Chlorella vulgaris* E-25, a freshwater green algae, traditionally used as a nutrition additive, especially as a source of vitamins, specific proteins and assimilated salts. E-25 strain is a mutant form of the heterotrophic type of *Chlorella vulgaris*, isolated by means of...
single cell isolation technique, and cloned by Ogaki and his team in Japan since 1978. The cell-wall of this strain has a very low content of α-cellulose which inhibits its digestibility and absorption in the intestine. In Japan, this algae is produced on a mass-scale by tank cultivation and is available as dry granules under the commercial name “Momotaro E-25”. Experimental and clinical studies over the past two years have indicated that consumption of E-25 green algae is beneficial in a number of pathological conditions such as hypertension, bronchial asthma, diabetes and late radiation injuries such as those resulting from radiotherapy. This paper reports an investigation of an effect of orally administered *Chlorella vulgaris* E-25 on the frequencies of micronucleated polychromatic erythrocytes (MnPCEs) in bone-marrow cells of mice subjected to acute whole-body irradiation with 1 Gy of 60Co-gamma-rays.

**MATERIALS AND METHODS**

**Mice**

Male Swiss albino mice, 6–8 weeks old, weighing 25–30 g were used in the experiments. The animals were housed in polypropylene cages at 25±2°C and allowed *ad libitum* access to tap-water and a standard mouse diet (Lipton India Ltd., India).

**Irradiation**

Mice were restrained within individual compartments in a well-ventilated acrylic cylinder and placed in a gamma-chamber [60Co source, 204 TBq (5500 Ci)] for whole-body irradiation. A total of 1 Gy was delivered at a dose-rate of 2.6 Gy/min as determined by Fricke’s dosimetry.

**Chlorella vulgaris** E-25

Dried samples of the algae E-25 strain were weighed and suspended in double-distilled water, shaken in a vortex mixer and administered to mice by gavage. Each mouse received the desired dose in a volume of 10 ml/kg body weight.

**Experimental design**

The first set of experiments was designed to assess the effect, if any, of *Chlorella vulgaris* E-25 administered orally to mice 1 hr before whole-body irradiation with 1 Gy of 60Co-gamma-rays. Groups of six mice were given various doses (10 to 500 mg/kg) of E-25 as acute pretreatment, and the frequencies of radiation-induced micronuclei in bone-marrow was determined by a micronucleus test. Based on the results (Table 1), the lowest dose of E-25 which elicits optimal radioprotection was determined. It must be mentioned that in these experiments, the maximum quantity of E-25 that could be administered as an acute dose was limited by the increased viscosity of the algal suspensions at higher concentrations. Therefore, in the next set of experiments, a single dose of *Chlorella vulgaris* E-25 was given by gavage to groups of six mice each, either once, or twice, or thrice every day for a period of 28 days before irradiation. During the entire course of this chronic pretreatment, the average amount of food consumed by the animals in various treatment groups and their individual body weights were recorded every day.
From this, the average food intake per mouse per day and average change in body weight per mouse per week were calculated (Figures 1 and 2). The effect of post-treatment with *Chlorella vulgaris* E-25 was also tested using a single effective dose administered at increasing time-intervals after irradiation. The results were compared with that obtained in pretreatment studies using the same dose (Table 3). In all of the experiments, control animals were given equal volumes of double-distilled water simultaneously.

### Micronucleus test

Animals were killed by cervical dislocation 24 hr after irradiation and bone-marrow cells were flushed out into 1 ml of fetal calf serum (Gibco, Scotland). The smears were prepared and stained according to the method described by Schmid. A single sampling time was used since it has been shown that in mouse bone-marrow cells, the frequency of MnPCEs induced by 1 Gy of

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### Table 1. Effect of acute pretreatment with *Chlorella vulgaris* (E-25) on gamma-ray induced micronuclei in mouse bone-marrow cells *in vivo*

<table>
<thead>
<tr>
<th>E-25 (mg/kg)</th>
<th>γ-rays (Gy)</th>
<th>MnPCEs/1000 PCEs (x±SE)</th>
<th>Protection (%)</th>
<th>PCE PCE ± NCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>—</td>
<td>—</td>
<td>3.5 ± 0.4</td>
<td>—</td>
<td>0.53</td>
</tr>
<tr>
<td>—</td>
<td>1.0</td>
<td>20.9 ± 1.0</td>
<td>—</td>
<td>0.51</td>
</tr>
<tr>
<td>10</td>
<td>1.0</td>
<td>20.5 ± 1.9</td>
<td>None</td>
<td>0.52</td>
</tr>
<tr>
<td>100</td>
<td>1.0</td>
<td>20.7 ± 1.8</td>
<td>None</td>
<td>0.50</td>
</tr>
<tr>
<td>200</td>
<td>1.0</td>
<td>16.5 ± 1.4</td>
<td>21</td>
<td>0.52</td>
</tr>
<tr>
<td>300</td>
<td>1.0</td>
<td>17.6 ± 1.0</td>
<td>16</td>
<td>0.54</td>
</tr>
<tr>
<td>400</td>
<td>1.0</td>
<td>14.4 ± 1.0**</td>
<td>31</td>
<td>0.51</td>
</tr>
<tr>
<td>500</td>
<td>1.0</td>
<td>13.9 ± 0.9**</td>
<td>34</td>
<td>0.50</td>
</tr>
<tr>
<td>500</td>
<td>—</td>
<td>2.2 ± 0.6</td>
<td>—</td>
<td>0.57</td>
</tr>
</tbody>
</table>

E-25 was fed orally 1 hr before whole-body irradiation and the mice were sacrificed 24 hr later. From each animal, 2500 PCEs were scored.

**p<0.01 (t-test).**

### Table 2. Effect of chronic pretreatment (for 28 days) with E-25 on gamma-ray induced micronuclei in mouse bone-marrow cells *in vivo*

<table>
<thead>
<tr>
<th>Dosage (mg/kg×n)</th>
<th>Total dose (mg/kg/day)</th>
<th>γ-rays (Gy)</th>
<th>MnPCEs/1000 PCEs (x±SE)</th>
<th>Protection (%)</th>
<th>PCE PCE ± NCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0</td>
<td>1.0</td>
<td>20.4 ± 1.2</td>
<td>—</td>
<td>0.52</td>
</tr>
<tr>
<td>500×1</td>
<td>500</td>
<td>1.0</td>
<td>11.0 ± 1.1**</td>
<td>46</td>
<td>0.54</td>
</tr>
<tr>
<td>500×2</td>
<td>1000</td>
<td>1.0</td>
<td>11.9 ± 0.7**</td>
<td>42</td>
<td>0.53</td>
</tr>
<tr>
<td>500×3</td>
<td>1500</td>
<td>1.0</td>
<td>19.9 ± 1.3</td>
<td>None</td>
<td>0.51</td>
</tr>
</tbody>
</table>

n: number of treatments per day.

Data are derived from groups of 6 mice; 2500 PCEs scored from each animal.

*Chlorella vulgaris* (E-25) was fed to mice by gavage once/twice/thrice every day (at 3 hr intervals) for 28 days. Animals were whole-body irradiated on day 29 and sacrificed 24 hr later.

From this, the average food intake per mouse per day and average change in body weight per mouse per week were calculated (Figures 1 and 2). The effect of post-treatment with *Chlorella vulgaris* E-25 was also tested using a single effective dose administered at increasing time-intervals after irradiation. The results were compared with that obtained in pretreatment studies using the same dose (Table 3). In all of the experiments, control animals were given equal volumes of double-distilled water simultaneously.
gamma-rays reaches a peak 24 hr after irradiation\textsuperscript{81}. The permanent slides were coded before scoring and for each experimental animal, 2500 polychromatic erythrocytes were scored from a single slide for determining the frequency of MnPCEs. In addition, the ratio of PCEs (polychromatic erythrocytes) to NCEs (normochromatic erythrocytes) in different regions of the slides prepared for each animal was also monitored in order to check cell proliferation in the bone-marrow of Chlorella treated animals.

**RESULTS**

Table 1 shows the results of the experiments to evaluate the incidence of MnPCEs in bone marrow cells of mice that received Chlorella vulgaris E-25 1 hr before exposure to 1 Gy of Gamma-rays. There is a highly significant reduction (P<0.01) in the frequency of radiation-induced MnPCEs by E-25 only at and above a dose of 400 mg/kg body weight. Further, there is no difference in the degree of radioprotective effect between the two effective doses (400 and 500 mg/kg body weight).

**Table 3. Radioprotective effect of E-25 administered as an acute dose (500 mg/kg) at various time intervals before/after whole-body irradiation (1 Gy)**

<table>
<thead>
<tr>
<th>Treatment time (hours ± T\textsubscript{a})</th>
<th>MnPCEs/1000 PCEs</th>
<th>Protection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Treated</td>
</tr>
<tr>
<td>-24 hr</td>
<td>20.0 ± 0.2</td>
<td>18.2 ± 0.7</td>
</tr>
<tr>
<td>-1 hr</td>
<td>19.8 ± 0.4</td>
<td>14.0 ± 1.0**</td>
</tr>
<tr>
<td>+0.4 hr</td>
<td>19.7 ± 0.6</td>
<td>15.2 ± 1.1*</td>
</tr>
<tr>
<td>+1.0 hr</td>
<td>20.4 ± 1.2</td>
<td>20.0 ± 1.0</td>
</tr>
<tr>
<td>+2.0 hr</td>
<td>20.4 ± 1.2</td>
<td>19.1 ± 1.8</td>
</tr>
<tr>
<td>+8.0 hr</td>
<td>20.4 ± 1.2</td>
<td>18.4 ± 1.2</td>
</tr>
</tbody>
</table>

\(T\textsubscript{a} = \)Time of irradiation.
Values are mean±SE (n=6); 2500 PCEs scored per animal.
\*p<0.05; **p<0.01 (t-test)

Chronic pretreatment with E-25 also resulted in an appreciable reduction in the radiation-induced MnPCEs (Table 2). There is significant radioprotection (P<0.01) in mice pretreated for 28 days with 500 mg/kg of E-25, either once or twice every day, but not in mice receiving 500 mg/kg thrice every day; a third dose of 500 mg/kg of E-25 results in abolition of the radioprotection observed at 1×500 mg/kg/day and 2×500 mg/kg/day. Further, the animals which received a third dose of E-25 everyday showed loss of body weight (Figure 2) although the amount of food consumed by them was not diminished in comparison with the others (Figure 1). It is also noted that the magnitude of radioprotection by a single dose of E-25 (500 mg/kg) is the same (35–45\%) whether it is administered acutely (one hour before irradiation) or chronically (once or twice every day for 28 days before irradiation).
The results of pre- and post-treatments with E-25 (500 mg/kg) on radiation-induced micronuclei are presented in Table 3. Clearly, E-25 exerts significant radioprotection (P<0.01) when it is administered 1 hr before irradiation. However, pretreatment with E-25 is not radioprotective if the time-interval between feeding and irradiation is 24 hr. Interestingly, E-25 exerts significant radioprotection (P<0.05) even when it is administered 0.4 hr after irradiation,
but not 1 hr, 2 hr or 8 hr later.

The effect of E-25 feeding on food consumption by mice during the period of treatment (28 days) is illustrated in Figure 1. Administration of E-25 to mice once or twice everyday results in a significant decrease ($P<0.05$) in the amount of food consumed by mice during the first and second weeks of treatment. There is a significant improvement in the same during the third week while the average for the fourth week shows no difference between the Chlorella treated groups and control. In contrast, data from animals receiving E-25 at 500 mg/kg thrice every day show no differences in amount of food consumed during the first and second weeks of treatment but show significant increase ($P<0.01$) during the third and fourth weeks of treatment.

The corresponding changes in the body weight of the mice in various treatment groups are shown in Figure 2. It is evident that up-to the second week of feeding, there are no differences in the average body weight of Chlorella pretreated mice in comparison with the control mice. However, during the third and fourth weeks of treatment, the average body weight of mice fed E-25 (500 mg/kg) thrice a day, registers a significant decrease ($P<0.05$) in comparison with the mice from the control group. A similar trend is not observed for the other two Chlorella treated groups.

**DISCUSSION**

The results (Tables 1, 2 and 3) show that Chlorella vulgaris E-25 exerts a radioprotective effect in whole-body irradiated mice. The frequency of radiation-induced MnPCEs is reduced by 25–40% when E-25 is given not earlier than 1 hr (acute or chronic) or not later than 15 min after irradiation. The noteworthy observation, however, is that E-25 is radioprotective only at and above 400 mg/kg body weight. In addition, at very high dosed (as in $3 \times 500$ mg/kg/day) its radioprotective effect disappears, coinciding with a significant loss of body weight in these animals (Table 2, Figure 2). This probably indicates the upper limit of tolerance of E-25 in mice. Under such situation, the cell proliferation of PCEs cells could be also influenced and the peak of the frequency of MnPCEs might appear later than 24 hr. Such delay in cell cycle progression may result in the observed decrease in the frequency of MnPCEs. Preliminary investigations by Japanese and Russian scientists on the effect of long-term E-25 consumption in human beings have indicated a good tolerance of therapeutic doses of E-25 in adults (80–130 mg/kg/day) and children (25–75 mg/kg/day). In a few cases, E-25 administration resulted in diarrhea, increased diuresis and loss of appetite. These symptoms disappeared when E-25 consumption was stopped or the dose reduced.

For mechanistic considerations, the chemical composition of E-25 is relevant. Each gram of E-25 contains, among others, chlorophyll (27 mg) and beta-carotene (0.11 mg). The anti-mutagenic activity of chlorophyll and beta-carotene present in vegetable extracts is now well established in different test systems. Negishi et al. (1989) have reported that the chlorophyll extracted from Chlorella vulgaris E-25 inhibits the activity of a known mutagen (3-amino-1-methyl-5H-pyrido[4,3-b]indole) in bacteria and Drosophila in a dose-dependent manner. These authors suggest that the protective effect is due to complex formation between
chlorophyll and the mutagen which renders the latter ineffective. In our study, E-25 afforded the same magnitude of protection in chronic pretreatment as in acute pretreatment and there was no dose-dependent increase in the protective effect.

Although there is yet no evidence that E-25 scavenges free radicals generated in vivo, at least some of its constituents are known to be good antioxidants. For example, beta-carotene, a singlet oxygen quencher\(^{12}\), can protect mouse bone-marrow cells from gamma-ray induced chromosomal damage in vivo\(^{13}\). Similarly, chlorophyllin, the sodium and copper salt of chlorophyll, has been reported to be an effective photoreducing agent\(^{14}\) and inhibitor of microsomal lipid peroxidation\(^{15}\). There is another possibility that E-25 might stimulate rejoining of the micronuclei to chromosomes. Since, in our present study, protection resulted even when E-25 was administered a few minutes after irradiation, the role of E-25, or its constituent(s), in bringing about "chemical repair" of long-life radical configurations involving target molecules, is an important consideration and needs further assessment. Recently long-life organic radical formation (with a half life of about one day) is reported by Yoshimura et al (in press) in a mammal cell system irradiated by \(\gamma\)-rays\(^{16}\).

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REFERENCES


