Protective Effect of Lycopene on Ethyl Methane Sulfonate–induced Chromosome Aberrations in Allium cepa

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Abstract — In this study, whether lycopene has protective effect on chromosome aberrations or not was investigated. For this purpose, Allium cepa were treated with 4 different concentrations (1 µM, 3 µM, 5 µM and 10 µM) of lycopene extracts for 24 h. Afterwards, plant roots were treated with 2 different concentrations (2x10⁻²M and 3x10⁻²M) Ethyl Methane Sulfonate for 2 h. 2x10⁻²M EMS and 3x10⁻²M EMS were used as positive control and tap water was used as negative control. Root tip meristematic cells were observed under the microscope. Mitotic index (MI), chromosomal aberrations (fragments, bridges, stickiness, polar deviation) and micronucleus formation were evaluated and statistically analyzed.

As a result, it was determined that lycopene reduced mitotic index (MI) in treatment groups in comparison with controls. 3 µM, 5 µM lycopene doses reduced total chromosome aberrations in treatment groups in comparison with positive control (p<0.05).

Key words: Allium cepa; carotenoid; chromosome aberration; EMS; lycopene

INTRODUCTION

The carotenoids are a group of over 600 naturally pigments in plants. Of these about 24 are common in human foods. These compounds have two major functions in plants: they act as accessory pigments in photosynthesis and in photoprotection (Hughes et al. 2000).

Lycopene is an acyclic carotenoid with 11 linearly arranged conjugated double bounds (Clinton 1998). Lycopene is present in tomatoes, processed tomato products and other fruits such as watermelon, pink grapefruit and guava. It is a natural pigment synthesized by plants and microorganisms but not by animals (Agarwal and Rao 2000). Since the human body does not produce lycopene, it is available through the diet (Slosmski 2000). Lycopene from tomatoes is absorbed much better into the blood stream if it is processed (Shi 2000). Lycopene is one of the most potent antioxidants with a singlet-oxygen quenching ability twice as high as that of β-carotene and 10 times higher than that of α-tocopherol (Miller et al. 1996; Woodall et al. 1997; Di Mascio et al. 1989).

Several epidemiological studies have suggested a role of tomato products in protecting cancer and chronic diseases (Porrini and Riso 2000). It has been hypothesized that lycopene prevent carcinogenesis by protecting critical cellular biomolecules, including lipids, lipoproteins, proteins and DNA (Agarwal and Rao 1998; Rao and Agarwal 1998; Pool-Zobel et al. 1997). Moreover, lycopene is exceptionally potent in protecting lymphocytes from NO₂ radical cell death and membrane damage (Hennekens et al. 1996). Recent studies reported antiproliferative effects of lycopene against cancer cells in culture (Stahl and Sies 1996; Gerster 1997). Lycopene reduces also cellular proliferation of various cancer cell lines induced by Insulin-like Growth Factor-I (IGF-I) and lycopene is more effective than β-carotene on cell cycle inhibition (Levy et al. 1995).

It is a very potent endometrial-, lung- and breast cancer cell growth inhibitor in comparison with α- and β-carotene (Sharoni and Levy 1995). Besides the preventive effects of lycopene on tumor formation and cancer; it can enhance resistance against bacterial infections (Hughes 2000), age-related macular degeneration and blindness (Shi 2000).

In this study, we investigated protective effect of lycopene on Ethyl Methane Sulfonate (EMS)-
induced chromosome aberrations in *Allium cepa* root tip meristem cells. It has been demonstrated that alkylating agent EMS is a potent mutagen on *Allium cepa* root tip meristematic cells (Rank and Nielsen 1997). Since the results of studies using *Allium cepa* fit well in a test composed of prokaryotes and/or eukaryotes (Fiskesjö 1993) and final it is very cheap and easy test to apply, we used this test material. This is the first study about protective effects of lycopene on chromosome aberrations using plant material.

**MATERIAL AND METHODS**

*Chemicals* - Petroleum ether (CAS No: 1.00909.5000), Tetrahydrofuran (THF) (CAS No: 1.08114.2500), Acetone (CAS No:1.00013.2500), Sodium Chloride (CAS No:1.06404.1000) and Potassium carbonate (CAS No:1.04928.1000) were purchased from Merck (Darmstadt, Germany) and Ethyl Methane Sulfonate (EMS) (CAS No:M-0880) was purchased from Sigma (Germany).

*Lycopene Extraction from Tomatoes* - For lycopene extraction, tomatoes were broken with a homogenisor (KIKAK Labortechnik T25 basic) and the pure lycopene extracted from pure by rinsing with a mixture of petroleum ether-acetone (50/50%). After extraction, liquid extract was filtered and washed with saturated sodium chloride solution, aqueous potassium carbonate and deionized water, respectively. Petroleum ether was evaporated using an evaporator with vacuum (KIKAWERKWE HB4 basic). After evaporation, pigments were dissolved in a small volume of Tetrahydrofuran (THF) to ensure dispersion of crystals and filtered through a filter paper. Concentrations were determined by using a spectrophotometer (Shimadzu UV-1601 UV-visible spectrophotometer). All the procedures were performed under dim lighting.

*Allium cepa Chromosome Aberration Test* - To perform this test, 12 commercial equal-sized *Allium cepa* onion bulbs of 3-4 g per concentration were used. They were carefully unscaled, placed on top of test tubes, including 4 different concentrations (1µM, 3 µM, 5 µM and 10 µM) of lycopene extracts and allowed to germinate in the dark at 22°C. After 48 h, two onions with the poorly growing roots were removed and the other healthy onion bulbs in lycopene extracts were treated with 2 different EMS concentrations (2.10−2 M and 3.10−2 M) for 2 h. After the treatment, onion bulbs were placed on top of test tubes filled with tap water for 24 h. EMS concentrations at 2.10−2 M and 3.10−2 M were used as positive control and tap water was used as negative control.

After the completion of treatment, the roots of each bulb were treated with 0.1% colchicine for 1 h and the roots were fixed in 3:1 (ethanol:acetic acid). After the fixation, the roots were hydrolyzed in 1N HCl for 2 min and stained with 2% aceto-orcein stain. Root tips were squashed in 45% acetic acid and examined microscopically for mitotic index (MI) and cells with chromosomal aberrations. Chromosomal aberrations were determined by scoring cells with bridges, fragments, sticky chromosomes, polar deviation and micronucleus formation in randomly picked 3 zones per slide. Five slides were examined per onion in each group included 10 onions.

*Statistical Analysis of Data* - The mean values were calculated for each group of concentrations and controls (negative and positive control). For the determination of the significance among the means, Independent Samples t-Test was applied (p<0.05).

**RESULTS**

The results of lycopene treatments (1µM, 3µM, 5 µM and 10 µM) before 2x10−2 M and 3x10−2 M EMS treatments are shown in Table 1 and 2, respectively. Although the experiment was performed in controlled conditions, we observed significant differences among mitotic index (MI) of the groups. When onion roots were treated with 4 different concentrations of lycopene (1µM, 3µM, 5 µM and 10 µM) before 2x10−2 M and 3x10−2 M EMS exposure, mitotic index (MI) rates were decreased significantly compared to negative and positive controls (p<0.05); even approached to zero in group treated with 10 µM lycopene before 3x10−2 M EMS.

Structural and behavioral changes in chromosomes were observed in addition to the mitotic index (MI). When control groups were compared with treatment groups, different results were obtained.

Fragments, which appear abnormally with physical and chemical mutagens, increased following treatment with EMS (2x10−2 M and 3x10−2 M). Fragment formation decreased significantly in the groups treated with 1µM, 3µM, 5 µM and 10
µM lycopene before 2x10⁻² M EMS exposure in the relation to the increase of concentrations of lycopene. Similar results were obtained in the groups treated with 1 µM, 3 µM, 5 µM and 10 µM lycopene treatment before 3x10⁻² M EMS exposure. It is also significant that the increase in lycopene concentration protect cells against the fragment formation.

Another chromosomal aberration observed in the experiment is chromatid bridges. The chromatid bridge frequency increased in positive control. Its frequency decreased significantly in 1 µM, 3 µM, 5 µM and 10 µM lycopene treatment before 2x10⁻² M EMS exposure similar to decrease in fragment formation values (p<0.05).

In groups treated with 3 µM lycopene before 2x10⁻² M EMS exposures and 1 µM, 5 µM and 10 µM lycopene before 3x10⁻² M EMS exposures no chromatid bridges were observed.

Chromosomal stickiness was also observed during the experiment. Chromosomal stickiness values were: 29 in negative control, 307 and 393 in positive controls. These values increased in the group treated with 1 µM lycopene before 2x10⁻² M EMS exposure, but in the groups treated with other lycopene concentrations before EMS were stickiness values decreased significantly. In statistical analysis made by comparing the values of groups, the differences were significantly important (p<0.05).

Polar deviations were also observed in our experiment. Polar deviation values were 19 in negative control, 51 and 203 in positive controls. These values showed that the decrease in lycopene treatment groups depended on increasing lycopene concentrations.

In the microscope observations, micronucleus formations were observed. There was only one micronucleus formation in negative control and its formation increased in positive controls, but in all treatment groups, the number of micronucleus showed decrease in accordance with doses.

When the total chromosomal aberrations were evaluated, chromosomal aberrations showed a decrease in 1 µM, 3 µM and 5 µM lycopene treated groups where they showed an increase in 10 µM lycopene treatment groups. Examples of EMS induced chromosomal aberrations observed in our study, are shown in Figure 1.

**DISCUSSION**

In this study, decreased mitotic index values at first seems to be due to toxic effect of lycopene, but it can be explained as lycopene suppresses the cell proliferation and prevents chro-

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**Table 1** — Effect of lycopene treatment before 2x10⁻² M EMS exposure on the mitotic index and chromosome aberrations in the root meristem cells in *Allium cepa*.

<table>
<thead>
<tr>
<th>Chemical concentration (µM)</th>
<th>Total cells</th>
<th>Dividing cells</th>
<th>MI % (±SD)</th>
<th>Fragment</th>
<th>Bridge</th>
<th>Stickness</th>
<th>Polar deviation</th>
<th>Micro-nuclei</th>
<th>Other</th>
<th>% Aberrant cells (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>13005</td>
<td>1443</td>
<td>11.1 (2.0)</td>
<td>2</td>
<td>11</td>
<td>29</td>
<td>19</td>
<td>1</td>
<td>2</td>
<td>4.4 (1.22)</td>
</tr>
<tr>
<td>Positive control</td>
<td>14025</td>
<td>1568</td>
<td>11.2 (2.6)</td>
<td>22</td>
<td>22</td>
<td>307</td>
<td>51</td>
<td>22</td>
<td>7</td>
<td>27.5 (3.05)</td>
</tr>
<tr>
<td>1 µM lycopene</td>
<td>20758</td>
<td>1713</td>
<td>8.3 (4.8)</td>
<td>6</td>
<td>5</td>
<td>442</td>
<td>14</td>
<td>11*</td>
<td>2</td>
<td>28.0 (6.81)</td>
</tr>
<tr>
<td>3 µM lycopene</td>
<td>19525</td>
<td>1229</td>
<td>6.3 (5.2)*</td>
<td>1</td>
<td>0</td>
<td>275</td>
<td>11</td>
<td>5*</td>
<td>3</td>
<td>24.1 (5.74)*</td>
</tr>
<tr>
<td>5 µM lycopene</td>
<td>19061</td>
<td>1259</td>
<td>6.6 (6.0)*</td>
<td>0</td>
<td>2</td>
<td>250</td>
<td>8</td>
<td>6*</td>
<td>1</td>
<td>21.2 (5.99)*</td>
</tr>
<tr>
<td>10 µM lycopene</td>
<td>17259</td>
<td>792</td>
<td>4.6 (5.3)*</td>
<td>1</td>
<td>2</td>
<td>211</td>
<td>4</td>
<td>2*</td>
<td>0</td>
<td>27.8 (5.54)*</td>
</tr>
</tbody>
</table>

*p<0.05 in independent samples t-test

**Table 2** — Effect of lycopene treatment before 3x10⁻² M EMS exposure on the mitotic index and chromosome aberrations in the root meristem cells in *Allium cepa*.

<table>
<thead>
<tr>
<th>Chemical concentration (µM)</th>
<th>Total cells</th>
<th>Dividing cells</th>
<th>MI % (±SD)</th>
<th>Fragment</th>
<th>Bridge</th>
<th>Stickness</th>
<th>Polar deviation</th>
<th>Micro-nuclei</th>
<th>Other</th>
<th>% Aberrant cells (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative control</td>
<td>13005</td>
<td>1443</td>
<td>11.1 (2.0)</td>
<td>2</td>
<td>11</td>
<td>29</td>
<td>19</td>
<td>1</td>
<td>2</td>
<td>4.4 (1.22)</td>
</tr>
<tr>
<td>Positive control</td>
<td>18261</td>
<td>1872</td>
<td>10.3 (1.9)</td>
<td>38</td>
<td>39</td>
<td>393</td>
<td>203</td>
<td>34</td>
<td>17</td>
<td>38.5 (4.05)</td>
</tr>
<tr>
<td>1 µM lycopene</td>
<td>19925</td>
<td>1042</td>
<td>5.2 (4.9)*</td>
<td>0</td>
<td>0</td>
<td>289</td>
<td>18</td>
<td>8</td>
<td>1</td>
<td>30.3 (6.65)*</td>
</tr>
<tr>
<td>3 µM lycopene</td>
<td>18117</td>
<td>353</td>
<td>1.9 (2.5)*</td>
<td>1</td>
<td>1</td>
<td>55</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>17.0 (2.25)*</td>
</tr>
<tr>
<td>5 µM lycopene</td>
<td>16130</td>
<td>453</td>
<td>2.8 (4.3)*</td>
<td>0</td>
<td>0</td>
<td>89</td>
<td>3</td>
<td>6</td>
<td>0</td>
<td>21.6 (3.94)*</td>
</tr>
<tr>
<td>10 µM lycopene</td>
<td>16951</td>
<td>204</td>
<td>1.2 (2.5)*</td>
<td>2</td>
<td>0</td>
<td>45</td>
<td>6</td>
<td>5</td>
<td>1</td>
<td>28.9 (3.21)*</td>
</tr>
</tbody>
</table>

*p<0.05 in independent samples t-test
mosomal aberration formation in organism. In vitro evidences indicate that lycopene reduces cellular proliferation of various cancer cell lines induced by Insulin-like growth factor-I (Levy et al. 1995).

Lycopene has been postulated to be the protective compound against prostate cancer. It has been reported that possible mechanisms of action for lycopene included the following: (a) inhibition of growth and induction of differentiation in cancer cells by modulating the expression of cell cycle regulatory proteins; (b) modulation of the IGF-I/IGFBP-3 system; (c) up regulation of tumor suppressor protein cx43 and increased gap-junctional intercellular communication; (d) modulation of redox signaling; (e) prevention of oxidative DNA damage and (f) modulation of carcinogen metabolizing enzymes (Kucuk et al. 2001).

Fig. 1 — Chromosomal aberrations observed in root tip cells of Allium cepa: A - Metaphase chromosomes; B - Cell with fragment; C - Chromatid Bridge; D - Stickiness; E - Polar deviation; F - Micronucleus.
Lycopene regulates gap-junction communication by inducing connexin 43 mRNA expression (Zhang et al. 1991; Zhang et al. 1992) moreover it is a potent inhibitor of endometrial cancer cell proliferation caused by IGF (Sharoni and Levy 1995). Lycopene inhibits also cell cycle progression by reducing cyclin-D level and retention of p27Kip1 in the Cyclin E-cdk2 complexes (Nahum et al. 2001).

In a recent study made on 9 adult women, it has been evaluated that the consumption of 25 mg tomato puree for 14 days increased plasma and lymphocyte concentration and this was related to an improvement in lymphocyte resistance to an oxidative stress (505 mumol hydrogen peroxide for 5 min). It was found that small amounts of tomato puree added to diet over a short period can increase carotenoid concentrations and the resistance of lymphocytes to oxidative DNA damage (Porrini and Risso 2000).

In our study, in addition to the changes in mitotic index values, aberration changes were also observed. Especially in positive control groups, due to EMS, which is an alkylating agent, most frequently observed aberrations were: fragments, chromatic bridges, polar deviations, stickiness and micronucleus formation (Fig.1).

When we have considered the total aberrations observed, they showed to decrease generally in lycopene treatment groups compared with control groups, while increased slightly in groups treated with 1µM and 10µM lycopene before 2x10^3M EMS exposure.

Therefore, our results show that, lycopene has protective effect on chromosome aberrations induced by EMS. According to our results and information in other reports about potential health benefits of lycopene; consumption of antioxidant-rich and especially lycopene-rich foods can be beneficial to health protection and increasing resistance against exogenous harmful effects. To obtain more information and to reach more definitive conclusions about this subject, further researches should be performed with different test systems.

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REFERENCES


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