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Preventive Effect of Lycopene on Chromosome Aberrations in *Allium cepa*

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Abstract: In this study, whether lycopene has preventive effect on chromosome aberrations or not was investigated. For this purpose, 2 different concentrations (0.02 and 0.03 M) of Ethyl Methane Sulfonate (EMS) which is known to induce chromosomal damage were treated on *Allium cepa* for 2 h. Afterwards, plant roots were treated with lycopene extracts in 4 different concentrations (1, 3, 5 and 10 μM) for 24 h. 0.02 and 0.03 M 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) and chromosomal aberrations (fragments, bridges, stickness, polar deviation) and micronucleus formation were evaluated and statistically analyzed. As a result; it was determined that depending on applied lycopene concentrations both in MI and chromosomal aberrations lycopene-caused some changes. It was seen that lycopene had preventive effect on chromosome aberrations particularly at 1 and 3 μM concentrations, but this effect decreased at 5 and 10 μM concentrations.

Key words: Lycopene, EMS, chromosome aberration, *Allium cepa*

INTRODUCTION

Lycopene is an acyclic carotenoid with conjugated 11 double bounds and it is a pigment which is commonly found in tomatoes (*Lycopersicum esculentum*) and processed tomato products[1]. When it is compared with other carotenoids such as β-carotene, α-carotene and lutein, it is known that lycopene is a strong antioxidant and it has preventive effects on free radicals[2].

Lycopene, which is commonly found in tomatoes, also found in watermelon, guava, pink grapefruit and rosehip in small amounts. Since the human body does not produce lycopene, it is available through the diet[3]. Lycopene from tomatoes is absorbed much better into the blood stream if it is processed[4].

Studies made in recent years have showed that lycopene can reduce DNA oxidation and DNA damage in lymphocytes, LDL oxidation and it can inhibit tumor formation or reduce tumor growth for several cancer types[5-8]. Lycopene delays cell cycle progression from G_1 to S stage interfering with Insulin-like growth factor-I receptor signals, thus lycopene reduces tumor growth inhibiting cell cycle[9].

Lycopene is more effective than β-carotene on cell cycle inhibition.

It is more potent endometrial, lung and breast cancer cell growth inhibitor than α- and β-carotene[10]. Besides the preventive effects of lycopene on tumor formation and cancer; it can enhance resistance against bacterial infections[11], age-related macular degeneration and blindness[4].

Up to now, studies related with potential benefits of lycopene have been done using animal test systems. However, these tests are difficult to apply and expensive, the use of plant test systems is more preferable. The purpose of this study was to determine, whether lycopene has preventive effect on Ethyl Methane Sulfonate (EMS)-induced chromosome aberrations in *Allium cepa* root tip meristematic cells. It has been demonstrated that alkyllating agent EMS is a potent mutagen on *Allium cepa* root tip meristematic cells[12]. Since the results of studies using *Allium cepa* fit in well in a test battery composed of prokaryotes and/or other eukaryotes[13] and it is very cheap and easy to apply, we used *Allium cepa* as a test material. This is a first study that is about preventive effects of lycopene on chromosome aberrations using plant material.

MATERIALS 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).

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Lycopene extraction from tomatoes: For lycopene extraction, tomatoes were broken with homogenisor (KIKA Labortechnik T25 basic) and the 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 evaporator with vacuum (KIKA-WERKWE HB4 basic). After evaporation, pigments were dissolved in a small volume of Tetrahydrohuran (THF) to ensure dispersion of crystals and filtered through a filter paper. Concentrations were determined by using spectrophotometer (Shimadzu UV-1601 UV-visible spectrophotometer). All of the procedures were performed under dim lighting.

***Allium cepa* chromosome aberration test:** To perform the test, 12 commercial equal-sized *Allium cepa* onion bulbs of 3-4 g per concentration were carefully unscaled, placed on top of test tubes filled with tap water and allowed to germinate in the dark at 22°C. After 48 h, two onions with the most poorly growing roots were removed and healthy onion bulbs rooted (1-2 cm long) in tap water were exposed to 2 different EMS concentrations (2.10^{-2} M and 3.10^{-2} M) for 2 h. After exposure, onion bulbs were treated with 4 different lycopene concentrations (1, 3, 5 and 10 μM) for 24 h. 2.10^{-2} M and 3.10^{-2} M EMS concentrations 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 fixation, the roots were hydrolyzed in 1 N HCl for 2 min and stained with 2% 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, multipolar anaphases, polar deviation and micronucleus formation in randomly picked 3 zones per slide. Five slides were examined per onion and each group included 10 onions.

Statistical analysis of data: For each group of concentrations and controls, the mean values were calculated. For the determination of the significance among the means, the Independent Samples t-test was applied ($p<0.05$).

RESULTS

Although the experiment was performed in controlled conditions, we observed significant differences between MI rates of groups. When onion roots were treated with 4 different concentrations (1, 3, 5 and 10 μM) after $2x10^{-2}$ M and $3x10^{-2}$ M EMS exposure, Mitotic Index (MI) rates were decreased significantly according to negative and positive controls ($p<0.05$) (Table 1 and 2).

Although there was a slight increase in MI of 5 and 10 μM lycopene applied groups after $2x10^{-2}$ M EMS exposure, MI value of negative control (11.1%) and positive controls (11.2 and 10.3%) decreased significantly in groups treated with lycopene ($p<0.05$) (Table 1 and 2).

Structural and behavioral changes in chromosomes, were observed in addition to the MI. When control groups were compared with treatment groups, different results were obtained. Although there were chromosomal aberrations, they were not changing in accordance with concentrations.

Table 1: Effect of lycopene treatment after $2x10^{-2}$ M EMS exposure on the mitotic index and chromosome aberrations in the root meristem cells in *Allium cepa*

Chemical concentration (μM)	Total cells	Dividing cells	MI % (±SD)	Fragment	Bridge	Polar Stickness	Deviation	Micronuclei	Other	% Aberrant cells (±SD)
Negative control	13005	1443	11.1(2.0)	2	11	29	19	1	2	4.4(1.22)
Positive control	14025	1568	11.2(2.6)	22	22	307	51	22	7	27.5(3.05)
1 μM lycopene	15109	822	5.4(3.5)*	7	17	67	59	11	1	19.7(2.54)*
3 μM lycopene	16300	797	4.9(2.8)*	14	6	78	24	84	1	6.8(2.53)*
5 μM lycopene	16308	1010	6.2(3.2)*	19	12	272	54	23	1	238.8(4.82)*
10 μM lycopene	16825	1193	7.1(3.2)*	16	23	291	109	16	5	38.6(5.35)*

*$p<0.05$ in independent samples t-test

Table 2: Effect of lycopene treatment after $3x10^{-2}$ M EMS exposure on the mitotic index and chromosome aberrations in the root meristem cells in *Allium cepa*

Chemical concentration (μM)	Total cells	Dividing cells	MI % (±SD)	Fragment	Bridge	Polar stickness	Deviation	Micronuclei	Other	% Aberrant cells (±SD)
Negative control	13005	1443	11.1(2.0)	2	11	29	19	1	2	4.4(1.22)
Positive control	18261	1872	10.3(1.9)	38	39	393	203	34	17	38.5(4.05)
1 μM lycopene	18950	817	4.3(3.1)*	1	8	89	42	5	21	8.0(2.54)*
3 μM lycopene	18408	663	3.6(2.8)*	2	5	73	18	12	2	16.9(2.88)*
5 μM lycopene	18052	719	4.0(2.0)*	9	22	222	43	29	3	45.6(3.93)*
10 μM lycopene	19746	664	3.4(2.4)*	16	17	211	63	24	6	51.0(4.91)*

*$p<0.05$ in independent samples t-test

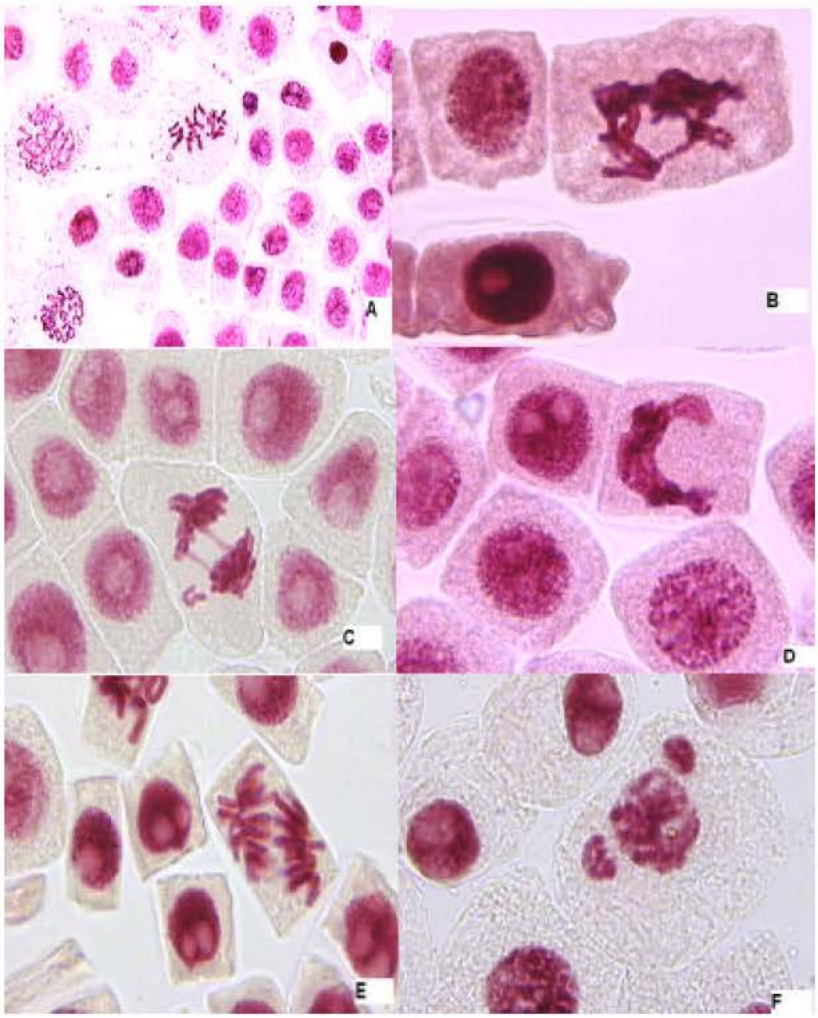

Fig. 1: Chromosomal aberrations observed in root tip cells of *Allium cepa*: A- Metaphase chromosomes; B- Cell with fragment; C- Chromatid bridge; D- Stickness; E- Polar deviation; F- Micronucleus

Fragments, which appear normally with physical and chemical agents, were increased with 2 applied concentrations of EMS ($2x10^{-2}$ M and $3x10^{-2}$ M). When 1 µM lycopene treated after $2x10^{-2}$ M EMS exposure, fragment formation decreased while it increased in other doses of lycopene. Similar results were obtained in 1 µM lycopene treatment after $3x10^{-2}$ M EMS exposure. It is also significant that the increase in lycopene concentration didn't prevent the formation of fragment.

Another chromosomal aberration observed in the experiment was chromatid bridges. The chromatid bridge frequency increased in positive control. Its frequency decreased in 1 and 3 µM lycopene treatment after $2x10^{-2}$ M EMS exposure while its frequency increased excitingly with the increase of lycopene dose. Similar increase was observed in $3x10^{-2}$ M EMS exposure in accordance with lycopene doses.

Chromosomal sticknesses were another chromosomal aberrations, which were observed during the experiment. Chromosomal stickness values were; 29 in negative control, 307 and 393 in positive controls. These values showed some differences in application groups regardless of doses (Table 1 and 2). In statistical analysis made by comparing the values of groups, the differences were important significantly ($p<0.05$).

Polar deviations were also observed in our experiment. Instead of going to polar deviation values were 19 in negative control, 51 and 203 in positive controls. These values showed decrease in 1 and 3 µM lycopene treatment groups while they showed increase in 5 and 10 µM lycopene treatment groups.

In the microscopic observations, micronucleus formations were also observed. There was only one micronucleus formation in negative control while in all

treatment groups number of micronucleus showed increase in accordance with doses.

When total chromosomal aberrations were observed, chromosomal aberrations showed decrease in 1 and 3 μM lycopene treatment groups where they showed increase in 5 and 10 μM lycopene treatment groups. Examples of EMS induced chromosomal aberrations, which observed in this study as shown in Fig. 1.

DISCUSSION

In this study, it is exciting that, mitotic index values showed decrease at first seems to be because of toxic effect of lycopene, but it can be explained as lycopene suppresses the cell proliferation and prevents chromosomal aberration formation in organism. These results are in accordance with the information in other literatures. Lycopene affects cell surface receptors and prevents intercellular communication so suppresses proliferation especially in cancer cells.

Lycopene regulates gap-junction communication by inducing connexin 43 mRNA expression[14,15] and lycopene is a potent inhibitor of endometrial cancer cell proliferation caused by IGF[16]. Lycopene inhibits also cell cycle progression by reducing cyclin-D level and retention of $p27^{Kip1}$ in the Cyclin E-cdk2 complexes[17].

Results from study made with MCF7 mammary cancer cells suggest that, lycopene decreased the activity of IGF-I receptors and prevented the cell growth. Lycopene didn't cause necrotic and/or apoptotic cell death and this effect is related with its effect on cell cycle progression but not with toxic effects of carotenoid[9].

In this study, in addition to the mitotic index values, aberration changes were also observed. Especially in positive control groups, with the effects of EMS, which is an alkyllating agent, most frequent aberrations were: fragments, chromatid bridges, polar deviations, stickness and micronucleus formation (Fig. 1).

When we considered totally observed aberrations, aberrations showed decrease in 1 and 3 μM lycopene treatment groups compared with control groups, while increased significantly in 5 and 10 μM lycopene treatment groups. This situation can be related to the physiological tolerance of the treated organism. Some properties of organism such as its simplicity or complexity, its physiological properties can affect antioxidant tolerance. Applied doses could be out of physiological dose intervals and it could behave as prooxidant. In a study made by Yeh and Hu[18], Hs68 cells were treated with lycopene and β-carotene after exposure to the different doses of oxidants and antioxidant and prooxidant properties of 2 carotenoids were compared. They showed that, both lycopene and β-carotene could be prooxidant in accordance with their applied dose and the type of used oxidant[18].

Present results show that, lycopene has preventive effect although its effects decrease above a particular dose level. To determine the decrease or absence of lycopene's preventive effect, further researches should be performed with different test systems.

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