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GENERAL GENETICS

The Mutagenic and Antimutagenic Effects of *Ecballium elaterium* Fruit Juice in Human Peripheral Lymphocytes*

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Abstract—The aim of this study was to investigate the mutagenic and antimutagenic effects of *Ecballium elaterium* (EE) fruit juice, which has an anti-inflammatory effect, using in vitro human peripheral lymphocytes. To investigate the mutagenic effects of the EE fruit juice, human peripheral lymphocytes were treated with three doses (18, 36, and 72 μl/l) of fruit juice alone for 24 and 48 h. For investigating the antimutagenic effects of the EE fruit juice, the human lymphocytes were also treated with the mixture of the fruit juice and 0.25 μg/ml MMC. The EE fruit juice induced the percentage of total CA when used alone (especially the percentage of structural CA than the percentage of the numerical CA) and synergically induced the percentage of total CA when used as a mixture with MMC. The EE fruit juice did not affect the SCE frequency for 24 and 48 h treatment time. In contrast, EE and MMC as a mixture sinergically induced the SCE frequency at the highest concentration for 48 h treatment time only. EE alone did not decrease the RI while it decreased the MI in a dose-dependent manner. EE and MMC as a mixture have a higher cytotoxic effect than the cytotoxic effects of EE alone. As a result, it can be concluded that EE had no antimutagenic effect while EE had a mutagenic and a cytotoxic effect in human peripheral lymphocytes.

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INTRODUCTION

Totally 35 genera belong to the Cucurbitaceae family; one of them is *Ecballium.* The genus *Ecballium* has only the *Ecballium elaterium* species. *Ecballium elaterium* (L.) A. Rich (EE) (synonymes: *Momordica elaterium, Elaterium cordifolium*), which is also known as squirting cucumber, spitting cucumber, and wild balsame apple, is a wild medicinal plant found in Mediterranian region. EE fruit juice is used for the treatment of sinusitis and for several illnesses in Turkish folk medicine [1, 2]. Yesilada et al. [3] reported that EE has an anti-inflammatory activity developed by cucurbitacin. Uslu et al. [4] reported that an aqueous solution of EE reduced the NO metabolites (NO_2, NO_3, NO) and reduced the activity of NOS enzyme and also suggested that EE extracts may have the potential to be used as an anti-inflammatory agent and can be used in the treatment of rhinosinusitis diseases. On the other hand, there are several reports on the uvular edema caused in humans by nasal administration of juice produced from the EE fruit [5–8]. However, it was reported that, the juice fruit of EE and its triterpenoid constituent, cucurbitacin B, dose-dependently inhibited the serotonin and bradykinin induced edema in mice [9]. In addition, cucurbitacin B had also preventive and curative effects against CCl_4 induced hepatoxicity [10].

Basaran et al. [11] reported that *E. elaterium* extracts were not mutagenic in *Salmonella typhimurium* TA98 and TA100 strains, however, it increased the DNA fractions in the comet assay. At present, there are no reports on mutagenicity and antimutagenicity of EE fruit juice. Thus, the aim of this study was to investigate mutagenic and antimutagenic effects of *Ecballium elaterium* fruit juice using chromosome aberration (CA) and sister chromatid exchange (SCE) tests in human peripheral lymphocytes in vitro.

MATERIALS AND METHODS

In this study, human peripheral blood was used as a test system and *Ecballium elaterium* (EE) fruit juice was used as a test substance.

Plant material. Ecballium elaterium fruits were collected in April from Adana province of Turkey located in East Mediterranian. The fruits were sterilized with 70% ethanol and squashed in sterile petri dishes to obtain fruit juice. The extract was filtered in sterile con-

* This article was submitted by the authors in English.

Table 1. Frequency of chromosome aberrations in cultured human lymphocytes treated with *E. elaterium* fruit juice alone and with the mixture of MMC+

Test substance	Treatment		Structural CA			Numerical CA ± SE(%)(P + E)*	Total CA ± SE (%)
	periods, h	concentrations	chromotid type	chromosome type	total ± SE (%)		
Control	–	–	7	1	4.00 ± 1.08	0.00 ± 0.00	4.00 ± 1.08
MMC	24	0.25 μg/ml	48	21	34.50 ± 1.89	0.00 ± 0.00	34.50 ± 1.89
EE	24	18 μl/l	6	3	5.50 ± 2.17^{b2}	2.50 ± 0.25	8.00 ± 2.66^{b1}
		36 μl/l	5	1	3.00 ± 0.62^{b3}	2.50 ± 0.62	5.50 ± 0.62^{a1b3}
		72 μl/l	17	3	10.00 ± 1.08^{a1b3}	4.00 ± 1.08	14.00 ± 1.08^{a2b2}
EE +	24	18 μl/l EE + 0.25 μg/ml MMC	33	10	21.50 ± 1.11^{a2b2c2}	2.50 ± 0.75	24.00 ± 1.75^{a1b1c1}
MMC		36 μl/l EE + 0.25 μg/ml MMC	18	16	17.00 ± 0.28^{a3b3c3}	3.00 ± 1.19	20.00 ± 2.87^{a1c1}
		72 μl/l EE + 0.25 μg/ml MMC^{d}	29	9	19.00 ± 1.50^{a2b2c1}	6.00 ± 1.47^{a1}	25.00 ± 2.53^{a1}
MMC	48	0.25 μg/ml	47	36	39.00 ± 4.03	0.50 ± 0.25	39.50 ± 4.11
EE	48	18 μl/l	7	2	4.50 ± 0.62^{b3}	1.50 ± 0.47	6.00 ± 0.57^{b3}
		36 μl/l	23	2	12.50 ± 1.25^{a1b2}	3.00 ± 0.50^{a1}	15.50 ± 1.25^{a1b2}
		72 μl/l	12	7	9.50 ± 0.47^{a2b3}	10.50 ± 1.11^{a2b1}	20.00 ± 0.70^{a3b2}
EE +	48	18 μl/l EE + 0.25 μg/ml MMC	45	34	39.50 ± 1.31^{a3b3}	0.00 ± 0.00^{b3}	39.50 ± 1.31^{a3b1}
MMC		36 μl/l EE + 0.25 μg/ml MMC	41**	15**	56.00 ± 0.40^{a3b3c3}	7.00 ± 0.28^{a3b2c1}	63.00 ± 0.64^{a3b3c3}
		72 μl/l EE + 0.25 μg/ml MMC	36**	13**	49.00 ± 1.44^{a3b3c1}	3.00 ± 0.57^{a1b1c1}	52.00 ± 1.68^{a3b2c2}

Notes: + A total 200 cell were scored.

* P, poliploidy; E, endoreduplication.

** Only 100 cells were scored due to excessive toxicity.

a: significant from the control; b: significant from the positive control (MMC); c: significance between the cultures treated with EE fruit juice and the cultures treated with the mixture of EE fruit juice and MMC (for example, significance between 18 μl/l EE and 18 μl/l EE + 0.25 μg/ml MMC).

a1b1c1: $P < 0.05$; a2b2c2: $P < 0.01$; a3b3c3: $P < 0.001$.

Table 2. Frequency of SCE, RI, and MI in cultured human lymphocytes treated with *E. elaterium* fruit juice alone and with the mixture of MMC+

Test substance	Treatment		min–max SCE	SCE/cell ± SE	RI ± SE	MI ± SE
	periods, h	concentrations				
Control	–	–	1–20	9.22 ± 0.65	1.93 ± 0.06	2.97 ± 0.13
MMC	24	0.25 μg/ml	11–42	24.74 ± 2.78	1.81 ± 0.08	2.41 ± 0.24
EE	24	18 μl/l	3–14	7.07 ± 0.38^{a1b3}	1.83 ± 0.11	1.80 ± 0.25^{a1}
		36 μl/l	2–15	7.53 ± 0.38^{a1b3}	1.85 ± 0.17	1.79 ± 0.29
		72 μl/l	3–14	7.86 ± 0.80^{b2}	1.80 ± 0.04	1.42 ± 0.15^{a1b1}
EE + MMC	24	18 μl/l EE + 0.25 μg/ml MMC	13–62	28.89 ± 4.32^{a1c1}	1.80 ± 0.04	1.76 ± 0.44^{a1}
		36 μl/l EE + 0.25 μg/ml MMC	11–64	22.20 ± 3.93^{a1c1}	1.73 ± 0.07	1.62 ± 0.17^{a2b1}
		72 μl/l EE + 0.25 μg/ml MMC^{d}	16–56	30.05 ± 3.99^{a1c1}	1.69 ± 0.10^{c3}	0.73 ± 0.17^{a3b2c1}
MMC	48	0.25 μg/ml	16–78	51.69 ± 6.68	1.56 ± 0.11	1.75 ± 0.33
EE	48	18 μl/l	4–19	8.94 ± 1.43^{b2}	2.08 ± 0.18	2.20 ± 0.40
		36 μl/l	3–28	9.73 ± 1.84^{b2}	1.92 ± 0.15	1.98 ± 0.41
		72 μl/l	4–15	7.82 ± 2.44^{b1}	1.54 ± 0.11	0.81 ± 0.35^{a1}
EE + MMC	48	18 μl/l EE + 0.25 μg/ml MMC	13–57	35.82 ± 5.59^{a1c1}	1.62 ± 0.04^{a2c2}	2.07 ± 0.48
		36 μl/l EE + 0.25 μg/ml MMC^{d}	10–91	52.00 ± 5.39^{a1c1}	1.24 ± 0.08^{a1c1}	1.57 ± 0.38^{a1}
		72 μl/l EE + 0.25 μg/ml MMC^{d}	50–80	69.33 ± 0.33^{a2b1c1}	1.28 ± 0.09^{a1}	0.56 ± 0.15^{a2b1}

Notes: + A total 50 cells were scored for the SCE assay; 200 cells were scored for RI.

a: significant from the control; b: significant from the positive control (MMC); c: significance between the cultures treated with EE fruit juice and the cultures treated with the mixture of EE fruit juice and MMC (for example, significance between 18 μl/l EE and 18 μl/l EE + 0.25 μg/ml MMC).

a1b1c1: $P < 0.05$; a2b2c2: $P < 0.01$; a3b3c3: $P < 0.001$.

ditions; then, this fruit juice was diluted with sterile distilled water before adding to the culture to obtain 18, 36, and 72 µl/l of the chromosome medium. The fruit extracts were prepared freshly before treatment.

Mutagenicity test. The technique of Evans [12] and Perry and Thompson [13] were followed for preparation of chromosomes with minor modifications. In addition, this study was prepared according to IPCS guidlines [14]. Whole blood (0.2 ml) from two healthy donors (one male and one female, nonsmokers, aged: 21 and 23) was added to 2.5 ml chromosome medium B (Biochrom, F5023) supplemented with 10 µg/ml bromodeoxyuridine (Sigma, B5002). The cultures were incubated at 37°C for 72 h. The cells were treated with 18, 32, and 72 µl/l concentrations of EE fruit juice for 24 h (fruit juice was added 48 h after initiating the culture) and 48 h (fruit juice was added 24 h after initiating the culture). A negative control and a positive control (mitomycin-C, MMC, Kyowa, Hakko, Japan) were used. No solvent control was used because the fruit juice of EE was diluted with sterile bidistiled water. Colchicine (0.06 µg/ml, Sigma C9754) was present for the last 2 h of culture. To collect the cells, the cultures were centrifuged (1200 rpm, 15 min), treated with hypotonic solution (0.4% KCl) for 13 min at 37°C, and then fixed in cold methanol : glacial acetic acid (3 : 1) for 20 min at room temperature. The treatment with fixative was repeated three times. Then the cells were spread on glass slides and air dried. The slides were stained with Giemsa according to the fluorescence plus Giemsa technique [15]. One hundred well-spread metaphases per donor (totally 200 metaphases per concentration) were examined at 1000× magnification for occurrence of different types of CA.

Antimutagenicity test. The modified method of Roncada et al. [16] and Mendelsohn [17] was used for evaluating antimutagenicity of the EE fruit extract. In the present study, mitomycin C was used as a mutagenic agent. To investigate the antimutagenic effect of EE fruit juice against the mutagenicity induced by mitomycin C, the cultures were treated with 0.25 µg/ml of MMC and with different concentrations of the EE fruit juice (18, 36, and 72 µl/l) for 24 and 48 h treatment times.

The number of CA was obtained by calculating the percentage of metaphases from each concentration and treatment period that showed the structural and numerical alterations. The CA was classified according to the ISCN (Paz-y-Miño et al., 2002 from Mitelman, 1995) [18]. The scoring of SCE was carried out according to Albertini et al. [14] as described earlier by Carrano and Natarajan (1988). For the occurrence of the number of SCEs, a total of 50 cells (25 cells from each donor) under second metaphases were examined. The results were used to determine the mean number of SCE (SCE/cell). In addition, a total of 200 cells (100 cells from each donor) were scored for determination of the replication index (RI). MI was also determined by scoring 3000 cells from each donor. The gaps were not evaluated as CA according to Mace et al. [19].

Statistical significance. The significance between the percentage of the structural and numerical CA, mean SCE, RI, and MI in treated cultures and their controls were determined using the *t*-test. Dose response relationships were determined from the correlation and regression coefficients for the percentage of the structural CA, numerical CA, total CA, mean SCE, RI, and MI.

At the antimutagenicity test, the results that obtained from the cultures treated with the mixture of EE fruit juice and MMC were compared with both the results of the cultures treated with MMC or with EE fruit juice alone.

RESULTS

Table 1 shows the percentage of structural and numerical CAs of EE fruit juice and also the CAs values of blanded EE fruit juice and MMC that compared with the control and positive control. EE fruit juice induced the structural chromosome aberrations (CAs) only at the two highest concentrations for 24 and 48 h treatment periods. However, EE fruit juice induced the numerical CA only at the highest concentration for 48 h treatment period when compared with the control group. EE fruit juice induced the total CA at a dose dependent manner for 48 h treatment period. EE fruit juice and MMC as a mixture induced CAs when compared with control; however, they decreased the total CAs when compared with mutagen MMC for 24 h treatment time. That means that EE fruit juice decreased the effect of MMC on CAs for 24 h treatment time; however, it did not show such effect in cultures treated for 48 h treatment time. EE fruit juice and MMC as a mixture induced the total CAs at all concentrations when compared with the cultures treated with EE fruit juice or MMC alone for 48 h treatment time without a dose-dependent manner (Table 1). At the 48 h treatment period, a synergic effect between EE fruit juice and MMC was shown.

As shown in Table 1, EE fruit juice caused both chromatid and chromosome type aberrations. EE fruit juice alone and as a mixture with MMC caused numerical CAs only at the highest concentrations for 48 h treatment time.

EE fruit juice decreased the SCE frequency only at 24 h treatment period when compared with control; however, EE fruit juice did not affect the SCE frequency for 48 h treatment period (Table 2). EE fruit juice and MMC as a mixture significantly increased the SCE frequency at all concentrations and treatment periods when compared with the control. EE fruit juice and MMC also synergically induced the SCE frequency only at the highest concentration for 48 h treatment time.

EE alone did not decrease the RI however, EE and MMC as a mixture decreased the RI. EE fruit juice

alone and the mixture with MMC decreased the MI in a dose-dependent manner (Table 2) and it showed a synergic effect between EE and MMC on decreasing the MI at the highest concentration for both treatment times.

DISCUSSION

In folk medicine in Turkey, EE was used for sinusitis and used as anti-inflammatory effect [3, 20]. Several forms of cucurbitacin were isolated from EE [1, 20] and it was reported that cucurbitacin has an anti-inflammatory effect [3, 9]. On the other hand, using EE for sinusitis treatment via nasal aspiration caused uvular edema and death in humans [5–8]. There are several reports on allergic complications caused by EE fruit juice in humans [8, 21, 22]. EE extracts was not mutagenic for *S. typhimurium* strains; however, it gave positive results in the comet assay [11].

In conclusion, EE fruit juice especially induced structural CAs at a frequency higher than numerical CAs. However, EE fruit juice did not decrease the mutagenicity of MMC; in contrast it induced the MMC mutagenicity. EE fruit juice showed a synergic effect with the MMC on induction of SCEs at the highest dose for 48 h treatment time in human lymphocytes and showed a cytotoxic effect. The cytotoxicity of EE caused from cucurbitacin E as described by Attarda et al. [23]. In addition, after nasal administration of EE fruit juice, the allergic complications were developed in humans. EE also caused the DNA fragmentations [11]. According to the Guidance of Committee on Mutagenicity [24], the chemicals should be tested with an in vitro test at first stage and if a positive result is obtained with any of these first step tests, the chemical should be considered as an in vitro mutagen. According to this report and our results, it can be concluded that EE fruit juice is an in vitro mutagen. In addition, it can also be concluded that EE fruit juice is capable to induce the mutagenicity of MMC. Thus, one must be careful when using EE fruit juice as a therapeutic substance.

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