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The Investigation of Antimutagenic Effects on Chromosomes and Cell Division Mechanisms against Mitomycine C in Human Lymphocyte Culture of Liquid Extracts Obtained from Blueberry (*Vaccinium myrtillus* L.) and Raspberry (*Rubus idaeus* L.)

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: In this study, we determined that whether the liquid extracts of the above-ground parts of *Vaccinium myrtillus L*. and *Rubus idaeus L.* have antimutagenic effects against mitomycin C in human peripheral lymphocytes, chromosome aberration (CA), micronucleus (MN) and mitotic index (MI) tests.
Methodology: Blood samples of the negative control group (Group I) were allowed to react in a cell culture medium without any treatment, while positive control group (Group II) was allowed to interact in a cell culture medium supplemented with mitomycin-C (MMC) at a dose of 0.3 µL /mL for each chromosome medium. Blood samples of the other groups were allowed to react for 24 hours

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with 0.3 μL /mL MMC and *V. myrtillus* L. (Group III, IV, V and VI), and *R. idaeus* L. (VII, VIII, IX and X) extracts in 0.2 μL /mL, 0.4 μL /mL, 0.8 μL /mL and 1.6 μL /mL doses for each of cell cultures. At the end of the incubation period, culture cells were evaluated by chromosomal aberrations, mitotic, micronucleus and nuclear division index tests.
Results: Compared with Group II, it was determined that mitotic, nuclear division and nuclear cytotoxic cleavage indices were increased when chromosome aberrations and micronucleus indexes were decreased in extract groups. When we compared to extract groups and group II, we observed that chromosome aberrations and micronucleus index decreased in extract groups, while mitotic, nuclear division and nuclear cytotoxic cleavage indices were increased.
Conclusion: It was concluded that aqueous extracts of *V. myrtillus* L. and *Rubus idaeus* L. had significantly antimutagenic effects on the human peripheral lymphocyte cells at the working doses.

Keywords: Vaccinium myrtillus L.; Rubus idaeus L.; chromosome aberration; mitotic index; micronucleus index; antimutagenity.

1. INTRODUCTION

Although berries of plants such as blueberries (*Vaccinium myrtillus*) and raspberries (*Rubus idaeus*) are very rich in phenolic compounds, they are often associated with the fact that these fruits are often consumed to preserve their overall health status and that they contain antioxidant substances in their bodies [1]. According to many recent *in-vivo* and *in-vitro* studies, blueberries have been reported to increase apoptosis and prevent heart disease, carcinogenesis and angiogenesis by reducing DNA damage and cancer cell proliferation [2]. Again, in the light of these data, high levels of anthocyanidins found in blueberry species such as delfinidine (30-40 μM), peonidine 3-glucuside (30-100 μM) and cyanidine 3-glucuside (30-100 μM) inhibits the growth of human tumor cells in HCT1 16 colon and HS578T breast cell lines by means of inducing apoptosis and stopping G2/M cell cycle. Anthocyanidins also reduce cell proliferation to increase lactate dehydrogenase activity (LDH) and aggregation of sub-G1 cells in CaCo-2 and Hep-G2 lethal cancer cell lines, as well as in HT-29 and HCT-116 colon cancer cell lines [3] and HL-60 in human leukemia cells have been shown to be effective in preventing the development of cancer cells by regulating apoptosis. As a result of all these data, anthocyanins against growing cancer cells are recommended as natural and potent inhibitors [4, 5]. Similarly, *Rubus idaeus* fruits contain high amounts of phenolic compounds, because of they have been reported to reduce lung, colon, human stomach and breast tumor cells by 20-54% [6] because they have potent antiinflammatory and antioxidant effects in connection with predominantly anthocyanins and elajitannins [7]. The wine produced by *R. idaeus* showed strong antiproliferative effects in *in-vitro* human breast (MCF-7), colon (CaCo-2) and cervical (HeLa) cancer cell lines depending on the dose [8]. mRNA expression of matrix metalloproteinase in 2 (MMP-2) was also suppressed by inhibition of focal adhesion kinase (FAK) and extracellular signal regulating kinase (ERK) enzyme and was found to be antimetastatic [9].

The rates of damage in DNA are determined by MN test, which is one of the most useful cytotoxicity tests *in vivo* and *in-vitro* [10,11]. The percentage of MN cells is determined in nucleus (binucleated) cells where nucleolysis occurs but no cytokinesis is present by applying certain amounts of Cyt-B to standard lymphocyte cultures [10,12]. In addition, the mitotic index (MI) is obtained by determining the number of mitosis cells in a cell population and calculating the ratio of this number to all cells. This test reflects the frequency of cell division [13]. As the toxicity of the administered agent increases, the MI value decreases [14]. Chromosomal aberration test, which is another cytotoxicity test with the principle that chromosomal aberration rate in peripheral lymphocyte cultured cells will reflect the general risk of cancer in the body, is used to evaluate the mutagenicity or carcinogenicity characteristics of external factors because chromosome aberrations are considered to have similar characteristics in various tissues and cell types of a living organism [15].

In this study, we investigated the antimutagenic effects of the plant extracts of blueberry (*Vaccinium myrtillus*) and raspberry (*Rubus idaeus*) on human peripheral lymphocyte cells and chromosomes against mitomycin C, based on the literature reviews.

2. MATERIALS AND METHODS

This study was carried out using blood samples taken with heparin injectors from healthy, non smoker adults aged 20-25 years, of whom 6 were female and 6 were male. The aqueous extracts of above-ground parts of *Vaccinium myrtillus* L. and *Rubus idaeus* L. (Cas no: 84082-34-8 and Cas no: 84929-76-0, Talya Herbal Products, Turkey) were purchased commercially.

2.1 Detection of Chromosome Aberrations (CA)

Heparinized whole blood (0.4 mL) was added to 5ml chromosome medium. Cell cultures were left in the incubator at 37 ± 1°C for 72 hours to proliferate the cells. To increase the number of metaphases and chromosome quality in each experimental group, 100 µl of solution A was added 48 h after incubating and 100 µl of solution B was added 65 h after incubating (Chromosome Synchro P Complete Medium, Italy). Experimental groups were constructed as follows using 0.4 mL heparinized bloods added to tubes containing 5 mL chromosome medium under sterile conditions (group I; negative control). It was applied 0.3 µg/mL mitomycin-C (MMC) to group II (positive control). It was added 0.3 µg/mL MMC plus 0.2, 0.4, 0.8 and 1.6 µL/mL of the *V. myrtillus* extract to heparinized blood samples of group III, IV, V and VI, respectively. It was added 0.3 µg/mL MMC plus 0.2, 0.4, 0.8 and 1.6 µL/mL of the *R. idaeus* extract to heparinized blood samples of group VII, VIII, IX and X, respectively. These processes were applied 48 hours after that transplantated 0.4 mL heparinized bloods to tubes containing 5 mL chromosome medium under sterile conditions.

Lymphocytes were cultured in the incubator for 72 h, and metaphases were blocked during the last 2 hours with colchicine at final a concentration of 0.06 µg/mL. Cells were collected by centrifuging (2000rpm, 10 min) and resuspended in KCl solution (0.075 M) for 30 min at 37°C. At the end of this procedure, cells were centrifuged again and fixed in a cold fixative made of methanol:acetic acid (3:1) mixture for10 min at +4°C. Following this process, cells were fixed at least twice until it appear clear. Slides were prepared by dropping concentrated cell suspensions onto the glass and very careful not to overlap the drops. Then all slides were left at room temperature for 24 hours to dry.

2.2 Drying and Preparation of Slides

The preparations were stained with 10% giemsa dye solution. The dried preparations were placed directly in the dye and left in solution for 11 minutes. The preparations were then removed from the dye solution, removed from distilled water in 3 separate wells, and removed from excess dyes and kept in a vertical position for drying. The dried slides were coated with the entellan and a permanent preparation was made. Following the these prosedures, slides were observed under a microscope. One hundred metaphases per culture were analysed for the presence of chromosomal aberrations (CA).The number of CAs was obtained by calculating the percentage of metaphases at each concentration and treatment period that showed structural or numerical chromosome aberrations. Chromosome fracture, chromatid fracture, fragment, polyploidy, dicentric chromosome brother chromatid association were screened at all treatment concentrations The observed chromosomal abnormalities were analyzed according to the International Human Cytogenetic Nomenclature (ISCN = International System for Human Cytogenetic Nomenclature) [16].

2.3 Determination of Mitotic Index (MI)

Mitotic index was calculated to determine the effects of *Vaccinium myrtillus* L. and *Rubus idaeus* L. plants on the mitosis of the cell cycle phases. A total of 2000 cells were observed from the prepared preparations of each group, and those from the metaphase stage were recorded. The percentage of metaphases was calculated and the mitotic index was determined.

2.4 Micronucleus Test

Micronucleus test developed by Rothfuss et al. It was modified and used [17]. The blood samples taken to determine the presence of micronuclei were grouped as described in the detection of chromosome aberrations. Blood samples were dropped into the cell culture medium and incubated at 37ºC for 72 hours. In the cell culture medium, 6 µg / ml (medium) of cytokalazine B agent was added to each tube 24 hours before at the end of the incubation period in order to induce the two nucleated cells. At the end of the incubation period (72 hours), the culture tubes were centrifuged at 1200 rpm for 10 min and the supernatants removed. Approximately 0.5-0.7 ml of the mixture containing the cells at the bottom of the tubes was thoroughly mixed by pipetting,

then KCL solution at 37° was added dropwise with 5 mL and with stirring. The tubes were closed and kept at 37°C for 20 min. The tubes were then heated at 1200 rpm for 10 min. centrifugation and supernatants removed. Cold fixative made of methanol:acetic acid (3:1) was added, such that the total volume of 5 ml was added slowly and with stirring in each tube. The fixative was obtained with 1/3 of acetic acid and methanol. The fixative treated cells were centrifuged at 1200 rpm for 10 min and the supernatants of the mixtures were removed. The fixative addition and centrifugation process was repeated 3 times. After the last centrifugation, the supernatant was removed in the bottom to approximately 0.5-0.7 mL of liquid. A homogeneous mixture was stirred by pipetting and about 4-5 drops of the pasteur pipette were vacuumed from this cell suspension. The suspension was dropped from this suspension at a certain distance onto the lamina and the cells spread on the slide were obtained. The obtained preparations were kept at 25°C for 24 hours to dry. The air-dried slides were stained for 10 minutes with 10% Giemsa stain (pH: 6.72) prepared in a Sorensen buffer. The dried preparations were coated with the entellan and the permanent preparation was formed.

2.5 Taking Pictures with a Microscope

Following the previous procedures, these slides were observed under binocular light microscope under 40 'objective. 2000 double-core cells were counted from each preparation and micronucleus-containing cells were determined and the percentages were calculated.

2.6 Calculation of Nucleus Division Index

The calculation of the nucleus cleavage index (NBI) was made according to the formula recorded by Fenech [18].

$$NBI = (1xN1 + 2xN2 + 3xN3 + 4xN4) / \text{Total Cell Count}$$

N1: a core,
N2: double core,
N3: Three cores and
N4: symbolizes the number of four-core cells. A total of 2000 cells were examined in each preparation to determine the NBI.

2.7 Calculation of Nuclear Cleavage Cytotoxicity Index

Calculation of Nuclear Cleavage Cytotoxicity Index (NSBI) was performed according to the formula recorded by Fenech [18].

$$NSBI = [Ap + Nec + M1 + 2 (M2) +3 (M3) +4 (M4)] / N$$

Ap: Apoptotic cell
Nec: Necrotic cell

2.8 Taking Pictures with a Microscope

In accordance with the purposes of the study, all photographs related to the cells, chromosomes and nuclei were obtained in a 10x100 magnification using an Olympus brand microscope.

2.9 Statistical Analysis

The statistical calculations of the obtained data were done by using software package program (SPSS 20.0 for Windows, IBM). Dunnett and Tukey tests were used to determine the differences between the groups.

3. RESULTS

Antimutagenic effects of Vaccinium myrtillus L. and Rubus idaeus L. extracts on chromosome aberration rates against MMC

The effects of different doses of *V. myrtillus* L. and *R. idaeus* L. extracts on aberration rates in human peripheral lymphocyte culture chromosomes were determined by evaluating chromosome fracture, chromatid fracture, fragment, polyploidy, dicentric chromosome and sister chromatid conjugation. When the extract groups were compared with the positive control, the difference was statistically significant ($P < 0.001$) and in the 0.2 µL /mL group with the lowest dose of *V. myrtillus*, it did not decrease the aberration rates according to the positive control group MMC group but in the other dose groups of both extract type aberrations decreased significantly (Table 1).

3.1 Effects of V. myrtillus L. and R. Idaeus L. extracts on Mitotic Index against MMC

It was determined that different doses of *V. myrtillus* L. and *R. idaeus* L. extracts increased significantly in human peripheral lymphocyte culture after 24 hours of administration compared to positive control group. The extracts of *V. myrtillus* L. were found to be more effective than the extracts of *R. idaeus* L. on MI ($P < 0.001$) (Table 2). A strong negative correlation between chromosome aberration rates including chromosome fracture, chromatid fracture, fragment, polyploidy, dicentric chromosome and

sister chromatid conjugation was also determined (P <0.01). At the dosing stage, when doses of *V. myrtillus* L. and *R. idaeus* L. were evaluated with mitotic index levels in 1 µg/mL MMC-treated human peripheral lymphocyte culture, doses in concentrations where intensifying effects on MI (for 0.2, 0.4, 0.8 and 1.6 µL / 5 mL media) were observed were included in the applications.

3.2 Effects of V. myrtillus L. and R. idaeus L. extracts on Micronucleus Index against MMC

The effects of *V. myrtillus* L. and *R. idaeus* L. extracts on micronucleus index were determined by taking micronucleus counts in nucleus and binuclear cells in cells after 24 hours of application in 2000 cells. According to this, the number of MN was significantly decreased with all doses of both plant extracts according to the positive control group MMC (P <0.001) (Table 3). However, these decreases were found to be more effective with doses of 0.8 and 1.6 µL /mL compared to 0.2 and 0.4 µL /mL doses. There was a positive correlation between the cell numbers of 2, 3 and 4 nucleus cells and the negative correlation between M1 values of 1 and 2 MN and cell numbers (P <0.001). There were strong negative correlation relationships between chromosome aberration rates including chromosome fracture, chromatid fracture, fragment, polyploidy, dicentric chromosome, and sister chromatid incorporation (P <0.001), including the same parameters as cell numbers 1 and 2, with 2 nucleus cells. chromosome aberration rates were found to be positively correlated with positive correlation (P <0.001).

Table 1. Effects of different doses of *V. myrtillus* L. and *R. idaeus* L. extracts on aberration rates in human peripheral lymphocyte culture chromosomes against MMC

Groups	Dose (24 h)	CF	Cf	F	P	DSC	SCC	Total (%)	± SEM (%)
Negative Control	-	0	1	1	0	0	0	2	0,07
MMC (µg/mL)	0.3	73	86	12	2	5	28	204	0,79
***V. myrtillus* (µL /mL)+ MMC (µg/mL)**	0,2	70	83	5	1	3	17	179	1,06*
	0,4	67	71	3	0	0	11	152	0,63*
	0,8	62	48	0	0	1	6	117	0,44*
	1,6	49	11	0	0	0	4	64	0,41*
***R. idaeus* (µL /mL) + MMC (µg/mL)**	0,2	67	76	1	0	1	9	154	0,45*
	0,4	63	64	0	1	0	10	138	0,50*
	0,8	50	45	0	0	0	3	98	0,33*
	1,6	36	11	0	0	0	4	51	0,41*

*CF: Chromosome fracture, Cf: Chromatid fracture, F: Fragment, P: polyploidy, DSC: Disentric chromosome SCC: Sister chromatid conjugation MMC: Mitomycin-C, ± SEM: Standard error of mean. .. *: P <0.001)*

Table 2. Effects of different doses of *V. myrtillus* L. and *R. idaeus* L. extracts on mitotic index in human peripheral lymphocyte culture against MMC

Groups	Dose (24h)	MI (% X)	± SEM (%)
Negative Control	-	7,37	0,07
MMC (µg/mL)	0.3	2,98	0,21
***V. myrtillus* (µL /mL) + MMC (µg/mL)**	0,2	6,23**	0,95
	0,4	5,14**	0,14
	0,8	5,56**	0,38
	1,6	6,71**	0,38
***R. idaeus* (µL /mL))+ MMC (µg/mL)**	0,2	3,95	0,10
	0,4	4,68*	0,19
	0,8	4,34*	0,14
	1,6	4,69*	0,15

*MMC: Mitomycine-C (positive control), MI: Mitotic index, X: Mitotic index percentage in 2000 cells, ± SEM: The standard error of mean, X * and X **: Compared to the positive control, the difference was statistically significant compared to the Tukey test (P <0.05 and P <0.001)*

Table 3. Effects of different doses of *V. myrtillus* L. and *R. idaeus* L. on micronucleus index in human peripheral lymphocyte culture against MMC

Groups	Dose (24 h)	Nucleus numbers in cells				MN Numbers in BN cells				MN/cell (%) ± SEM
		1	2	3	4	1	2	3	4	
Negative Control	-	725	1022	71	182	1	0	0	0	0,05±0,01
MMC (µg/mL)	0.3	1315	617	34	41	155	27	2	0	9,20±0,11
V. myrtillus	0,2	1121	719	69	93	19	6	1	0	1,30±0,05*
(µL /mL) +	0,4	1143	730	61	66	20	4	0	0	1,20±0,06*
MMC (µg/mL)	0,8	1157	734	51	56	6	1	0	0	0,35±0,03*
	1,6	1179	724	48	52	4	1	0	0	0,25±0,02*
R. idaeus	0,2	2602	752	63	88	24	7	0	0	1,55±0,05*
(µL /mL) +	0,4	1134	754	46	72	15	5	0	0	1,00±0,05*
MMC (µg/mL)	0,8	1175	724	46	55	3	0	0	0	0,15±0,01*
	1,6	1188	741	39	53	2	0	0	0	0,10±0,01*

*MMC. Mitomycin-C (Positive control), BN: Binuclear, MN: Micronucleus, SEM: Standard deviation of mean, X *: Compared to the positive control, the difference was statistically significant compared to the Dunnett test (P <0.001)*

3.3 The Effects of Extracts of *V. myrtillus* L. and *R. idaeus* L. on Nuclear Cleavage and Nuclear Cytotoxic Cleavage Index against MMC

Effects of *V. myrtillus* L. and *R. idaeus* L. extracts on NBI were evaluated by multiplying the numbers of 1, 2, 3 and 4 nucleated cells by 1, 2, 3 and 4, respectively. Their effects on NSBI were evaluated by adding apoptotic and necrotic cell numbers. The extracts of both NBI and NSBI from *V. myrtillus* L. and *R. idaeus* L. were found to be low in all dose groups compared to the negative control group and high in the positive control group. In the positive control group MMC group these levels were found to be lower than the negative control group (P <0.001) (Table 4). A strong positive correlation between both NBI and NSBI and MI, strong negative correlation between chromosome aberration rates including chromosome fracture, chromatid fracture and sister chromatid incorporation, strongly correlated with chromosome aberration rates including NSBI and chromosome fracture and sister chromatid incorporation. negative correlation relationships were determined (P <0.001).

Table 4. The effects of different doses of *V. myrtillus* L. and *R. idaeus* L. on the nuclear division and nuclear cytotoxic cleavage index in human peripheral lymphocyte culture against MMC

Groups	Dose (24 h)	NBI	AHS	NHS	NSBI
Negative Control	-	1,87±0,04	0	0	1,87±0,04
MMC (µg/mL)	0.3	1,41±0,04**	36	48	1,45±0,03**
***V. myrtillus* (µL /mL) +**	0,2	1,55±0,05*	8	13	1,56±0,05*
MMC (µg/mL)	0,4	1,56±0,03*	5	3	1,57±0,03*
	0,8	1,58±0,04*	4	3	1,59±0,04*
	1,6	1,54±0,04*	1	2	1,54±0,05*
R. idaeus	0,2	1,52±0,05*	8	12	1,53±0,04*
(µL /mL))+ MMC	0,4	1,55±0,04*	3	3	1,56±0,02*
(µg/mL)	0,8	1,53±0,03*	2	1	1,53±0,02*
	1,6	1,56±0,02*	1	0	1,56±0,02*

*MMC: Mitomycin-C (Positive control), NBI: Nuclear cleavage index, AHS: Apoptotic cell count, NHS: Necrotic cell count, NSBI: Nuclear cytotoxic cleavage index, SEM: Standard deviation of the mean, X * and X **: with negative control The difference compared to the negative control was statistically significant compared to the Dunnett test (P <0.01 and P <0.001, respectively)*

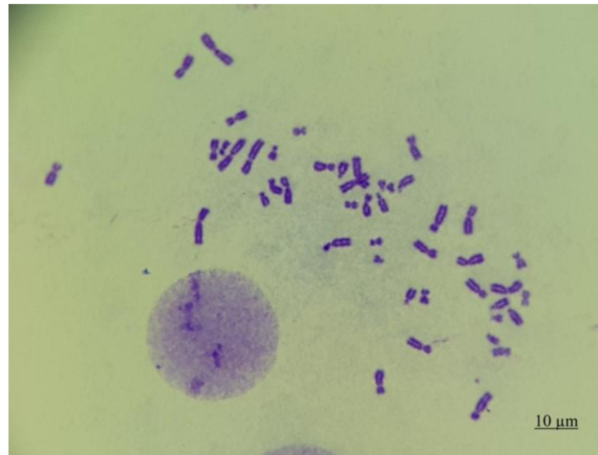

Fig. 1. Untreated human chromosome set (X1000)

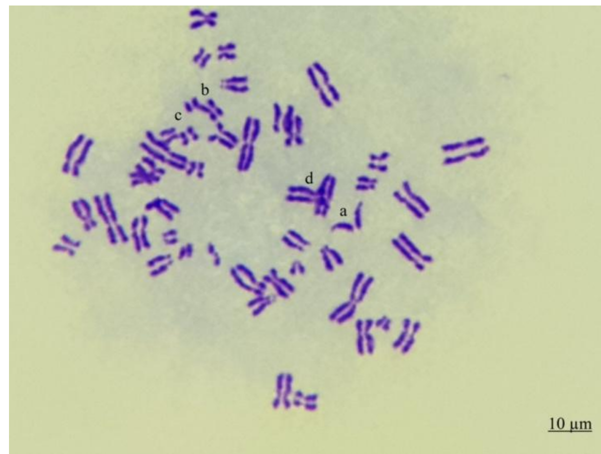

Fig. 2. MMC (0.3 µg/mL; positive control) intensive centromeric disruption, acentric fragment (a), chromatid break (b), chromatid fracture (c) and triradial chromosome formation (d) (X1000)

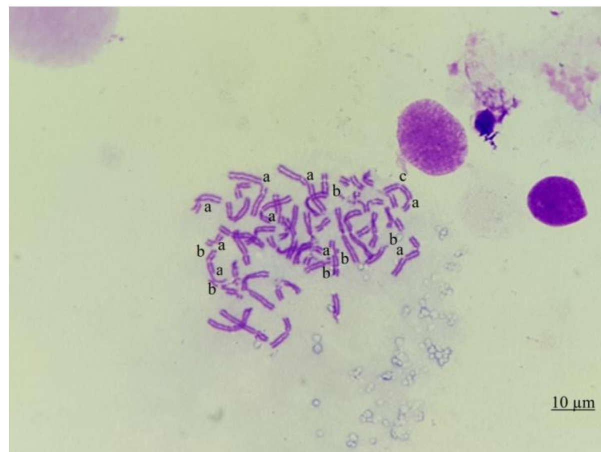

Fig. 3. *R. idaeus* extract (0,2 µL /mL) + MMC (0.3 µg/mL) single chromatid break (a), double isochromatid break (b) and centromere break (c) (X1000)

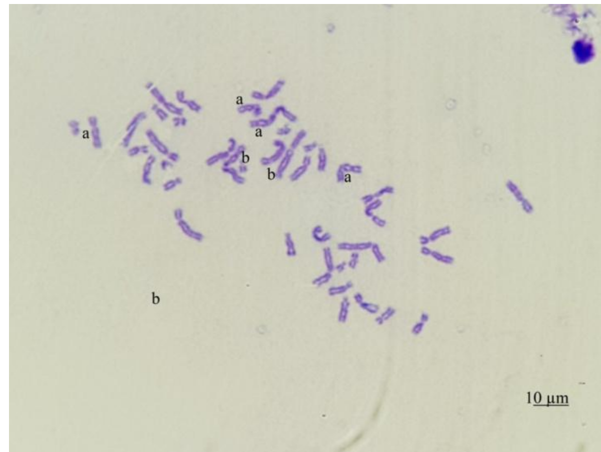

Fig. 4. *R. idaeus* extract (0,4 µL /mL) + MMC (0.3 µg/mL) dicentric chromosome (a), sister chromatid union (b) (X1000)

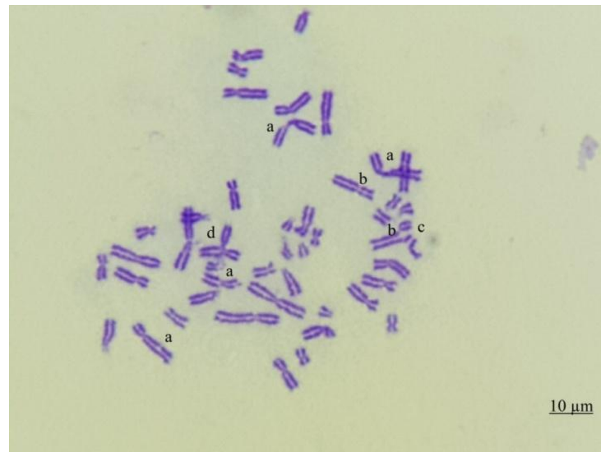

Fig. 5. *V. myrtillus* extract (0,2 µL /mL) + MMC (0.3 µg/mL) single chromatid break (a), double chromatid break (b), chromatid fracture (c) and triradial chromosome formation (d) (X1000)

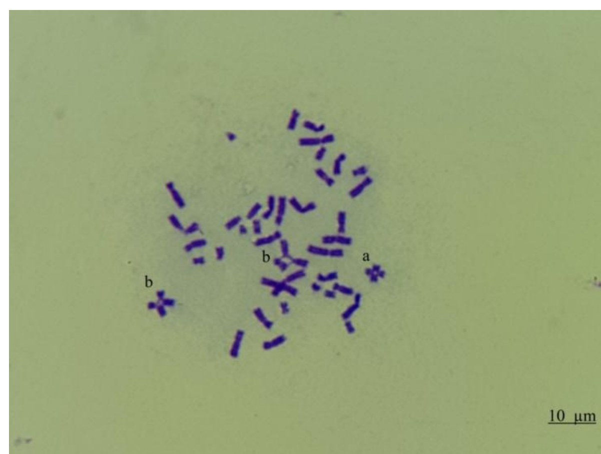

Fig. 6. *V. myrtillus* extract (0,2 µL /mL) + MMC (0.3 µg/mL) quatriradial chromosome formation (a), centromere elongation (b) (X1000)

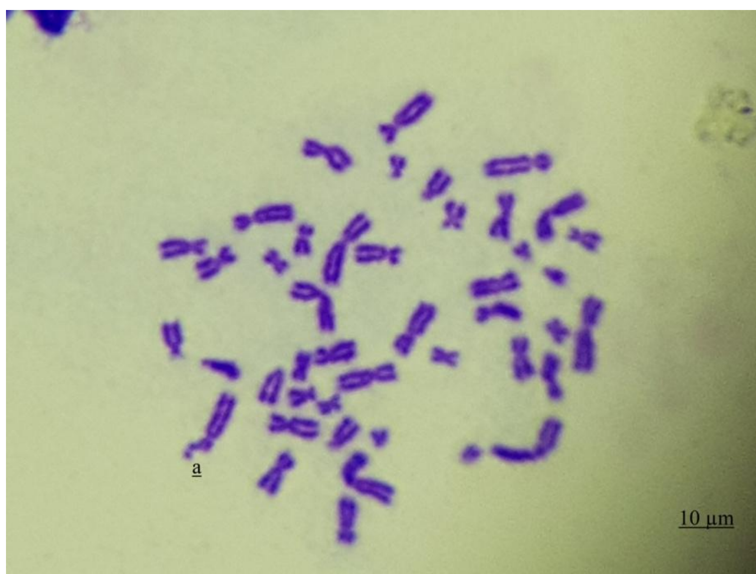

Fig. 7. ***V. myrtillus*** **extract (0,8 µL /mL) + MMC (0.3 µg/mL) a single broken (a) chromatid seen on a large chromosome of group A (X1000)**

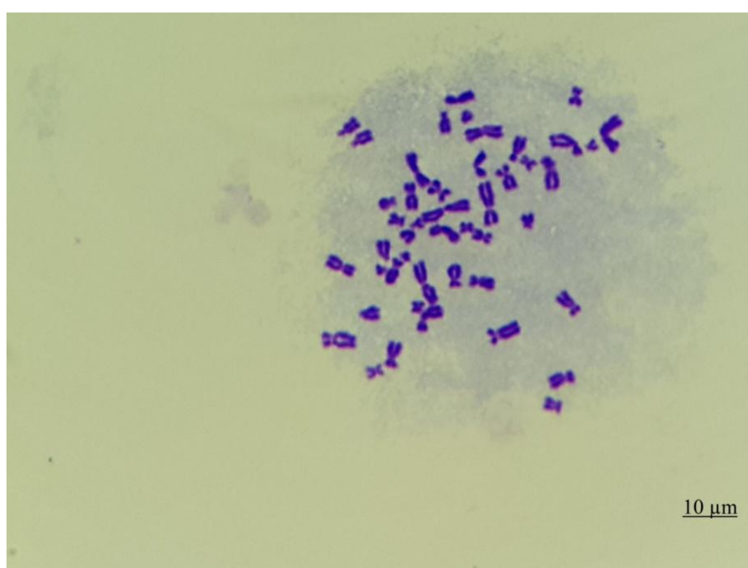

Fig. 8. ***V. myrtillus*** **extract (1,6 µL /mL) + MMC (0.3 µg/mL) chromosome set without any abnormality (X1000)**

4. DISCUSSION

It is believed that secondary metabolites may be important on the healing potential of some plants used in traditional drugs [19,20,21]. For this reason, it is very important that they are subjected to dose determination and genotoxicity tests in order to reveal the effects of health-related compounds on medicinal and aromatic plants on hereditary material. In this study, it was determined that plant extracts of *V. myrtillus* L. and *R. idaeus* L. significantly increased and maintained MI compared to the MMC group, which was evaluated as positive control (Table 2). It was determined that all of the plant extracts of *V. myrtillus* and *R. idaeus* increased the MI in 0.2, 0.4, 0.8 ve 1.6 µL /mL concentrations compared to the MMC group during experimental applications, and both extracts applied with 1.6 µL /mL concentration increased the MI more. In

the preliminary studies to determine the doses of both *V. myrtillus* L. and *R. idaeus* L. extracts, the fruits and leaf extract doses at the 0.2, 0.4, 0.8, 1.6, 2.4, 3.2, 4 ve 4,8 µL /mL concentration were tried, and in the trials of up to 1.6 µL /mL doses, there was an increase in the mitotic index (in the number of metaphases), but from 1.6 µL /Ml. There was a negative correlation between the dose increase and the mitotic index and an increase in chromosomal abeations. At a dose of 4.8 µL /mL, the chromosome medium was found to deteriorate shortly after dark brown coloration. In an *Allium cepa* plant cell division analysis conducted with a concentration of 400, 800 and 1200 µg/mL (For a 5 mL medium, 0.4, 0.8 and 1.2 µL / mL) of the aqueous leaf extract of *Rubus fruticosus* from the same family as *V. myrtillus* and *R. idaeus*, an accumulation has been reported in the prophase phase of mitosis and the MI decreased [22]. *R. idaeus* and *Rubus coriifolius* extract from the same family in a study comparing 1.5 and 3.0 mg / mL dose of cell culture in the 3.0 mg / mL dose of *Rubus coriifolius* extract has also been reported to show strong cytotoxic effects [23]. Therefore, in the study, it was found appropriate to undergo genotoxicity and cytotoxicity tests of 0.2, 0.4, 0.8 and 1.6 µL / mL.

The elagic acids obtained from *R. idaeus* show dose-dependent and apoptosis-inducing properties against CaCo-2 human adenocarcinoma colon cancer cell lines at doses ranging from 2.5 to 160 µg/mL and against positive control groups [24]. In one study, *R. idaeus* extracts have been shown to be very effective in reducing the volume of tumors and tumor cell proliferation by inducing apoptosis, and the fact that *R. idaeus* plant fruit extract can be a potential anticancer drug because it has a cytotoxic effect in HepG2 and L20B cancer cell lines [25]. In another study, polyphenolic profile and antioxidative properties of *R. idaeus* leaf extract were determined and investigated for potential biological activity. The effects of cytotoxicity, antioxidative/proximal effect and total glutathione concentration on human laryngeal carcinoma (HEp2) and colon adenocarcinoma (SW480) cell lines were investigated. SW480 cells are more susceptible to *R. idaeus* leaf extract compared to HEp2 cells. Unlike SW480 cells, it was observed that *R. idaeus* leaf extract induced antioxidative structure induced the formation of reactive oxygen species in HEp2 cells treated with hydrogen peroxide. *R. idaeus* leaf extract increased the level of total glutathione in HEp2 cells. This effect was detected after a 24-hour improvement, and as a result, it is thought that the compounds present during the cellular metabolism of the compounds present in the extract are caused. The results obtained from both cell lines confirm the biological activity of *R. idaeus* leaf polyphenols and this suggests that the traditional plant can support the daily intake of valuable natural antioxidants that show beneficial health effects [26]. The other study, *R. idaeus* and strawberry extracts were evaluated for anticancer activities in baseline stages of initiation, progression and invasion using *in-vitro* colorectal cancer models; at the physiologically relevant dose range (µg/mL gallic acid equivalent 0-50 µg) showed significant antitoxic, antimutagenic and antiinvasive activity on cancer cells in the colony. This study also demonstrates that phenolic compounds obtained from *R. idaeus* extract undergo significant structural modifications during the passage of the gastrointestinal tract, but that they retain biological activities of degradation products and metabolites and can modulate cellular processes associated with colon cancer. Again, *R. idaeus* fruit extract maintained its activity after fermentation and significantly inhibited the invasion of HT115 cells [27]. In another study, the mutagenic potential of the *R. idaeus* acetone extract was investigated *in-vitro* using the ames test, and according to the results obtained, *R. idaeus* acetone extract did not show any mutagenic effect or cytotoxicity against CaCo-2 cells [28]. It is also reported that acetone as a solvent does not have toxicity in the negative control group consisting of certain doses of lymphocytes in an *in-vitro* antimutagenicity study [29], using a comet analysis method *R. niveus* extract (500, 1000 and 2000 mg / kg) showed no genotoxic and mutagenic effects [30]. The results described above are consistent with the results obtained from our study that confirmed the absence of genotoxic, mutagenic and cytotoxic effects for *R. idaeus*. However, also reported an increase in the frequency of MN after treatment with *R. imperialis* extracts (250 and 500 mg / kg) [31]. In our study, it was determined that R. idaeus extract increased the mitotic index, nuclear division and nuclear cytotoxic index in the group of MMC given to mutagen in human peripheral lymphocyte cells (Tables 1-4). R. idaeus extract was found to decrease chromosomal aberration rates, micronucleus index, apoptopic cell and necrotic cell numbers in, human peripheral lymphocyte cells according to the MMC group known as mutagen. Microscopic examination showed that intensive

asentric chromosome, chromatid separation, resiprocal translocation, chromatid fracture and ring chromosome status were decreased with *R. idaeus* extract in MMC group (Figs. 1-4). When all parameters are considered, it can be suggested that R. idaeus extract has an important antimutagenic effect at administered doses.

In addition to the effects of *V. myrtillus* on the reduction of ROS's involved in the pathogenesis of many diseases, including cancer, there is much evidence that it prevents the progression of cancer in particular. One of these possible mechanisms is to prevent their growth by inducing apoptosis of cancer cells. Anticancer properties are associated with bioactive phytochemicals, particularly polyphenols [32]. In a study aimed at inducing apoptosis in cancer cells, extract of *V. myrtillus* was found to be effective on inhibition of growth of *in vitro* HCT116 human colon cancer cells and HL60 human leukemia cells. *V. myrtillus* extract was shown to induce nucleosomal DNA degradation and apoptotic cells in HL60 cells, and the ratio of *V. myrtillus* extract-induced apoptotic cells in HCT116 cells was significantly lower than HL60 cells, and the reason for the inhibitory effect on the growth of HL60 and HTC116 cells was found mainly in its content and the anthocyanidins delfinidine was bound to the phenolic compound and glycosides [5]. *V. myrtillus* has been shown to have many protective roles in the maintenance of healthy life in humans due to cardiovascular disorders, increased age-induced oxidative stress, inflammatory response and various biomedical effects in degenerative diseases, as well as initiating genetic signals against diseases. *V. myrtillus*, an anthocyanin-rich fruit, is commercially available as a pharmaceutical preparation for the treatment of both ophthalmic diseases and blood vessel disorders. The anthocyanin components exhibit high *in-vitro* and *in-vivo* antioxidative capacity and also inhibit low-density lipoprotein oxidation [33]. It is reported that against cisplatin-induced oxidative stres and DNA damage consumption of 200 mg/kg bilberry for 10 days, effective on antioxidant enzyme systems and MDA level and significantly decrease the comets. These results show that bilberry is capable of preventing genotoxic and cytotoxic damage caused by cisplatin in peripheral blood cells in rats [34]. V. myrtillus extract has been reported to have cytoprotective effect in various models in vivo. After oral administration of V. myrtillus in 50, 100 and 200 mg / kg mice for 5 days, it has been reported that remarkably decreased plasma alanine amino transferase (ALT) level and thus alleviated stress-induced liver damage. In addition, bilberry extracts increased glutathione (GSH) and vitamin C levels and significantly decreased malondialdehyde (MDA) and nitricoxide (NO) levels in the liver tissues. These results suggest that bilberry extract plays an important role in protecting against restraint stress-induced liver damage by both scavenging free radicals activity and lipid peroxidation inhibitory effect [35]. Cardiovascular disease is characterized by an increased proatherogenic state and highlevels of circulating microvesicles. In a study conducted with patients with myocardial infarction, blueberry extract was added to the diet of patients for 8 weeks, and it was found that the endothelium-derived microvesicle levels in their blood were improved by measuring flow cytometry at the end of the period. In addition, they examined the effect of blueberry extract on *in vitro* endothelial vesiculation with western blot and qRT-PCR and found that both platelet-derived micro particles and endothelial-derived microparticles decreased, and they brought a new perspective to the cardioprotective effect of blueberries [36]. In another study in which the protective effects of anthocyanin-richblueberry extracts at 50 mg / L and 100 mg / L doses against the toxicity of 20 µM copper (II) chloride (CuCl2) on *Allium cepa* L., micronucleus (MN) as cytogenetic parameters, mitoticindex (MI) and chromosomal abnormality (CA) frequency were studied. As a result, they found that blueberry extract decreased germination percentage, weight gain, root length and MI ratio against CuCl2 application, and increased the frequency of micronucleus (MN) and chromosome aberration (CA) [37]. In our study, it was found that *V. myrtillus* extract increased the number of metaphase count, mitotic index, nuclear division and nuclear cytotoxic cleavage in human peripheral lymphocyte cells compared to the MMC group which was known to be mutagen (Tables 1-4). It was determined that *V. myrtillus* extract reduced the number of chromosomal aberration rates, micronicleus index, apoptotic cell and necrotic cell numbers in human peripheral lymphocyte cells compared to the MMC group known to be mutagen. Microscopic examination showed that intensive asentric chromosome, chromatid cleavage, resiprocal translocation, chromatid fracture, and ring chromosome status decreased in the MMC group (Figs. 1, 2, 5-8). Considering all parameters and previous genotoxicity and cytotoxicity studies, it was concluded that *V.*

myrtillus extract had a significant antimutagenic effect at the doses administered.

5. CONCLUSION

As a result of our study, it has been found that blueberry and raspberry liquid extracts reduce chromosome aberration rate and micronucleus formation against MMC known to be mitogen. Mitotic, nuclear division and nuclear cytotoxic cleavage indices were found to be increased. The antimutagenic and antigenotoxic effects of the extract doses were found to be significant in light of all these datas. In addition, it was concluded that further studies should be done on whether these plants should be consumed more than doses used in study.

CONSENT

It is not applicable.

This study was produced from the first author's doctoral thesis prepared under the supervision of the second author.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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