In vitro genotoxic and antigenotoxic effects of delphinidin chloride on human peripheral blood lymphocytes

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ABSTRACT: Delphinidin is an anthocyanidin which is found in fruits and vegetables as a primary plant pigment used for various purposes. The purpose of this study was to investigate the in vitro genotoxic and antigenotoxic effects of delphinidin chloride (DC) in human peripheral blood lymphocytes. Final concentrations of 25 µM, 50 µM, 75 µM and 100 µM of DC were tested for 24 or 48 hours treatment periods. For detection of possible antigenotoxic potential of DC, human peripheral lymphocytes were co-treated with DC and a known clastogenic agent mitomycin-C (MMC). Genotoxic and antigenotoxic effects of DC were determined with the chromosome aberration (CA) and micronucleus (MN) tests. Cytotoxic effect of DC was determined by measuring the nuclear division index (NDI) and mitotic index (MI). Additionally, total oxidant and antioxidant values were determined by a spectrophotometric method. CA variations resulting from DC treatments did not reveal statistical significance as compared with controls. In tubes treated with DC and MMC together, DC significantly decreased the CA frequency caused by MMC (P≤0.01). This decrease was almost 50% as compared to the positive control MMC. In this study, DC alone did not lead to the CA and MN formation in all culture tubes. DC did not cause a significant oxidative stress. DC has an antigenotoxic effect against the mutagenic effects of MMC.

KEYWORDS: Delphinidin chloride, genotoxicity, antigenotoxicity, chromosome aberration, oxidative stress.

1. INTRODUCTION

Anthocyanins, which was derived from the Greek words (Anthos, meaning flower, and kyanos meaning blue) are composed of water-soluble pigments that change colour to red, purple and blue depending on the pH. Anthocyanins belong to a main group of flavonoids, which are biologically synthesized by phenylpropanoid pathway. They are almost tasteless and odorless, giving a sensation of slightly bitter feeling. Anthocyanins can be present in all tissues of higher plants, however, they are particularly found in leafs, stems, roots, flowers and fruits.

Anthocyanins are in a class of polyphenols and it protects us from adverse effect of many agents (chemical, UV, etc.) [1]. Flavonoids are the most common group of polyphenolic plant compounds and a large number of these molecules exist in most of the tissues of many plants [2]. DC (E163b) (2-(3,4,5-trihydroxyphenyl) chromenylium-3,5,7-triol), the test substance of this study has a polyphenolic diphenylpropan-based ring structure and it is available in the epidermal tissue layer of the flower [3].

In addition to the above mentioned features of Delphinidin, cytotoxic, mutagenic, apoptotic and oxidative effects of Delphinidin on healthy or cancer cell lines are remarkable. One study has indicated that although Delphinidin was a weak mutagen according to DNA repair test, it did not significantly induce point or frameshift mutations. In addition, it mildly increased the frequency of mutations in the D5 strain of Saccharomyces cerevisiae. However, in V79 Chinese hamster cells, it has been demonstrated that Delphinidin is an effective inducer of micronuclei. On the other hand, addition of S9 mix hardly caused any effect on the induction of micronuclei [4]. It was expressed that Delphinidin might be partially effective on preventing the tumor invasion process in a way by inhibiting the activities of matrix metalloproteinase MMP-2 and MMP-9 enzymes which are secreted by fibrosarcoma (HT-1080) cells and degrade extracellular matrix [5]. In another study performed by Meiers et al. [6], it was found that delphinidin-rich foods inhibit the in vitro development of human tumor cells (epidermoid carcinoma) at micromolar levels. It was found that pure form of delphinidin, which is isolated from blueberry extract, induced apoptosis in HL60 (human promyelocytic leukemia) cells and inhibited growth of human colon carcinoma (HCT116) cells [7].

Delphinidin is an antioxidant, which belongs to the group of anthocyanin basic plant pigments [3]. It is a colorant, which gives blue hues in flowers of *Viola tricolor* (pansy) and delphinium (larkspur, palace flower). At the same time, it

gives the blue-red color in fragrant grapes, which are used in the production of Cabernet Sauvignon wines [8]. Short-term genotoxicity tests are generally preferred to describe genotoxic, anti-genotoxic and/or cytotoxic effects of plant extracts, food additives, food colorings or any drug substances [9, 10, 11, 12, 13].

Therefore, this study was conducted with the intention of investigating the in vitro genotoxic and antigenotoxic effects of DC, which is the main compound of dye-stuff of various plants, on human peripheral blood lymphocytes. For this purpose, genotoxic and antigenotoxic effects of DC were determined using the CA and MN tests. Total oxidant and total antioxidant status (TOS and TAS) values were also determined in order to measure the oxidative stress.

2. RESULTS

CA were determined with human peripheral lymphocytes treated with increasing concentrations (25 µM, 50 µM, 75 µM and 100 µM) of DC for 24 or 48 hours treatment periods. Most common chromosomal abnormalities were chromatid type (B') breaks (Figure 1). This was followed by chromosome type breaks (B''), chromatid exchange (CE) (Figure 2), chromatid type fragment (F'), chromosome type fragment (F''), sister union (SU) and translocation (T) abnormalities.
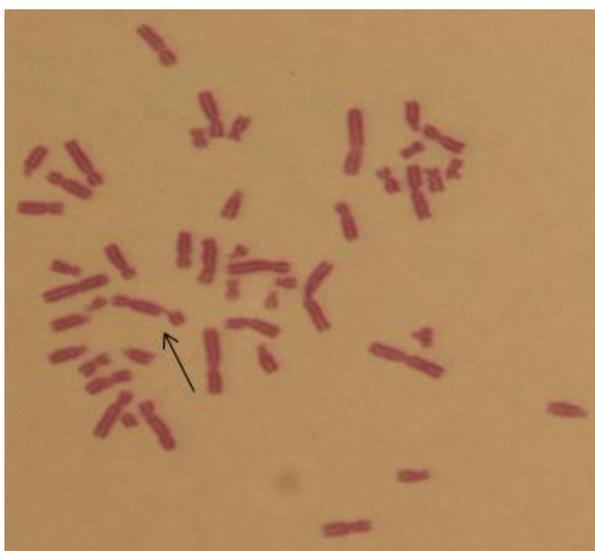

Figure 1. Chromatid type fracture in human peripheral lymphocytes treated with Delphinidin chloride and MMC (x1000) (100 µM, 48h, ♂)
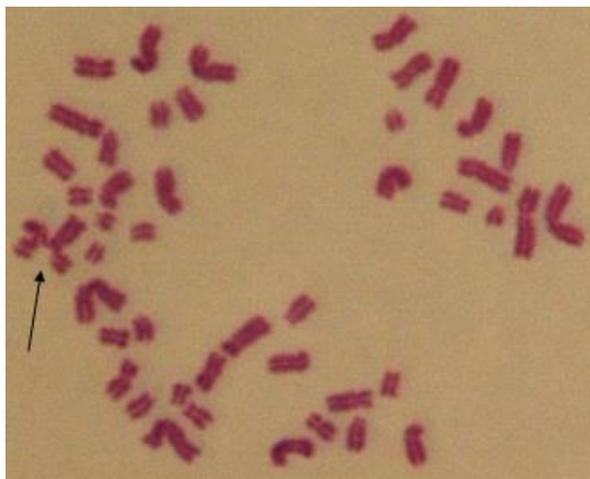
Figure 2. Chromatide exchange in human peripheral lymphocytes treated with Delphinidin chloride and MMC (x1000) (100 µM, 48h, ♂)

In cultures, there was no significant difference (P>0.05) between CA findings obtained from control and solvent control (DMSO) and chromosomal abnormality (CA) findings occured by the clastogenic effect of DC.

We have observed that the ratios of CA per cells were not affected from DC as in the CA results. CA variations resulting from DC treatments did not reveal statistical significance as compared with controls (Table 1).

Table 1. Chromosome Aberration* (CA) and cytotoxicity** indexes caused by Delphinidin chloride in human peripheral lymphocyte culture

Test substance	Time (h)	Conc. (µM)	CCA±SE	CA/Cell±SE
Control	-	0	1.5±0.5	0.015±0.01
DMSO	24	3 µL	2.5±1.5	0.025±0.02
MMC	24	0.75	30.5±5.5	0.425±0.03
	24	25	$0.5±0.5c_3$	$0.050±0.05c_3$
DC	24	50	$1.5±0.5c_2$	$0.015±0.01c_3$
	24	75	$4.0±1.0c_2$	$0.040±0.01c_3$
	24	100	$1.5±1.5c_2$	$0.015±0.02c_3$
DMSO	48	3 µL	1.5±0.5	0.015±0.01
MMC	48	0.75	47.5±7.5	0.995±0.03
	48	25	$2.5±0.5c_3$	$0.025±0.01c_3$
DC	48	50	$4.0±1.0c_3$	$0.040±0.01c_3$
	48	75	$2.5±1.5c_3$	$0.040±0.03c_3$
	48	100	$2.0±0.0c_3$	$0.025±0.01c_3$
MMC	24	0.75	30.5±5.5	0.425±0.03
DMSO+ MMC	24	3µL+0.75 µM	33.0±3.0	0.465±0.06
	24	25	$15.5±0.5c_2d_2$	$0.165±0.01c_1d_2$
DC+ MMC	24	50	$16.5±0.5c_2d_2$	$0.215±0.03d_2$
	24	75	$13.5±2.5c_2d_2$	$0.155±0.04c_1d_2$
	24	100	$16.0±2.0c_2d_2$	$0.185±0.03c_1d_2$
MMC	48	0.75	47.5±7.5	0.995±0.03
DMSO+ MMC	48	3µL +0.75 µM	39.0±1.0	0.310±0.04
	48	25b	43.0±27.0	1.060±0.89
DC+ MMC**	48	50	43.5±1.5	0.570±0.01
	48	75c	64.0±21.0	0.560±0.13
	48	100d	25.5±16.5	0.445±0.36

*   In total. 200 well-spread metaphases were evaluated.
** Cytotoxic effect (a:56. b: 124. c:175. d: 111 metaphases were evaluated). a: Significant from control; b: Significant from solvent control; c: Significant from MMC (positive control); d: Significant from solvent control+ MMC. $a_1b_1c_1$: P≤0.05; $a_2b_2c_2$: P≤0.01; $a_3b_3c_3$: P≤0.001

In tubes treated with DC and MMC together (24-hour treatment period), DC significantly decreased the CA frequency caused by MMC (P≤0.01). This decrease was almost 50% as compared to the positive control MMC. In addition, CA/Cell frequency displayed a similarity with the frequency of abnormal cells. When compared to the control and solvent control, there was no statistical significance found in abnormal cells or CA/Cell frequency at all concentrations treated for 48 hours (Table 1) in DC treated groups.

Different sizes and number of micronuclei (MN) formation (Figure 3) were detected in human peripheral lymphocytes treated with different concentrations (25 µM, 50 µM, 75 µM and 100 µM) of DC for 24 or 48 hour periods. We also found that the test substance did not significantly increase MN formation as compared to control and solvent control (DMSO) for 24 or 48-hour treatment periods (Table 2).
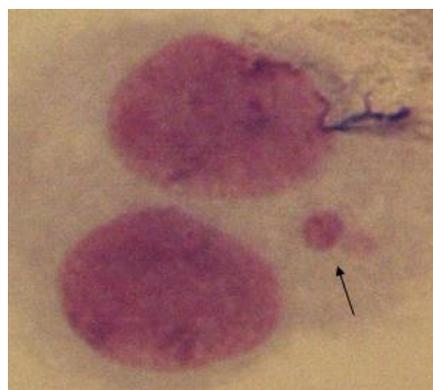

Figure 3. Micronucleus formation in human peripheral lymphocytes treated with positive control (MMC) (x1000) (0,25 µL/mL, 48h, ♀).

To determine the antigenotoxic effect of DC, 0.75 µM MMC was added to tubes that were also treated with the test substance. In this comparison, significant decreases in micronuclei frequency were observed only at 50 and 75 µM concentrations of DC for 48 h treatment as compared with MMC (Table 2).

To sum up the results, test substance did not significantly increase micronuclei formation, which is almost similar to CA findings. However, antigenotoxic potential of DC was detected at 50 and 75 µM concentrations for 48 hour treatment.

Table 2. Micronucleus (MN) and cytotoxicity* indexes caused by Delphinidin chloride in human peripheral lymphocyte culture

Test substance	Time (h)	Conc. (µM)	‰MN±SE	MI±SE	NDI±SE
Control	-	0	1.5±0.5	4.62±0.19	1.578±0.074
DMSO	24	3 µL	4.0±3.0	4.07±0.59	1.559±0.059
MMC	24	0.75	14.0±1.0	1.90±0.57	1.393±0.020
DC	24	25	$1.5±0.5c_2$	4.26±1.30	1.531±0.004
	24	50	$1.5±1.5c_2$	3.96±0.60	1.562±0.157
	24	75	$3.0±3.0c_1$	2.83±0.13	1.670±0.038
	24	100	$2.0±1.0c_2$	3.20±0.17	1.465±0.032
DMSO	48	3 µL	1.5±0.5	2.85±0.79	1.509±0.089
MMC	48	0.75	53.5±26.5	1.13±0.07	1.227±0.081
DC	48	25	$0.5±0.5c_3$	$4.75±1.18c_1$	$1.511±0.014c_1$
	48	50	$3.5±1.5c_3$	3.35±0.82	$1.563±0.080c_1$
	48	75	$2.5±0.5c_3$	3.05±0.29	$1.497±0.019c_1$
	48	100	$2.5±0.5c_3$	3.87±0.37	1.435±0.026
MMC	24	0.75	14.0±1.0	1.90±0.57	1.393±0.020
DMSO+ MMC	24	3µL+0.75 µM	16.5±0.5	1.58±0.15	1.477±0.010
DC+ MMC	24	25	11.5±10.5	2.15±0.42	1.426±0.007
	24	50	20.0±0.0	2.16±0.30	1.483±0.001
	24	75	10.5±9.5	1.93±0.23	1.549±0.107
	24	100	7.5±1.5	2.70±0.47	1.438±0.011
MMC	48	0.75	53.5±26.5	1.13±0.07	1.227±0.081
DMSO+ MMC	48	3µL+0.75 µM[a]	$190.5±3.5a_3$	0.30±0.00	1.231±0.003
DC+ MMC**	48	25	$55.5±33.5d_1$	0.85±0.35	1.150±0.007
	48	50	$33.0±30.0d_1$	1.26±0.30	1.146±0.002
	48	75[b]	$24.0±20.0d_1$	1.10±0.06	$1.073±0.050 d_1$
	48	100	$72.0±37.0a_1$	0.72±0.32	1.133±0.030

*   In total. 2000 well-spread interphase were evaluated.
**   Cytotoxic effect (a:1841. b: 1610. binuclear cell were evaluated). a: Significant from control; b: Significant from solvent control; c: Significant from MMC (positive control); d: Significant from solvent control+ MMC. $a_1b_1c_1$: P≤0.05; $a_2b_2c_2$: P≤0.01; $a_3b_3c_3$: P≤0.001

NDI values in cultures treated only with DC did not show any difference when compared to the control or solvent control (P>0.05). In cultures co-treated with DC and MMC, reduction in NDI were not statistically significant except at 75 µM concentration for 48-hour treatment period (P≤0.05). Similar results were also obtained for MI (Table 2).

TOS were determined by spectrophotometric measurements of supernatants of 72-hour CA cultures. Decreases in TOS were significantly higher than control levels at highest concentration (100 µM) in 24 and 48-hour treatment periods (P≤0.01). Moreover, the value measured at the highest concentration for 48 hour treatment period was higher than positive control. At other concentrations, TOS values were found equal to the level of controls.

In cultures treated with DC and MMC, TOS value measured at highest concentration for 24 hour treatment was statistically higher than the control (Solvent control + Positive control) (P≤0.05). Other variants remained in control levels with slight increases. All the obtained TOS values in 48 hour treatment period were statistically significant at several levels. In this period, the highest increases were observed also at 100 µM concentration (Table 3).

Test substance significantly increased total antioxidant status at all concentrations for both treatment periods (24 and 48 h). In cultures treated only with DC for 24 hours, TAS values were significantly higher than each of the controls (except the lowest concentration). As compared with their controls, TAS values exhibited very significant increase in cultures treated with both test substance and positive control (MMC).

DC did not cause a significant oxidative stress. Unlike DMSO, DC reduced the oxidative stress. OSI values measured in cultures treated with DC and MMC did not show significant differences as compared to their controls (Table 3).

Table 3. Effect of the delphinidin chloride (DC) to oxidative stress in human cultured lymphocytes for 24 or 48-hour treatment periods

Test substance	Time (h)	Conc. (μM)	TOS ± SE	TAS ± SE	OSI ± SE
Control	-	0	3.891±0.58	0.593±0.015	6.545±0.80a_3
DMSO	24	3 μL	6.165±0.39	0.558±0.036	11.14±1.42
MMC	24	0.75	4.193±0.70	0.588±0.024	7.101±0.90
DC	24	25	5.000±1.05	0.709±0.018b_1c_1	7.020±1.31
	24	50	4.841±0.76	0.902±0.023$a_3b_3c_3$	5.350±0.70b_1
	24	75	5.838±0.76	1.230±0.021$a_3b_3c_3$	4.737±0.54b_1
	24	100	7.694±0.81a_2	1.931±0.026$a_3b_3c_3$	3.981±0.37b_2
DMSO	48	3 μL	5.808±0.11	0.558± 0.015	10.420±0.09a_3
MMC	48	0.75	3.928±0.66	0.561±0.015	6.975±0.98
DC	48	25	4.982±0.74	0.736±0.040	6.769±0.64b_1
	48	50	4.852±0.83	0.844±0.017$a_3b_1c_1$	5.735±0.87b_2
	48	75	5.789±0.85	1.135±0.043$a_3b_3c_3$	5.081±0.56b_2
	48	100	7.725±0.13a_2c_1	1.816±0.077$a_3b_3c_3$	4.266±0.25b_2
MMC	24	0.75	4.193±0.70	0.588±0.024	7.101±0.90
DMSO+ MMC	24	3μL+0.75 μM	3.194±0.97	0.377±0.083a_2	9.510±4.67a_2
DC+ MMC	24	25	5.341±0.58	0.780±0.003$a_1c_2d_2$	6.854±0.77
	24	50	4.716±0.55	0.937±0.018$a_3c_3d_3$	5.048±0.67
	24	75	6.072±0.86	1.239±0.015$a_3c_3d_3$	4.893±0.64
	24	100	7.236±0.64a_1d_1	1.923±0.045$a_3c_3d_3$	3.758±0.24
MMC	48	0.75	3.928±0.66	0.561±0.015	6.975±0.98
DMSO+ MMC	48	3μL+ 0.75 μM	1.584±0.07	0.378±0.048a_2	4.237±0.34
DC+ MMC**	48	25	4.644±0.86d_1	0.755±0.013$a_1c_2d_3$	6.140±1.04
	48	50	5.324±0.49d_2	0.871±0.012$a_3c_2d_3$	6.105±0.48
	48	75	5.762±0.42d_2	1.148±0.002$a_3c_2d_3$	5.020±0.37
	48	100	7.539±0.36$a_1c_1d_3$	1.803±0.048$a_3c_2d_3$	4.190±0.31

a: Significant from control; b: Significant from solvent control; c: Significant from MMC (positive control); d: Significant from solvent control+ MMC.
$a_1b_1c_1$: P≤0.05; $a_2b_2c_2$: P≤0.01; $a_3b_3c_3$: P≤0.001

3. DISCUSSION

In this study, DC, the test substance was a blue-purple dyestuff found in flowers and fruits, and some other organs of plants. The lack of data on the genotoxic effects of this compound led us to perform this study. According to our results, DC did not induce chromosome damage in cells at any concentration for any treatment periods. In addition, it reduced the genotoxic effects of MMC, which is a well-known clastogen. Moreover, it increased the antioxidant status and reduced the oxidative stress significantly as comparison to the solvent control. Similar to our findings, it has been reported that various anthocyanins similar to delphinidin exhibited protective effects against DNA damage caused by tert-butyl-hydroperoxide (TBHP) [26]. In another study, it has been shown that purified delphinidin, extracted from eggplant (*Solanum melanogena*), decreased the micronuclei frequency caused by cyclophosphamide in polychromatic erythrocytes of mice (10 and 20 mg/kg b.w.). However, delphinidin did not present any mutagenic or genotoxic effect in mice bone marrow and peripheral blood cells [27]. However, delphinidin was cytotoxic in metastatic colorectal cancer cell lines (LoVo and LoVo/ADR) according to Cvorovic *et al*. [15]. Singletary *et a*l. [28] have reported that 0.6 μM concentration of delphinidin significantly blocked the formation of DNA adducts caused by benzo[a]pyrene (BP) in non-cancerous (benign) human mammary epithelial cell line. Fritz *et al*., [29] have revealed that delphinidin has protective effects against oxidative DNA damage caused by menadione in human colon carcinoma cells (HT29), which also supports our findings. However, in the same study, delphinidin did not display antioxidant effect in the absence of catalase. In addition, it has been found that delphinidin exhibits growth inhibitory activity in breast cancer cells of various molecular subtypes [30]. In another study, it has been revealed that delphinidin was protective against the effects of topoisomerase II poison on DNA in a

concentration-dependent manner *in vitro* [31]. The protective effects of delphinidin against DNA damage caused by cyclophosphamide have been also reported by other researchers [32].

A large number of in vitro animal models showed that flavonoids modulate important cellular and molecular mechanisms related to carcinogenesis also epidemiological studies have affirmed that, among many flavonoids, delphinidin appears to be an efficacious compound seen in a diverse manner in different stages of carcinogenesis [33]. Domitrović and Jakovac [34] proposed that delphinidin revealed therapeutic effects of in CCl(4)-induced liver fibrosis by inciting extracellular matrix degradation. Delphinidin exerts HATi activity against anti-inflammatory signaling by blocking p65 acetylation, which is a compound that may prevent inflammatory arthritis [35]. Moreover, the photoprotection of delphinidin against the adverse effects of UV radiation has been reported by Afaq and Katiyar [36].

4. CONCLUSION

In conclusion, the results of this study indicate similarities with the findings of other studies. In this study, we have found some evidence that DC has antigenotoxic and antioxidative potential. Furthermore, it was determined that the test substance had no significant cytotoxic or proliferative effect in human peripheral lymphocytes. Therefore, this study suggests that DC can be used as a important protective agent against tumor growth. Additionally, *in vivo* studies are warranted to determine the optimal antigenotoxic concentrations.

5. MATERIALS AND METHODS

5.1. Chemical Substances

DC was purchased from Cayman Chemical Company (*CAS* No: *528-53-0*) (Figure 4). The molecular structure of DC is illustrated in Figure 4. Purity of DC was ≥ 97%. Dimethyl sulfoxide (DMSO) (CAS No. 67-68-5. Merck, Darmstadt, Germany) was used as solvent control. Gibco PB-MAX Karyotyping Medium was used for the short-term culture of peripheral blood lymphocytes for cytogenetic studies (Gibco, CAT No 12557-013). Colchicine (CAS No. 64-86-8), cytochalasin B (Sigma-Aldrich CAS No. 14930-96-2) and MMC (Sigma CAS No: 50-07-7) were purchased from supplier companies.

5.2. Experimental Design

This study was carried out with the confirmation of Cukurova University Ethics Committee (33/10). Human blood samples were supplied from two healthy volunteers who are non-smokers, and have no alcohol and drug use, one male and one female (n=2), whose ages are 22 and 24, respectively and treated with four different final concentrations of DC (25 μM, 50 μM, 75 μM, 100 μM). DMSO was used as a solvent control (3 μl/2.7 ml chromosome medium) in two different treatment periods (24 or 48 h).
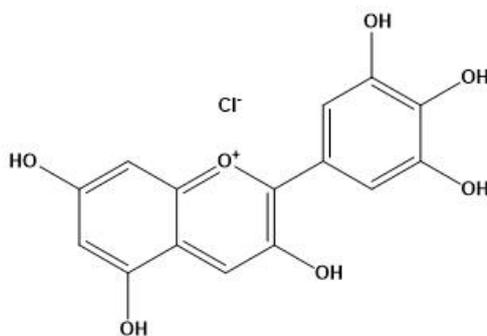

Figure 4. Molecular structure of delphinidin chloride

5.3. Chromosome Aberration (CA) Test

1/10 heparinized peripheral blood (0.2 ml) was added to each tube of 2.5 ml PB-max chromosome medium [14]. Cells were cultured in an incubator at 37 °C for 72 hours. Four non-toxic concentration (25 μM, 50 μM, 75 μM ve 100 μM) were chosen according to Cvorovic *et al*. [15]. Delfinidin chloride was dissolved in DMSO and used max 3 μL for 2.7 ml medium. To reveal genotoxic effects of Delfinidin chloride, the cultures were treated with Delfinidin chloride during 24 or 48 hours after the start of incubation (24 or 48 hour treatment periods). In addition, solvent control (DMSO) and positive control (MMC, 0.75 μM) were also used in the culture tubes. To reveal the effect of DC against the effects of MMC, 0.75 μM of MMC was added to each culture tube together with a test substance in a different culture series. Other processes related to the method were performed with minor modifications according to Evans [16] and Perry and Thompson [17].

For each slide, 100 metaphases (200 metaphases for two donors) were examined to detect the chromosomal abnormalities caused by DC. Chromosome number and structural abnormalities that we observed in the studied cells were evaluated in accordance with International System for Human Cytogenetic Nomenclature (ISCN) [18]. The number of structural and numerical CAs per cell and the percentage of cells containing abnormalities (CA/cell) were calculated.

For determination of the effects of DC on cell division, MI was calculated. A total of 3000 cells for each slide (6000 cell for two donor) were examined and the cells at the metaphase stage were determined and recorded. MI= ([The number of dividing cells]/3000) × 100.

5.4. Micronucleus (MN) Test

MN test developed by Rothfuss *et al.* [19] was carried out with some modifications. Donor selection and the set up of human lymphocyte cultures were almost the same as in the CA methods. However, in this method, incubation time was 68 hours. To block cytokinesis, 6 µg/ml cytochalasin B was added 44 h after the initiation of culture time. Likewise, solvent control (DMSO) and positive control (0.75 µM MMC) were included in different test tubes. In this test, cultured cells were treated with the test substance for 24 or 48 hours as in the previous test.

Slides were stained for 4 min. 1000 binucleated lymphocytes of each donor (total 2000 binucleated cells) were analyzed to calculate the percentage of MN. Percentage of micronucleated binuclear cells were calculated by dividing total binuclear cells (1000) by micronucleated binuclear cell number. Also, a total of 1000 cells were scored to determine the frequency of the cells with 1, 2, 3, or 4 nuclei. Based on this ratio, NDI was calculated [20].

NDI was calculated according to the following formula;

NDI= $(1×N1 + 2×N2 + 3×N3 + 4×N4)/N$

    N1: Number one nucleated cells

    N2: Number two nucleated cells

    N3: Number three nucleated cells

    N4: Number four nucleated cells

    N: Total cell number

5.5. Total Oxidant and Total Antioxidant Status Test

Total oxidant and antioxidant level tests were performed using 72 hour cultured peripheral blood donors treated by controls and DC. Culture tubes were centrifuged at 2000 rpm for 5 min and supernatant gathered. Then, it was stored at -80 °C for spectrophotometric analysis. TOS and TAS values were determined using commercial TOS and TAS kits (Relassay, Turkey). Calculation of TOS values is based on the oxidization of divalent iron (ferrous ion=Fe^{+2}) to trivalent iron (ferric ion=Fe^{+3}) by oxidant if it is present in the samples. Ferric ions form color complex in acidic medium. This color density is proportional to the amount of oxidizing molecules and was measured spectrophotometrically (530 nm). Test was calibrated with hydrogen peroxide and the results were expressed as per litre of hydrogen peroxide micro molar (mmol H_2O_2 Equiv/L) [21]. Determination of TAS values is made based on the bleaching of ABTS (2,20-azino-bis [3-ethylbenzothiazol-6- sulfonic acid]) radical cation by antioxidants. Therefore, TAS values were measured spectrophotometrically (660 nm). Results were expressed as mmol Trolox equivalent/L [22]. Oxidative stress index (OSI) was determined using TOS and TAS values and calculated using the following formula [23, 24, 25].

$$OSI = TOS / TAS$$

5.6. Statistical Analysis

All the data were represented as the mean ± standard error. The data displayed a normal distribution (Shapiro–Wilk). One-way analysis of variance (ANOVA) and post hoc Dunnett's tests were performed using SPSS software (Chicago, IL). Concentration-effect relationship was investigated by Pearson correlation. In statistical comparison between the control, positive control and treated groups, the value of " ≤0.05 " was determined as statistically significant.

REFERENCES

[1] Welch CR, Wu Q, Simon JE. Recent Advances in Anthocyanin Analysis and Characterization. Curr Anal Chem. 2008; 4(2): 75–101.

[2] Spencer JPE. Flavonoids: modulators of brain function? Br J Nutr. 2008; 99(1) 60-77.

[3] Afaq F, Syed DN, Malik A, Hadi N, Sarfaraz S, Kweon MH, Khan N, Zaid MA, Mukhtar H. Delphinidin, an anthocyanidin in pigmented fruits and vegetables, protects human HaCaT keratinocytes and mouse skin against UVB-mediated oxidative stress and apoptosis. J Invest Dermatol. 2007; 127(1): 222-232.

[4] Ferguson LR, van Zijl P, Holloway WD, Jones WT. Condensed tannins induce micronuclei in cultured V79 Chinese hamster cells. Mutat Res. 1985; 158(1-2): 89-95.

[5] Nagase H, Sasaki K, Kito H, Haga A, Sato T. Inhibitory effect of delphinidin from Solanum melongena on human fibrosarcoma HT-1080 invasiveness *in vitro*. Planta medica. 1998; 64(3): 216-219.

[6] Meiers S, Kemeny M, Weyand U, Gastpar R, Angerer EV, Marko D. The anthocyanidins cyanidin and delphinidin are potent inhibitors of the epidermal growth-factor receptor. J Agric Food Chem. 2001; 49(2): 958-962.

[7] Katsube N, Iwashita K, Tsushida T, Yamaki K, Kobori M. Induction of apoptosis in cancer cells by Bilberry (*Vaccinium myrtillus*) and the anthocyanins. J Agric Food Chem. 2003; 51(1): 68-75.

[8] Ribéreau-Gayon J, Ribéreau-Gayon P. The Anthocyans and Leucoanthocyans of Grapes and Wines. Am J Enol Vitic. 1958; 9: 1-9.

[9] Kayraldiz A, Kocaman AY, Rencuzogullari E, İstifli ES, İla HB, Topaktaş M, Dağlıoğlu YK. The genotoxic and antigenotoxic effects of Aloe vera leaf extract *in vivo* and *in vitro*. Turk J Biol. 2010; 34: 235-246.

[10] Tazehkand MN, Topaktas M. The in vitro genotoxic and cytotoxic effects of remeron on human peripheral blood lymphocytes. Drug Chem Toxicol. 2015; 38(3): 266-271.

[11] Karatepe R, Akal ZU, Alpsoy L. Genotoxic and cytotoxic effects of *Prunus armeniaca* seed extract *in vitro*. Fresen Environ Bull. 2015; 24(8): 2549-2555.

[12] Timocin T, Ila HB. Investigation of flurbiprofen genotoxicity and cytotoxicity in rat bone marrow cells. Drug Chem Toxicol. 2015; 38(3): 355-360.

[13] Alam-Escamilla D, Estrada-Muniz E, Solis-Villegas E, Elizondo G, Vega L. Genotoxic and cytostatic effects of 6-pentadecyl salicylic anacardic acid in transformed cell lines and peripheral blood mononuclear cells. Mutat Res Genet Toxicol Environ Mutagen. 2015; 777(1): 43-53.

[14] Rencüzoğulları E, Topaktaş M. The relationship between quantities of bromodeoxyuridine and human peripheral blood with determination of the best differential staining of sister chromatids using Chromosome Medium-B. J Sci Eng Çukurova University Insitute of Natural and Applied Science. 1991; 5(3): 19-24.

[15] Cvorovic J, Tramer F, Granzotto M, Candussio L, Decorti G, Passamonti S. Oxidative stress-based cytotoxicity of delphinidin and cyanidin in colon cancer cells. Arch Biochem Biophys. 2010; 501(1): 151-157.

[16] Evans HJ. Human Peripheral Blood Lymphocytes For The Analysis Of Chromosome Aberrations In Mutagen Tests. In: Kilbey, BJ, Legator M, Nichols W, Ramel C. (eds). Handbook of Mutagenicity Test Procedures. Elsevier Sciences, Amsterdam, 1984, pp. 405-427.

[17] Perry PE, Thomson EJ. The Methodology Of Sister Chromatid Exchanges In: Kilbey, BJ, Legator M, Nichols W, Ramel C. (eds). Handbook of Mutagenicity Test Procedures. Elsevier Sciences, Amsterdam, 1984, pp. 495-529.

[18] Paz-y-Miño C, Bustamante G, Sánchez ME, Leone PE. Cytogenetic monitoring in a population occupationally exposed to pesticides in Ecuador. Environ Health Perspect. 2002; 110(11): 1077-1080.

[19] Rothfuss A, Schütz P, Bochum S, Volm T, Eberhardt E, Kreienberg R, Vogel W, Speit G. Induced micronucleus frequencies in peripheral lymphocytes as a screening test for carriers of a BRCA1 mutation in breast cancer families. Cancer Res. 2000; 60(2): 390-394.

[20] Fenech M. The in vitro micronucleus technique. Mutat Res. 2000; 455(1-2): 81-95.

[21] Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem. 2005; 38(12): 1103-1111.

[22] Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem. 2004; 37(4): 277-285.

[23] Harma M, Harma M. Erel O. Increased oxidative stress in patients with hydatidiform mole. Swiss Med Wkly. 2003; 133(41-42): 563-566.

[24] Kosecik M, Erel O, Sevinc E, Selek S. Increased oxidative stress in children exposed to passive smoking. Int J Cardiol. 2005; 100(1): 61-64.

[25] Yumru M, Savas HA, Kalenderoglu A, Bulut M, Celik H, Erel O. Oxidative imbalance in bipolar disorder subtypes: a comparative study. Prog Neuro-Psychopharmacol Biol Psychiatry. 2009; 33(6): 1070-1074.

[26] Lazzé MC, Pizzala R, Savio M, Stivala LA, Prosperi E, Bianchi L. Anthocyanins protect against DNA damage induced by tert-butyl-hydroperoxide in rat smooth muscle and hepatoma cells. Mutat Res. 2003; 535(1): 103-115.

[27] Azevedo L, Alves de Lima PL, Gomes JC, Stringheta PC, Ribeiro DA, Salvadori DM. Differential response related to genotoxicity between eggplant (Solanum melanogena) skin aqueous extract and its main purified anthocyanin (delphinidin) *in vivo*. Food Chem Toxicol. 2007; 45(5): 852-858.

[28] Singletary KW, Jung KJ, Giusti M. Anthocyanin-rich grape extract blocks breast cell DNA damage. J Med Food. 2007; 10: 244-251.

[29] Fritz J, Roth M, Holbach P, Esselen M, Marko D. Impact of delphinidin on the maintenance of DNA integrity in human colon carcinoma cells. J Agric Food Chem. 2008; 56(19): 8891-8896.

[30] Ozbay T, Nahta R. Delphinidin Inhibits HER2 and Erk1/2 Signaling and Suppresses Growth of HER2-Overexpressing and Triple Negative Breast Cancer Cell Lines. Breast Cancer: Basic Clin Res. 2011; 5: 143-154.

[31] Esselen M, Fritz J, Hutter M, Marko D. Delphinidin modulates the DNA-damaging properties of topoisomerase II poisons. Chem Res Toxicol. 2009; 22(3): 554-564.

[32] Khandelwal N, Abraham SK. Protective effects of common anthocyanidins against genotoxic damage induced by chemotherapeutic drugs in mice. Planta Med. 2014; 80:(15) 1278–1283.

[33] Clere N, Faure S, Martinez MC, Andriantsitohaina R. Anticancer properties of flavonoids: roles in various stages of carcinogenesis. Cardiovasc Hematol Agents Med Chem. 2011; 9: 62-77.

[34] Domitrović R, Jakovac H. Antifibrotic activity of anthocyanidin delphinidin in carbon tetrachloride-induced hepatotoxicity in mice. Toxicology. 2010; 272: 1-10.

[35] Seong AR, Yoo JY, Choi K, Lee MH, Lee YH, Lee J, Jun W, Kim S, Yoon HG. Delphinidin, a specific inhibitor of histone acetyltransferase, suppresses inflammatory signaling via prevention of NF-kappaB acetylation in fibroblast-like synoviocyte MH7A cells. Biochem Biophys Res Commun. 2011; 410: 581-586.

[36] Afaq F, Katiyar SK. Polyphenols: skin photoprotection and inhibition of photocarcinogenesis. Mini-Rev Med Chem. 2011; 11: 1200-1215.