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PROPERTIES OF GRAPE SEED PROANTHOCYANIDINS AND QUERCETIN IN HUMAN LYMPHOCYTES

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Abstract — This study addresses the in vitro protective effects of proanthocyanidins and quercetin on the frequency of micronuclei with reference to the antioxidant status of cultured human lymphocytes also subjected to 2 Gy of γ-radiation. Treatment of lymphocytes with proanthocyanidins induced a significant decrease in the frequency of micronuclei and reduction of malonyldialdehyde production, as well as increased catalase and glutathione S-transferase activity. Quercetin induces a higher incidence of micronuclei and production of malonyldialdehyde. The seven-fold increase of micronuclei frequency induced by a therapeutic dose of γ-radiation was decreased in the presence of proanthocyanidins. These results demonstrate that proanthocyanidins may be important in the prevention of ROS-induced oxidative lymphocyte damage by decreasing DNA damage, lowering membrane lipid peroxidation, and increasing the activity AOP enzymes, as well as by reducing the level of γ-radiation-induced DNA damage. Our results support the potential benefits of proanthocyanidins as efficient antioxidants and radioprotectors.

Key words: Micronucleus test, flavonoids, malonyldialdehyde, catalase, glutathione S-transferase, γ-radiation, human lymphocytes

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INTRODUCTION

Ionizing radiation generates reactive oxygen species (ROS) such as superoxide anions, hydrogen peroxide, and hydroxyl radicals, which show high reactivity to a variety of cellular macromolecules, including DNA, lipids, and proteins (Anderson et al., 2001; Bianchini et al., 2001). One of the first events that can be seen after irradiation is induction of DNA damage. It is known that hydroxyl radicals induce chromosome breaks and formation of a great number of micronuclei in dividing cells (Fenech and Morley, 1985). The cell membrane is known to be a secondary target for ionizing radiation. Membrane degradation results essentially from attack of the hydroxyl radical on polyunsaturated fatty acid residues of phospholipids, which results in the formation of malonyldialdehyde (MDA) or 4-hydroxynonenal, leading to induction of DNA-protein cross-links (Tominaga et al., 2004). MDA is a toxic and mutagenic metabolite produced by lipid peroxidation and prostaglandin biosynthesis (Marnett, 1999). For this reason, measurement of MDA is widely used as an indicator of lipid peroxidation (Prasad et al., 2006).

To minimize the harmful effects of an oxidative load induced by endogenous as well as exogenous impact, cells have evolved a variety of defense mechanisms. Antioxidant systems, including some antioxidants produced in the body (endogenous) and others obtained from the diet (exogenous), comprise protection against free radical-mediated injury. The first include antioxidant enzymes that convert the superoxide anion ($O_2^{\cdot -}$) to another oxidant (H_2O_2), e.g., superoxide dismutase (SOD), after which H_2O_2 is chemically neutralized by catalase (CAT) (Ishige et al., 2001; Ma et al., 2006). CAT is one of the most efficient enzymes known. CAT protects cells from hydrogen peroxide generated within them. Even though CAT is not essential for some cell types under normal conditions, it plays

an important role in the acquisition of tolerance to oxidative stress in the adaptive response of cells (Hunt et al., 1998; Oral et al., 2000). The third enzyme involved in antioxidant defense is glutathione S-transferase (GST). GST is thought to play a physiological role in initiating the detoxication of potential alkylating agents, including pharmacologically active compounds. These enzymes catalyze the reaction of such compounds with the –SH group of glutathione, thereby neutralizing their electrophilic sites and rendering the products more water-soluble (Wiencke et al., 1990; Nielsen et al., 1996).

Flavonoids are phenolic substances isolated from a wide range of vascular plants, and many studies suggest that flavonoids possess biological activities, including antiallergenic, antiviral, anti-inflammatory, and vasodilatory action. Most attention has been devoted to the antioxidant activity of flavonoids, due to their ability to reduce free radical formation and scavenge free radicals (Nijveldt et al., 2001). The basic flavonoid structure is the flavan nucleus, which consists of 15 carbon atoms arranged in three rings (C_6-C_3-C_6) labeled A, B, and C. The various classes of flavonoids differ in the level of oxidation as a pattern of substitution of the C ring, while individual compounds within a class differ in the pattern of substitution of the A and B rings. The flavonoid quercetin (QC) is predominant in the human diet, and estimates of human consumption are in the range of 4-68 mg per day (Knekt et al., 1997; Rimm et al., 1996). Early studies of flavonoids first documented the mutagenicity of QC (Sahu and Gray, 1996), while QC - which has methoxy groups instead of hydroxyl groups at the 3,7,3',4'-positions - showed anticlastogenic and antioxidative activity. Dietary grape seed proanthocyanidins (PC) are a mixture of several polyphenols/flavanols and contain mainly proanthocyanidins (89%), which constitute dimers, trimers, tetramers, oligomers, and monomeric flavanol (6,6%) (Sato et al., 2001; Khanna et al., 2002). Grape seed proanthocyanidins markedly inhibited photocarcinogenesis in mice (Mitall et al., 2003). They also reduced UVB-induced increases in the levels of IL-10 in the skin and enhanced the production of IL-12 (Sharma and Katiyar 2006; Katiyar, 2007).

In the present study, we used the micronucleus (MN) assay to detect DNA damage and production of MDA as an indicator of lipid peroxidation and employed assays for activities of CAT and GST as enzymes which decrease the content and protect cells from ROS damage in human lymphocyte culture treated *in vitro* with PC and QC with the aim of determining protective effects of these two flavonoids. Also, the ability of flavonoids to protect cells from γ-irradiation-induced oxidative damage was examined in human lymphocyte cultures treated with PC and QC for 19 h after irradiating blood samples with 2 Gy of γ-rays.

MATERIALS AND METHODS

Subjects

Venous blood samples were obtained with heparinized sterile vacutainers from 45 healthy male volunteers who had not been exposed to chemicals, drugs, or other substances. The volunteers gave the investigators their permission to use their blood for the experiment. Two aliquots of blood (5 ml each) were obtained from each subject.

The study complied with the code of ethics of the World Medical Association (Helsinki Declaration of 1964, as revised in 2002). The blood samples were obtained at the medical unit of our institute in accordance with current (2005) Serbian health and ethical regulations.

Chemicals

Lymphocyte cultures were treated with PC or the flavonoid QC. Chemically pure QC (*3,3',4',5,7-pentahydroxyflavone* $C_{15}H_{10}O_7 \times 2H_2O$, *FW 338,3*) was used (Sigma-Aldich, Vienna, Austria). PC *(proanthocyanidin[(-)-epycatechin-3-O-galate])* is the extract obtained from grape seeds by a method patented by Pekić and Kovač *(Yu. Pat. P-205/93).* Flavonoids were added to cell cultures at concentrations of 0.87 or 4.37 nmol/L.

Irradiation

Whole blood samples of volunteers were obtained by venepuncture, put in sterile plastic

test-tubes in a 15x15 cm Plexiglas container, and irradiated using a ^{60}Co γ-ray source. The radiation dose was 2 Gy (therapeutic dose), the dose-rate 0.45 Gy/min. Radiation was delivered to a field of 20 cm x 20 cm, the distance from the source being 75 cm. Blood samples were irradiated at room temperature. Irradiated and unirradiated blood samples were set up in cultures two hours after irradiation. PC or QC were added to cell cultures of human lymphocytes two hours after irradiation, while in the non-irradiated cultures, they were added immediately.

One cell culture served as the control, to which PC or QC were not added. All cultures were incubated in a thermostat at 37°C. Treatment with flavonoids lasted for 19 h, after which all cultures were rinsed with pure medium, transferred to 5 ml of fresh RPMI 1640 medium (RPMI 1640 Medium + GlutaMAX + 25mM HEPES) (Invitrogen - Gibco-BRL, Vienna, Austria), and incubated for additional 72 h (MN) and 48 h (for antioxidative enzymes). Treatment with flavonoids lasted 19 h because it has been calculated that this is the time necessary for flavonoids ingested by the organism *per se* to be metabolized and excreted from the organism.

Micronucleus Analysis

The extent of damage to DNA is successfully measured using the cytokinesis-block micronucleus assay. The cytokinesis-block micronucleus technique enables micronuclei to be specifically scored in cells that have completed nuclear division and are therefore not influenced by variations in cell division kinetics (Fenech and Morley, 1991). This technique has been repeatedly shown to be a sensitive and reliable index of chromosome damage.

About 2 x 10^6 of blood lymphocytes were set up in 5 ml of RPMI-1640 medium supplemented with 15% calf serum and 2.4 μg/ml of phytohemagglutinin (Invitrogen - Gibco-BRL, Vienna, Austria). One hour after the start of cell stimulation, 0.87 nmol/L or 4.37 nmol/L of aqueous extracts of PC or QC were added to both the irradiated and the unirradiated samples. The incidence of spontaneously occurring MN in control samples as well as of radiation-induced MN there was scored. For MN preparation, the cytokinesis block method of Fenech and Morely (1993) was used with some modifications. Cytochalasin B (Invitrogen- Gibco-BRL, Vienna, Austria) at a final concentration of 6 μg/ml was added to the samples after 44 h of culture, and the lymphocyte cultures were incubated for a further 24 h. After 72 h of culture, the cells were washed with 0.9% NaCl (Merck, Sharp, and Dohme GMBH., Vienna, Austria), collected by centrifugation, and treated with hypotonic solution at 37°C. The hypotonic solution consisted of 0.56% KCl + 0.9% NaCl (mixed in equal volumes). The cell suspension was prefixed in methanol/acetic acid (3:1), washed three times with fixative, and dropped onto a clean slide (Fenech and Morely, 1993). The slides were air dried and stained with alkaline Giemsa (Sigma-Aldrich, Vienna, Austria) (2%). At least 1000 binucleated (BN) cells per sample were scored, registering MN according to the criteria of Countryman and Heddle (1976) and Fenech and Morely (1993).

Enzyme assay

To determine the production of MDA and activities of antioxidative enzymes (CAT and GST), preparations were carried out after 48 h of incubating the cell culture. They involved centifugation (1200 rpm, 10 min) of the cell culture and separation of the supernatant into plastic tubes for measurement of CAT and GST. Physiological solution was added to the cell suspensions (~1 ml), and lymphocytes were separated on a gradient of Lymphoprep (PAA Laboratories, Pasching, Austria) (1 ml). Enzyme activities were measured on the separated lymphocytes.

Malonyldialdehyde (MDA) is the end-product of lipid peroxidation of cell membranes due to oxygen-derived free radicals and is considered a reliable marker of myocardial cell damage. It was determined by measuring chromogen obtained from the reaction of MDA with 2-thiobarbituric acid (Sigma-Aldrich, Vienna, Austria) according to Arouma et al. (1989). The values are expressed as nmol of thiobarbituric acid-reactive matter (MDA equivalent)/mg of protein, using the curve of 1,1,3,3-tetramethoxypropane as a standard. Protein concentration

was determined according to Bradford (1976).

Glutathione S-transferase (GST) activity in the supernatant was determined spectrophotometrically at 37°C according to the method of Habig et al. (1974). The reaction mixture (3 ml) contained 1.7 ml of 0.1 M phosphate buffer (pH 6.5) and 0.1 ml of 30 mM CDNB. After incubating the reaction mixture at 37°C for 5 min, 0.1 ml of diluted cytosolic fraction was added and the change in absorbance at 340 nm was followed for 3 min. Reaction mixture without the enzyme was used as the blank. The specific activity of GST was calculated as nM of GSH-CDNB conjugate formed/min/mg of protein using an extinction coefficient of 9.6 mM^{-1}/cm. The results were expressed as change of activity in relation to the control.

Catalase (CAT) has a dual function: decomposition of H_2O_2 to give H_2O and O_2 (catalytic activity); and oxidation of H donors, e.g., methanol, ethanol, formic acid, and phenols with consumption of 1 mol of peroxide (peroxidic activity).

In the ultraviolet range, H_2O_2 shows a continual increase of absorption with decreasing wavelength. Decomposition of H_2O_2 can be followed directly from the decrease in absorbance at 240 nm. The difference in absorbance (ΔA_{240}) per unit of time is a measure of catalase activity (Aebi, 1984).

Statistics and index calculations

Statistical analysis was performed using the Origin software package, version 7.0. The statistical significance of differences between data pairs was evaluated by analysis of variance (one-way ANOVA), followed by the Tukey test. A difference was considered significant at $p<0.05$. The results are presented as the % of change compared to the control.

RESULTS

Effects of grape seed proanthocyanidins and quercetin on control lymphocytes

The treatment of human lymphocyte cell cultures with PC at concentrations of 0.87 or 4.37 nmol/L gave a significant decrease in the frequency of MN - by 40 and 10%, respectively - and reduced the production of MDA by 30 and 15%, respectively (Table 1, Fig. 1).

Activities of the antioxidant enzymes CAT and GST in cell cultures of human lymphocytes treated with PC at a concentration of 0.87 nmol/L were similar to those in control human lymphocytes. However, activities of CAT and GST in cell cultures of human lymphocytes treated with PC at a concentration of 4.37 nmol/L were significantly higher (by 10 and 15%, respectively) than in control human lymphocytes (Table 1, Fig. 2).

The treatment of cell cultures with 0.87 or 4.37 nmol/L of the flavonoid QC increased the frequency of MN by 2 and 22%, respectively, as well as the production of MDA by 11 and 18%, respectively, as compared with control human lymphocytes (Table 1, Fig. 1). Activities of the antioxidant enzymes CAT and GST increased by 6 and 5.6% in the presence of 0.87 nmol/L QC, which is not significant in relation to the control value. A higher concentration of QC (4.37 nmol/L) induced a significant increase of CAT (12%) and GST (8%) activities in human lymphocytes compared to the control (Table 1, Fig. 2).

Difference in the frequency of MN are statistically significant in the ANOVA -Tukey test ($F_{(1.10)}$ = 17.82, $p<0.01$) between cell cultures control of the group and cell cultures treated with PC at a concentration of 0.87 nmol/L (Table 3).

The production of MDA also differs significantly according to the ANOVA-Tukey test ($F_{(1.10)}$ = 11.79, $p<0.01$) between cell cultures of the control group and cell cultures treated with PC at a concentration of 0.87 nmol/L (Table 3). Comparing the effects of control cell cultures and cell cultures treated with PC at a concentration of 4.37nmol/L, we see a statistically significant difference in the activity of GST ($F_{(1.10)}$ = 8.17, $p<0.05$) (Table 3). A significant difference is also evident between the control cultures and ones treated with PC (0.87 or 4.37 nmol/L) ($F_{(1.10)}$ = 7.52, $p<0.01$) (Table 3). Comparing the effects of PC at a concentration of 0.87 nmol/L and QC at a concentration of 0.87 nmol/L, we see a statistically significant difference in the frequency of MN and the production of MDA (Table 3).

Table 1. Incidence of MN, production of MDA, and activity of enzymes CAT and GST in cell cultures of human lymphocytes treated with 0.87 or 4.37 nmol/L of aqueous extracts of PC or QC. Abbreviations: MN - MN/1000 binucleated cells, MDA - nmol of MDA/mg of protein, CAT - ΔA240/s/mg of protein, GST – nmol of GSH/min/ml of lymphocytes, PC - proanthocyanidins, QC - quercetin.

	MN	MDA	CAT	GST
	Mean±SEM	*Mean±SEM*	*Mean±SEM*	*Mean±SEM*
Control cells	21.87±1.63	21.48±1.64	6.07±0.29	6.28±0.23
PC, 0.87 nmol/L	13.10±1.29	14.85±1.02	5.87±0.26	6.05±0.26
PC, 4.37 nmol/L	19.58±1.36	18.13±1.06	6.70±0.26	7.22±0.24
QC, 0.87 nmol/L	22.33±1.69	23.78±1.25	6.43±0.19	6.63±0.26
QC, 4.37 nmol/L	26.62±1.90	25.32±1.16	6.80±0.19	6.80±0.18

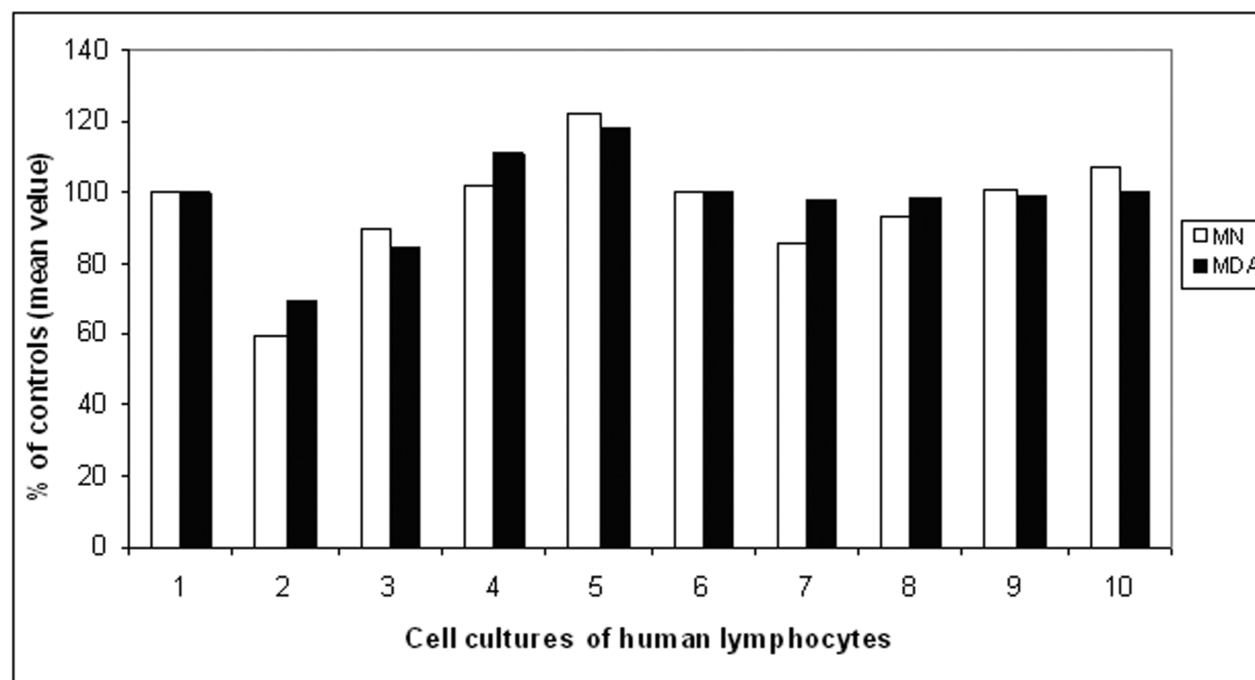

Fig. 1. Incidence of MN and production of MDA in human lymphocytes treated with PC or QC. Human lymphocytes in culture were treated with 0.87 or 4.37 nmol/L of aqueous extracts of PC or QC for 19 h. Incidence of MN and production of MDA were measured as indicated in Materials and Methods. Obtained results are presented as means ± S.E.M. of measurements from six cell cultures of human lymphocytes. 1. Controls; 2. PC (0.87 nmol/L); 3. PC (4.37 nmol/L); 4. QC (0.87 nmol/L); 5. QC (4.37 nmol/L); 6. Irradiated cells; 7. Irradiated PC (0.87 nmol/L); 8. Irradiated PC (4.37 nmol/L); 9. Irradiated QC (0.87 nmol/L); 10. Irradiated QC (4.37 nmol/L). MN - micronuclei; MDA - malonyldialdehyde.

Table 2. Incidence of MN, production of MDA, and activity of enzymes CAT and GST in irradiated cell cultures of human lymphocytes treated with 0.87 or 4.37 nmol/L of aqueous extracts of PC or QC. Abbreviations: MN - MN/1000 binucleated cells, MDA - nmol of MDA/mg of protein, CAT - ΔA240/s/mg of protein, GST – nmol of GSH/min/ml of lymphocytes, PC - proanthocyanidins, QC - quercetin.

	MN	MDA	CAT	GST
	Mean±SEM	*Mean±SEM*	*Mean±SEM*	*Mean±SEM*
Irradiated cells	164.2±13.16	18.75±0.14	5.25±0.31	5.43±0.17
Irradiated PC, 0.87 nmol/L	139.93±5.84	18.29±0.14	5.16±0.35	5.37±0.16
Irradiated PC, 4.37 nmol/L	152.26±7.55	18.43±0.16	5.18±0.24	5.39±0.17
Irradiated QC, 0.87 nmol/L	165.17±8.12	18.62±0.05	5.20±0.27	5.42±0.19
Irradiated QC, 4.37 nmol/L	174.83±12.25	18.74±0.09	5.17±0.21	5.45±0.15

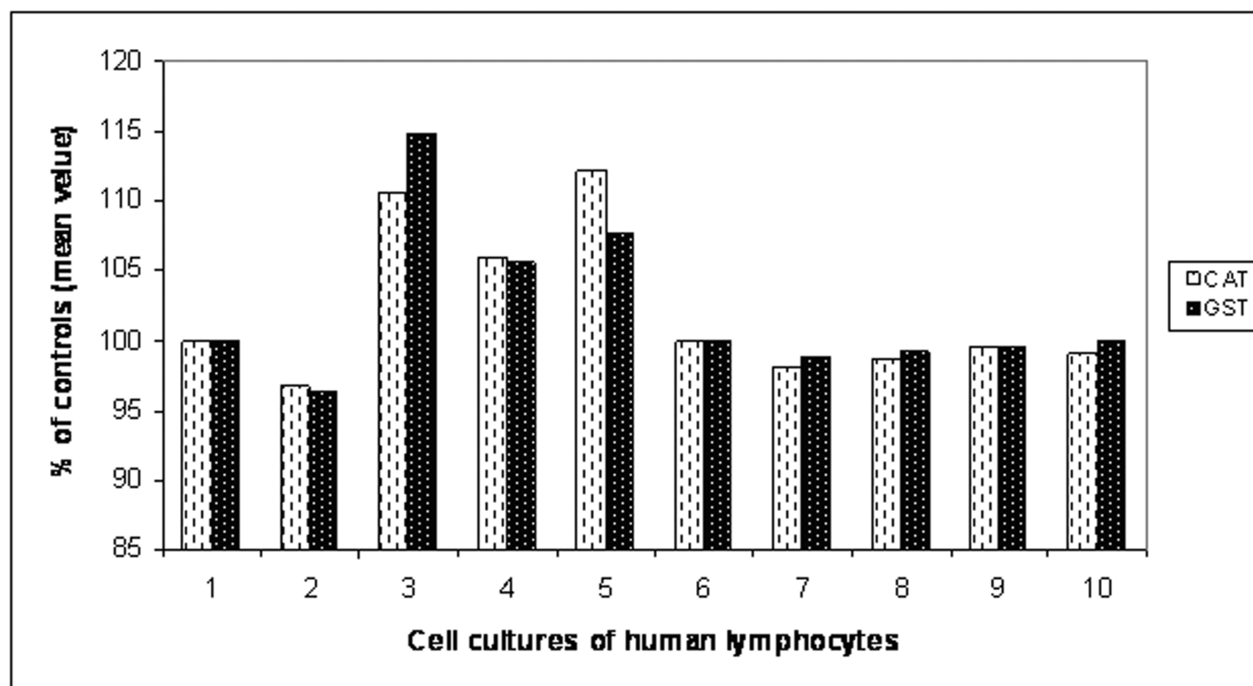

Fig. 1. Activity of enzymes CAT and GST in irradiated cell cultures of human lymphocytes treated with PC or QC. Human lymphocytes in culture were treated with 0.87 or 4.37 nmol/L of aqueous extracts of PC or QC for 19 h. Activity of enzymes CAT and GST were measured as indicated in Materials and Methods. Obtained results are presented as means ± S.E.M. measurements of six irradiated cell cultures of human lymphocytes. 1. Controls; 2. PC (0.87 nmol/L); 3. PC (4.37 nmol/L); 4. QC (0.87 nmol/L); 5. QC (4.37 nmol/L); 6. Irradiated cells; 7. Irradiated PC (0.87 nmol/L); 8. Irradiated PC (4.37 nmol/L); 9. Irradiated QC (0.87 nmol/L); 10. Irradiated QC (4.37 nmol/L). CAT - catalase; GST - glutathione S-transferase.

Table 3. Statistical significance of differences between data pairs (frequency of MN, production of MDA, and activity of enzymes CAT and GST) evaluated by analysis of variance (one-way ANOVA) followed by the Tukey test. Results are presented as cell cultures of human lymphocytes treated with 0.87 or 4.37 nmol/L of aqueous extracts of PC or QC. n.s.- no statistical difference, $p>0.05$.

	PC, 0.87 nmol/L				***PC, 4.37 nmol/L***			
	MN	MDA	CAT	GST	MN	MDA	CAT	GST
Control cells	**$p<0.01$**	**$p<0.01$**	n.s	n.s	n.s	n.s	n.s	**$p<0.05$**
PC, 0.87 nmol/L					**$p<0.05$**	**$p<0.05$**	**$p<0.05$**	**$p<0.01$**

	QC, 0.87 nmol/L				***QC, 4.37 nmol/L***			
	MN	MDA	CAT	GST	MN	MDA	CAT	GST
Control cells	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
PC, 0.87 nmol/L	**$p<0.01$**	**$p<0.01$**	n.s.	n.s.				
PC, 4.37 nmol/L					n.s	n.s	n.s	n.s
QC, 0.87 nmol/L					**$p<0.05$**	**$p<0.05$**	n.s	n.s

Table 4. Statistical significance of differences between data pairs (frequency of MN, production of MDA, and activity of enzymes CAT and GST) evaluated by analysis of variance (one-way ANOVA) followed by the Tukey test. Results are presented as irradiated cell cultures of human lymphocytes treated with 0.87 or 4.37 nmol/L of aqueous extracts of PC or QC. n.s.- no statistical difference, $p>0.05$.

	Irradiated PC, 0.87 nmol/L				***Irradiated PC, 4.37 nmol/L***			
	MN	MDA	CAT	GST	MN	MDA	CAT	GST
irradiated cells	n.s	n.s	n.s	n.s	n.s	n.s	n.s	**$p<0.05$**
PC, 0.87 nmol/L					n.s	n.s	n.s	**$p<0.01$**

	Irradiated QC, 0.87 nmol/L				***Irradiated QC, 4.37 nmol/L***			
	MN	MDA	CAT	GST	MN	MDA	CAT	GST
irradiated cells	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
PC, 0.87 nmol/L	**$p<0.05$**	n.s						
PC, 4.37 nmol/L				n.s	n.s	n.s	n.s	n.s
QC, 4.37 nmol/L				n.s	n.s	n.s	n.s	n.s

Effects of grape seed proanthocyanidins and quercetin on irradiated lymphocytes

As can be seen from Table 2, irradiation increased MN frequency seven-fold (statistic significance of $F_{(1.10)} = 180.31$, $p<0.01$) and decreased the activity of GST (statistic significance of $F_{(1.10)} = 7.39$, $p<0.05$) compared to the control lymphocyte culture.

Treatment of irradiated lymphocytes with 0.87 or 4.37nmol/L of PC induced a decrease in the frequency of MN by 14.6 and 7.3%, respectively, compared to irradiated cell cultures not treated with PC, while no changes of MDA production or CAT and GST activities were detected (Table 2, Fig. 2).

Treatment of irradiated cell cultures with QC at a concentration of 4.37 nmol/L increased the frequency of MN by 6% compared to untreated irradiated cell cultures (Table 2, Fig. 1). The production of MDA (Table 2, Fig. 1) in the presence QC and activities of antioxidant enzymes (CAT and GST) in the presence of both PC and QC measured in irradiated cell cultures of human lymphocytes are similar to these in irradiated human lymphocyte cell cultures not treated with flavonoids (Table 2, Fig. 2).

Comparing the effects of 0.87 nmol/L of PC or QC on irradiated cell cultures, we see a statistically significant difference in the frequency of MN ($F_{(1.10)} = 6.37$, $p<0.05$) (Table 4).

Comparison of the activity of GST in irradiated cell cultures with that in irradiated cell cultures treated with PC at a concentration of 4.37 nmol/L reveals a statistically significant difference ($F_{(1.10)} = 6.60$, $p<0.05$). Such a difference is also evident between cell cultures treated with 0.87 or 4.37 nmol/L of PC ($F_{(1.10)} = 7.48$, $p<0.01$) (Table 4).

However, in irradiated lymphocytes there were no significant differences in the obtained parameters between cultures treated with low and high concentrations of either PC or QC.

DISCUSSION

In this study we found that a lower concentration of PC (0.87 nmol/L) exerts a beneficial effect on control lymphocyte cell culture by decreasing the frequency of MN and reducing MDA production. Since the number of micronuclei serves as an indicator of DNA damage and the quantity of MDA as a measure of membrane lipide peroxidation, these results indicate that PC protects DNA and decreases lipid peroxidation of lymphocytes, which is mostly induced by superoxide anion radicals. Free radicals disturb cellular homeostasis through peroxidation of membrane lipids, oxidation of proteins, base damage, and adduct formation in DNA, which ultimately leads to cell death if the damage is beyond the cell's repair capacity (Szumiel, 1994; Samanta et al., 2004). The inherent antioxidant defense system of the cell, which includes glutathione-S-transferase and catalase, competitively counteracts oxidative stress. These results are in agreement with findings of other authors who showed that PC scavenge the superoxide anion radical and inhibit lipid peroxidation induced by superoxide in human lymphocyte cells (Ohshima et al., 1998; Konopacka and Rogolinski, 2004; Mantena et al., 2006). We observed that QC exerts an opposite effect, inducing an increase of DNA damage and lipid peroxidation with a stronger pro-oxidant effect at a higher concentration. The increase of DNA damage in control lymphocytes induced by QC might result from interaction of QC with metals of DNA-binding proteins. It has been shown that QC generates oxidative DNA damage in the presence of Cu(II), probably by producing free radicals with complexes of quercetin, Cu(II), and DNA (Sirinivasan et al., 2002; Moridani et al., 2003; Filipe et al., 2004). DNA injuries were determined as thymine and cytosine residues (Moskauge et al., 2004). The site specificities and inhibitory effects suggested that the DNA-copper-oxygen complex rather than the free hydroxyl radical induced the DNA damage. By producing free radicals, QC may induce lipid peroxidation and the formation of MDA we detected in lymphocyte cell culture.

The obtained results suggest that PC and QC at higher concentrations can induce activation of antioxidant enzymes (e.g., CAT and GST), which may act against the toxic and neoplastic effects of carcinogens. In our study, we found that QC added

to cell cultures after irradiation increased the levels of MN, but with no changes in MDA production or CAT and GST activities. The pro-oxidant action of QC, followed by the induction of free radicals by radiation, may account for increased DNA damage and hence increased frequency of micronuclei.

Exposing lymphocyte culture to gamma-radiation of 2 Gy induces severe DNA damage and sevenfold increase of MN incidence 72 h after irradiation. All other parameters we investigated were slightly decreased. Ionizing radiation has been shown to generate free radicals, almost immediately inducing lipid peroxidation, which can lead to DNA damage and cell death (Sun et al., 1998). These free radicals initiate chain reactions leading to the production of secondary radicals, which keep on accumulating with the passage of time. CAT and GST are endogenous antioxidant enzymes that protect cells from oxidative stress, and flavonoids are known to activate the glutathione-synthesizing enzyme (Kondalarao and Shaha, 2000; Myhrstad et al., 2002).

Our results provide evidence for radioprotective effects of PC against γ-radiation-induced cytogenetic and biochemical damage in human lymphocytes treated *in vitro.* In our study, we found that PC reduced the frequency of MN, this protection being dependent on the concentration of PC, the lower concentration being more effective. This result is in accordance with the data of Teissedre et al. (1995), who found that grape seed proanthocyanidins (18 mg/l) protect human low-density lipoproteins if added to cell cultures. It is known that PC are capable of scavenging ROS. Several earlier studies demonstrated that PC protect against oxygen species-mediated DNA damage (Shao et al., 2001; Fan and Lou, 2004). This result is important in view of the fact that synthetic protectors such as Amifostin WR 2721 used in the treatment of humans after irradiation decrease the frequency of MN by about 5% (Girard, 1995; Kumar and Goel, 2000). In the majority of these studies, the doses of grape seed proanthocyanidins ingested were between 75 and 400 mg or those present in 1-3 glasses of wine per day. As this study suggests, consumption of food and foodstuffs with increased concentrations of proanthocyanidins may contribute to human health.

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АНТИОКСИДАТИВНА СВОЈСТВА ПРОАНТОЦИЈАНИДИНА И КВЕРЦЕТИНА ИЗ ГРОЖЂА КОД ХУМАНИХ ЛИМФОЦИТА

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Циљ овог рада је био да се у *in vitro* условима испитају протективни ефекти проантоцијанидина и кверцетина на учесталост појаве микронуклеуса као и антиоксидативног статуса хуманих лимфоцита у култури изложених дејству γ-зрака од 2 Gy. Третман лимфоцита проантоцијанидинима доводи до значајног смањења у учесталости микронуклеуса, продукције малондиалдехида и повећања активности каталазе и глутатион-С-трансферазе. Кверцетин индукује већу инциденцу микронуклеуса и продукцију малондиалдехида. Седам пута повећана фреквенције микронуклеуса у лимфоцитима након излагања јонизујућем зрачењу у присуству проантоцијанидина је смањена. Добијени резултати указују да проантоцијанидини могу бити значајни у превенцији оксидативних оштећења хуманих лимфоцита насталих повећањем продукције реактивних кисеоникових врста, смањујући оштећења ДНК, липидну пероксидацију мембране и повећавајући активност антиоксидативних ензима, као и оштећења ДНК изазваних деловањем јонизујућег γ-зрачења. Ови резултати потврђују улогу проантоцијанидина као ефикасног антиоксиданта и радиопротектора.