Radioprotective properties of *Gentiana dinarica* polyphenols on human lymphocytes *in vitro*

Sandra Petrovic*, Andreja Leskovac and Gordana Joksic

Vinca Institute of Nuclear Sciences, POB 522, 11001, Belgrade, Serbia

The radioprotective properties of polyphenols from the plant *Gentiana dinarica* Beck. (family Gentianaceae) were evaluated in cultured human peripheral blood lymphocytes exposed to 2 Gy of gamma radiation *in vitro*. Major constituents of *G. dinarica* roots include tetraoxygenated xanthones (gentiacaulein and gentiakochianin), tetraoxygenated xanthone glycosides (norswertianin-1-*O*-primveroside and norswertianin-8-*O*-primveroside), C-glucoflavones (isoorientin and isoorientin-4′-*O*-glycoside) and secoridoids. The incidences of micronuclei, cell proliferation, apoptosis and lipid peroxidation products were examined. Isoorientin, isoorientin-4′-*O*-glycoside and norswertianin-1-*O*-primveroside showed antioxidant properties, whereas norswertianin-8-*O*-primveroside and gentiacaulein exhibited pro-oxidative effects. Gentiakochianin exhibited cytostatic effects in irradiated lymphocytes. These results contribute to the search for novel radioprotective agents.

Keywords: *Gentiana dinarica*, human lymphocytes, polyphenols, radioprotection.

IONIZING radiation damages cells by direct ionization of DNA and other cellular targets and indirectly via reactive oxygen species (ROS). ROS attack lipids, proteins and DNA, and induce oxidation and membrane damage, enzyme inactivation and DNA damage[1,2]. The radiation-induced alteration of intracellular redox homeostasis leads to oxidative stress. Lipid peroxidation is a key marker of oxidative stress and often causes extensive membrane damage, leading to cell death. The highly reactive hydroxyl radical attacks the DNA and causes single- and double-strand breaks and oxidative damage to sugar and base residues that can later be converted to strand breaks[3]. DNA damage leads to genomic instability, which may result in mutagenesis and carcinogenesis[4]. Humans are intentionally exposed to ionizing radiation for diagnostic or therapeutic purposes. The primary goal of radiotherapy is to increase DNA damage in tumour cells. Because ROS produced during radiotherapy perturb the integrity and survival of the surrounding normal cells, other methods of cancer treatment could modify signal transduction pathways, redox states, and the disposition of cells to apoptosis[5].

In recent years, extensive studies have been conducted to evaluate the potential beneficial effects of natural phytochemicals in radiorecovery and the protection of normal tissue during exposure to radiation. Naturally occuring polyphenols comprise a wide variety of compounds divided into several classes that occur in fruits and vegetables, wine and tea, and chocolate and other cocoa products[6]. The beneficial effects of polyphenols are mainly attributed to their antioxidant properties, since they can act as chain breakers or radical scavengers depending on their chemical structures[7]. Polyphenols might also trigger changes in the signalling pathways and subsequent gene expression[8,9]. It is possible that the distinct chemical and receptor-mediated activities of polyphenols might result in similar outcomes via different pathways[10]. Under some circumstances, polyphenols can exhibit pro-oxidative effects.

The use of plants in traditional medicine is widespread and still serves as a source of leads for the development of novel pharmacological agents[11]. Many such medicinal plants have hepatoprotective, neuroprotective, anti-inflammatory and antioxidant/radical-scavenging properties[12,13]. Several species from the family Gentianaceae are used in Serbian traditional medicine. *Gentiana lutea* L., *Gentiana punctata* L. and *Centaurium erythraea* Pers. are used in the treatment of digestive disorders, whereas *Gentianella austriaca* (A. Kern. & Jos. Kern.) Holub has shown radioprotective effects in human lymphocytes *in vitro*[14]. The aim of the present study was to evaluate the radioprotective properties of polyphenols from *Gentiana dinarica* Beck. (family Gentianaceae). *G. dinarica* is a rare perennial plant that grows in the subalpine and alpine regions[15]. Compounds isolated from *G. dinarica* roots include tetraoxygenated xanthones (gentiacaulein and gentiakochianin), tetraoxygenated xanthone glycosides (norswertianin-1-*O*-primveroside and norswertianin-8-*O*-primveroside), C-glucoflavones (isoorientin and isoorientin-4′-*O*-glycoside) and secoridoids[16]. The polyphenols gentiacaulein, gentiakochianin and isoorientin are found in a number of *Swertia* plants (*S. japonica*, *S. chirata*, *S. thomsonii*, *S. punctata* and *S. davidi*), which also belong to the Gentianaceae family, and are used in traditional medicine in India, China, Pakistan, Japan and other Asian countries in the treatment of liver disorders, fever, diar-

*For correspondence. (e-mail: sandra@vin.bg.ac.yu)

rhoea and dysentery[17]. Tetraoxygenated xanthones have been reported to exhibit anti-hepatotoxic, anti-inflammatory, antioxidant and antitumour properties[18,19]. In this study, we examined the effects of *G. dinarica* polyphenols on radiation-induced micronuclei formation, cell proliferation and induction of apoptosis in cultured human peripheral blood lymphocytes. In addition, we measured malondialdehyde levels as a marker of oxidative stress in irradiated cells.

Materials and methods

Plant material

G. dinarica Beck. (Gentianaceae) was collected from Mt. Tara in Serbia. The voucher specimen (accession number Gd072001) is deposited in the herbarium at the Faculty of Biology, University of Belgrade-Herbarium, Code BEOU. Polyphenols isolated from *G. dinarica* root include tetraoxygenated xanthones (gentiacaulein and gentiakochianin), tetraoxygenated xanthone glycosides (norswertianin-1-*O*-primveroside and norswertianin-8-*O*-primveroside), C-glucoflavones (isoorientin and isoorientin-4′-*O*-glycoside) and secoridoids. Details of their extraction and chemical structure have been described elsewhere[16].

Irradiation

Blood samples were obtained from three healthy, non-smoking young male volunteer donors at the Medical Unit in accordance with current health and ethical regulations in Serbia[20]. Heparinized whole blood was aliquoted into sterile plastic test tubes, placed in a 15 × 15 cm Plexiglas container and irradiated using a ^{60}Co γ-ray source at room temperature. The radiation dose employed was 2 Gy, the dose rate was 0.45 Gy/min, the dimensions of the radiation field were 20 × 20 cm, and the distance from the radiation source was 74 cm.

Cell culture

One hour after irradiation, 0.5 ml aliquots of whole blood were added to culture tubes containing 4.5 ml of PBmax karyotyping medium (Invitrogen-Gibco, Paisley, UK). Duplicate lymphocyte cultures were established to enable examination of micronuclei and malondialdehyde respectively. Irradiated and positive control cultures were established.

Preparation of plant extracts

Air-dried and powdered plant extracts were dissolved in sterile double-distilled water, kept for 30 min at room temperature and filtered using 0.45 µm membrane filters (Millipore Co Ltd.). Doses of 50 µl of the initial dilution (10 mg/ml) of each plant extract and plant infusion were added to cell cultures to obtain a final concentration of 0.1 mg/ml. Amifostine (WR-2721) was used as a positive control[21]. A dose of 50 µl of amifostine (final concentration 1 µg/ml) was added to irradiated cells.

Optimum dose selection

The optimum dose for evaluation of radioprotection was selected based on previously conducted experiments employing different concentrations (0.1–0.4 mg/ml) of plant extracts for the treatment of irradiated human lymphocytes. Because 0.1 mg/ml of plant extract provided the greatest radioprotection, it was considered the optimum radioprotective concentration and was used for this study[14].

Micronucleus assay

For micronuclei preparation, the cytokinesis block method of Fenech *et al.*[22] was used. For each sample, at least 1000 binucleate cells were scored and micronuclei were recorded using an AxioImager A1 microscope (Carl Zeiss, Jena, Germany) with 400× or 1000× magnification. The ability of cells to proliferate *in vitro* was evaluated by counting the number of cells with one to four micronuclei on the same slides. The results of these analyses are presented as a cytokinesis-block proliferation index (CBPI). CBPI was calculated as follows: $\mathrm{CBPI} = M\mathrm{I} + 2M\mathrm{II} + 3[M\mathrm{III} + M\mathrm{IV}]/N$, where $M\mathrm{I}$–$M\mathrm{IV}$ represent the number of cells with one to four nuclei respectively, and N is the number of cells scored[23].

Thiobarbituric acid assay

After 72 h of incubation, parallel cultures were separated on Lymphoprep (lymphocyte separation medium, PAA Laboratories GmbH, Austria). Lymphocytes were collected by centrifugation, washed in physiological saline (0.9% NaCl) and frozen at –20°C. A thawed lymphocyte suspension was treated with thiobarbituric acid (TBA) and used to determine malondialdehyde (MDA) levels spectrophotometrically[24] at 532 nm. Values are expressed as nmol TBA-reactive material (MDA equivalent)/mg protein, using a standard curve of 1,1,3,3-tetramethoxypropane. Protein concentration was determined according to Lowry *et al.*[25].

Apoptosis

For apoptosis assays, the method of Crompton *et al.*[26] was followed. Samples were stained with propidium iodide and analysed by flow cytometry (Becton Dickenson,

Table 1. Incidence of micronuclei (MN), proliferation index, level of malondialdehyde and percentage of apoptotic cells in irradiated control samples and irradiated samples treated with infusion and pure polyphenols of *Gentiana dinarica*

Irradiated cultures treated with infusion and pure polyphenols of *G. dinarica*	Incidence of MN/ 1000 BN cells	Mean ± SD	CBPI	Mean ± SD	nM MDA/mg of proteins	Mean ± SD	Percentage of apoptotic cells	Mean ± SD
Control	216.71		1.53		6.83		2.96	
	212.23	211.03 ± 6.37	1.55	1.55 ± 0.02	5.98	6.44 ± 0.43	2.99	3.01 ± 0.06
	204.15		1.56		6.51		3.08	
Gentiacaulein	214.67		1.43		7.30		2.94	
	207.22	209.29 ± 4.70[c,e]	1.45	1.44 ± 0.01[a,d]	6.63	6.92 ± 0.35[a,d]	3.07	2.99 ± 0.07[c,e]
	205.99		1.44		6.82		2.96	
Gentiakochianin	171.91		1.37		4.45		1.86	
	168.69	165.56 ± 8.37[b,e]	1.43	1.40 ± 0.03[a,f]	3.81	4.15 ± 0.32[b,d]	1.84	1.87 ± 0.04[b,e]
	156.07		1.39		4.18		1.91	
Norswertianin-1-*O*-primveroside	169.77		1.35		4.74		5.45	
	166.50	165.32 ± 5.15[b,e]	1.41	1.38±0.03[b,f]	4.45	4.31±0.52[a,d]	5.60	5.58 ± 0.12[b,e]
	159.68		1.39		3.73		5.69	
Norswertianin-8-*O*-primveroside	159.67		1.26		8.29		3.52	
	155.93	154.22 ± 6.48[a,e]	1.25	1.27 ± 0.03[a,d]	7.37	7.75 ± 0.48[b,d]	3.43	3.52 ± 0.09[a,e]
	147.06		1.30		7.58		3.61	
Isoorientin	205.31		1.50		6.16		3.22	
	201.54	200.54 ± 5.35[a,e]	1.53	1.54 ± 0.04[c,d]	6.19	5.94 ± 0.41[c,f]	3.26	3.28 ± 0.07[b,e]
	194.76		1.58		5.47		3.36	
Isoorientin-4′-*O*-glycoside	167.36		1.29		4.11		4.29	
	151.74	156.38 ± 9.54[a,e]	1.30	1.30 ± 0.02[b,d]	3.75	3.88 ± 0.20[a,e]	4.47	4.38 ± 0.09[a,e]
	150.05		1.32		3.77		4.38	
Infusion	308.16		1.41		5.96		10.33	
	304.08	305.20 ± 5.46[b,f]	1.44	1.44 ± 0.03[a,f]	5.52	5.70 ± 0.23[a,f]	10.70	10.52 ± 0.19[b,f]
	303.35		1.46		5.63		10.53	
Amifostine	183.87		1.52		6.19		3.09	
	186.85	176.25 ± 15.84[a]	1.50	1.50 ± 0.02[c]	6.34	6.13 ± 0.25[c]	3.33	3.20 ± 0.12[c]
	158.04		1.48		5.85		3.19	

[a]$P < 0.05$; [b]$P < 0.001$; [c]Nonsignificant compared to control; [d]$P < 0.05$; [e]$P < 0.001$; [f]Nonsignificant compared to infusion.

Heidelberg, Germany). The apoptotic population was calculated using CellQuest software (Becton Dickenson).

Statistical analysis

Statistical analysis was performed using the statistical software package Statistica 6.0 for Microsoft Windows. Statistical analysis was done using Student's *t*-test and a *P* value <0.05 was considered to be significant.

Results

Results are presented in Table 1 and Figure 1.

Effect of G. dinarica polyphenols on the incidence of radiation-induced micronuclei in irradiated human lymphocytes

Pure polyphenols isolated from *G. dinarica* reduced the incidence of radiation-induced micronuclei (MN). Maximal reduction of MN was observed after treating irradiated human lymphocytes with norswertianin-8-*O*-primveroside (a reduction of 26.92% compared to control cells). Radiation-induced MN were also significantly reduced by isoorientin-4′-*O*-glycoside, norswertianin-1-*O*-primveroside, gentiakochianin and isoorientin (25.90, 21.66, 21.55 and 4.97% respectively). Treatment of cells with gentiacaulein resulted in marginal, non-significant decreases in radiation-induced MN. Addition of whole plant infusion significantly increased the incidence of MN (44.62% higher than controls).

Effects of G. dinarica polyphenols on MDA levels in irradiated human lymphocytes

Maximal inhibition of lipid peroxidation was observed after treating irradiated human lymphocytes with isoorientin-4′-*O*-glycoside (39.75% lower levels compared to controls). MDA levels were significantly reduced by gentiakochianin, norswertianin-1-*O*-primveroside and plant infusion (35.56, 33.07 and 11.49% respectively). Isoori-

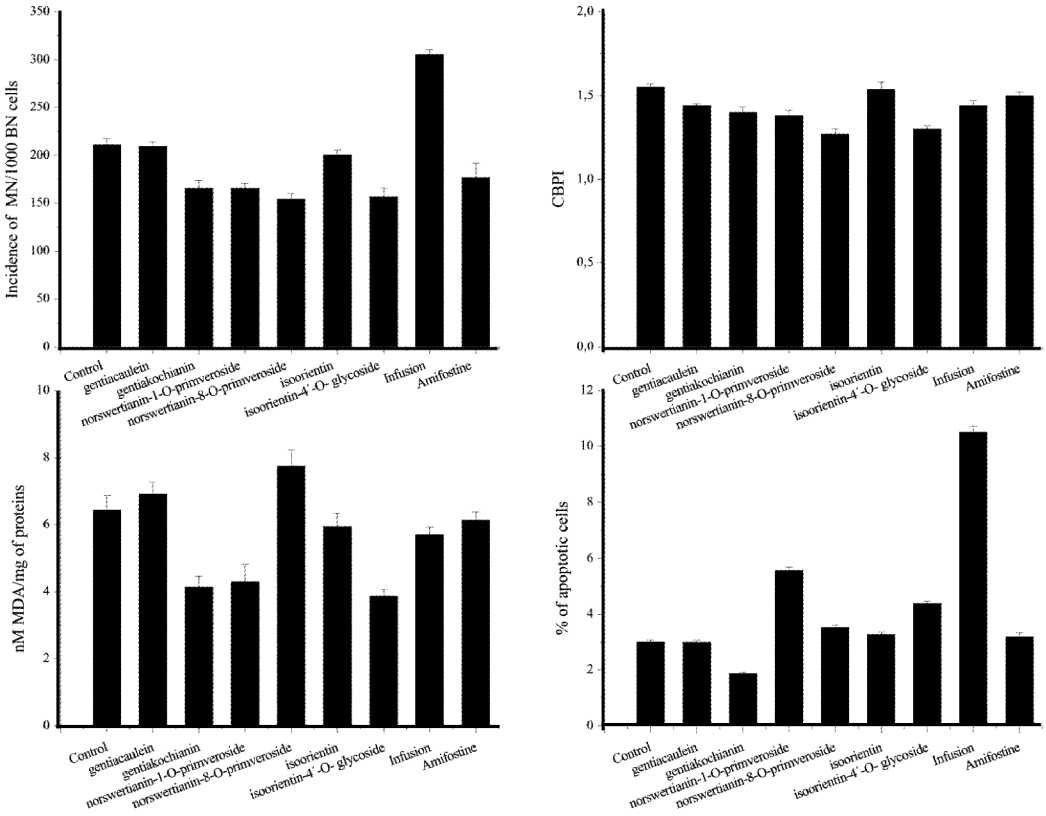

Figure 1. Effect of *Gentiana dinarica* infusion and pure polyphenols on incidence of micronuclei, proliferation index, level of malondialdehyde and percentage of apoptotic cells in irradiated human lymphocytes (mean ± SD).

entin induced marginal, non-significant reduction in MDA levels. Norswertianin-8-*O*-primveroside and gentiacaulein induced significant increases in MDA levels (20.24 and 7.45% respectively).

Effect of G. dinarica polyphenols on apoptotic cell death in irradiated human lymphocytes

Apoptosis was maximally induced by treating cells with plant infusion (249.50% higher than controls). Significant increase in the percentage of apoptotic cells was induced by norswertianin-1-*O*-primveroside, isoorientin-4′-*O*-glycoside, norswertianin-8-*O*-primveroside and isoorientin (85.49, 41.29, 16.94 and 8.97% respectively). Gentiakochianin decreased the percentage of apoptotic cells by 37.87, whereas treating cells with gentiacaulein did not significantly alter the percentage of apoptotic cells.

Effect of G. dinarica polyphenols on cell proliferation in irradiated human lymphocytes

Treatment of irradiated human lymphocytes with *G. dinarica* polyphenols inhibited cell proliferation, as revealed by significant reductions in the CBPI. The maximal reduction of cell proliferation was observed in cells treated with norswertianin-8-*O*-primveroside (18.06% lower than controls). Significant decrease in the proliferation index was observed for isoorientin-4′-*O*-glycoside, norswertianin-1-*O*-primveroside and gentiakochianin (16.13, 10.97 and 9.68% respectively). CBPI was 7.10% lower in cells treated with either gentiacaulein or plant infusion. Isoorientin caused no significant change in the cell proliferation index.

Effect of amifostine in irradiated human lymphocytes

Compared to control cells, amifostine reduced the frequency of MN by 16.48%, MDA levels by 4.82%, and increased apoptosis levels by 6.00%. Amifostine did not significantly influence the cell proliferation index.

Discussion

Endogenous antioxidants such as cellular thiols and antioxidant enzymes provide protection against free radicals produced by the body's normal use of oxygen during respiration and some cell-mediated immune functions[27]. Additional production of free radicals by ionizing radiation overwhelms the antioxidant capacity of the cell, intracel-

lular redox homeostasis is disrupted, and oxidative stress ensues[28]. Excessive increase in ROS production has been implicated in the pathogenesis of cancer, atherosclerosis, neurodegenerative diseases and other diseases[29]. A number of medicinal plants have shown radioprotective and radiorecovery effects[21]. The radioprotective efficacy of *Ginkgo biloba*, *Podophyillum hexandrum*, *Mentha piperica* and *Mangifera indica* has been attributed to the antioxidant activities of polyphenols contained in these plants[21,30]. In this study, we examined the radioprotective properties of *G. dinarica* polyphenols and their influence on different end-points of radiation damage. The C-glucoflavones isoorientin and isoorientin-4′-*O*-glycoside possess two to three double bonds in conjugation with 4-oxo function that are responsible for electron delocalization from the B ring. The electron- and H^+-donating capacities of these compounds contribute to the termination of lipid peroxidation chain reactions based on their reducing power, allowing them to act as antioxidants. Treatment of irradiated cells with these flavones decreased the incidence of radiation-induced MN and MDA levels. The maximum inhibition of lipid peroxidation (39.75%) was observed in cells treated with isoorientin-4′-*O*-glycoside. Isoorientin has a catechol moiety in its structure. Miura and co-workers[31] have suggested that flavonoids with catechol moieties in their structure can generate hydrogen peroxide by donating hydrogen from the catechol group to oxygen through a superoxide (O_2^-) anion radical. Increased H_2O_2 generation leads to more potent radical trapping. The response of cells to H_2O_2 depends on cell type and the level of antioxidant enzymes such as catalase and other H_2O_2-removing enzymes[27]. It has been suggested that H_2O_2 might contribute to apoptosis[32]. These C-glucoflavones significantly increased the percentage of apoptotic cells and inhibited cell proliferation, although treatment of cells with isoorientin-4′-*O*-glycoside provided better radioprotective effects than treatment with isoorientin. It is possible that isoorientin-4′-*O*-glycoside induces cell-cycle arrest, allowing cells to complete DNA repair before replication or division. It has been reported that polyphenols can induce cell-cycle arrest and apoptosis[33,34]. Prolonged time for DNA repair and elimination of heavily damaged cells via apoptosis might enable the maintenance of homeostasis in irradiated tissue.

The tetraoxygenated xanthone glycoside norswertianin-1-*O*-primveroside acted as an antioxidant, as evidenced by significant decrease in radiation-induced MN and MDA levels. It also inhibited cell proliferation and enhanced apoptosis. The exact mechanisms underlying its activity are not known. Most data regarding the antioxidant activities of xanthone glycosides are derived from studies of mangiferin. It has been reported that mangiferin inhibits the activation of NFκB, which plays a pivotal role in suppressing apoptosis[35]. It was also shown that mangiferin has an antiproliferative effect on leukaemia cells by inducing apoptosis, probably through down-regulation of *bcr/abl* expression[36]. It is possible that norswertianin-1-*O*-primveroside behaves in a similar fashion.

Unexpectedly, the tetraoxygenated xanthone glycoside norswertianin-8-*O*-primveroside exhibited pro-oxidative effects. Similar results were observed after treatment of cells with the tetraoxygenated xanthone gentiacaulein. These compounds stimulated oxidative stress, resulting in increased lipid peroxidation in the cell membrane and decreased cell proliferation. This cytotoxicity might be due to the inhibition of redox reactions by these polyphenols[37]. It was shown that polyphenols may decrease the activities of key antioxidant enzymes[38,39]. Polyphenolic compounds might form complexes with proteins through hydrogen and covalent bonds to cause enzyme inhibition, thereby promoting oxidative stress[37]. The induction of MDA by norswertianin-8-*O*-primveroside resulted in growth inhibition and led to apoptosis rather than reproductive cell death. In contrast, the antiproliferative effect of gentiacaulein was not accompanied by the enhancement of apoptosis.

The tetraoxygenated simple xanthone gentiakochianin exhibited cytostatic effects in irradiated lymphocytes. It significantly reduced reproductive cell death and lipid peroxidation. The antiproliferative effect of this xanthone was associated with the suppression of apoptosis. Tetraoxygenated xanthones have been reported to suppress apoptosis by influencing calcium signalling mechanisms[40]. Blocking plasma membrane calcium channels protects cells from apoptosis[41,42], and elevated intracellular calcium can activate the transcription of NFκB, which, as mentioned before, suppresses apoptosis[43]. The reduction of radiation-induced apoptosis has also been demonstrated in mouse crypt cells treated with *P. hexandrum* extracts[44], and in rat cerebellar neuronal cell cultures treated with *G. biloba* leaf extract[45]. The mechanisms underlying the activity of gentiakochianin require further detailed investigation, since it may serve as a novel powerful antitumour agent and free-radical scavenger.

The results presented here indicate that better understanding of the structure–activity relationship of these polyphenols and their different mechanisms of action might provide better therapeutic applications. The complexity of the action of the polyphenols tested was evident upon treatment of irradiated lymphocytes with *G. dinarica* infusion. Administration of plant infusion cannot be expected to produce the same effects as the administration of pure compounds. Interactions between individual polyphenols might determine the overall outcome of treatment with a mixture of these compounds. Because of this, results obtained with crude plant extracts must be considered with caution[46].

G. dinarica infusion significantly decreased lipid peroxidation and had antiproliferative effects associated with enhanced apoptosis. It also led to increased reproductive cell death. One possible explanation for the higher inci-

dence of MN in infusion-treated cells than in controls is that the synergistic and/or balanced action of its polyphenols modulates the radiobiological response of cells by influencing antioxidant enzymes, possibly decreasing their activity. The polyphenols quercetin, morin, narnigenin and hesperetin have been shown to decrease glutathione transferase activity, resulting in DNA damage in human lymphocytes[47]. These observations emphasize the importance of developing therapeutic formulations with appropriate proportions of bioactive constituents to achieve the desired beneficial effects.

Conclusion

Our results clearly indicate that *G. dinarica* polyphenols have significant potential to protect cells from radiation-induced damage. This study provides insights that might aid in identifying better and safer therapies which can be used to protect against the harmful effects of radiation.

1. Halliwell, B. and Gutteridge, J. M. C., Oxidative stress: Adaptation, damage, repair and death. In *Free Radicals in Biology and Medicine*, Oxford University Press, New York, 1999, pp. 246–351.
2. Valko, M., Izakovic, M., Mazur, M., Rhodes, C. J. and Telser, J., Role of oxygen radicals in DNA damage and cancer incidence. *Mol. Cell. Biochem.*, 2004, **266**, 37–56.
3. Wallace, S. S., Detection and repair of DNA base damages produced by ionizing radiation. *Environ. Mol. Mutagen.*, 1988, **12**, 431–477.
4. Turner, N. D., Brady, L. A., Ford, J. and Lupton, J. R., Opportunities for nutritional amelioration of radiation-induced cellular damage. *Nutrition*, 2002, **18**, 904–912.
5. Borek, C., Antioxidants and radiation therapy. *J. Nutr.*, 2004, **134**, 3207–3209.
6. Manach, C., Scalbert, A., Morand, C., Remesy, C. and Jimenez, L., Polyphenols: Food sources and bioavailability. *Am. J. Clin. Nutr.*, 2004, **79**, 727–747.
7. Rice-Evans, C., Flavonoid antioxidants. *Curr. Med. Chem.*, 2001, **8**, 797–809.
8. Chen, P. C., Wheeler, D. S., Malhotra, V., Odoms, K., Denenberg, A. G. and Wong, H. R., A green tea-derived polyphenol, epigallocatechin-3-gallate, inhibits IkappaB kinase activation and IL-8 gene expression in respiratory epithelium. *Inflamation*, 2002, **26**, 233–241.
9. Pfeilschifter, J., Eberhardt, W., Beck, K. F. and Huwiler, A., Redox signaling in mesangial cells. *Nephron Exp. Nephrol.*, 2003, **93**, 23–26.
10. Weiss, J. F. and Landauer, M. R., Protection against ionizing radiation by antioxidant nutrients and phytochemicals. *Toxicology*, 2003, **189**, 1–20.
11. Jagetia, G. C., Baliga, M. S. and Venkatesh, P., Influence of seed extract of *Syzygium cumini* (Jamun) on mice exposed to different doses of γ-radiation. *J. Radiat. Res.*, 2005, **1**, 59–65.
12. Perry, E. K., Pickering, A. T., Wang, W. W., Houghton, P. J. and Perru, N. S., Medicinal plants and Alzheimer's disease: From ethnobotany to phytotherapy. *J. Pharm. Pharmacol.*, 1999, **51**, 527–534.
13. Lin, C. C. and Huang, P. C., Antioxidant and hepatoprotective effects of *Acathopanax senticosus*. *Phytother. Res.*, 2000, **14**, 489–494.
14. Leskovac, A., Joksic, G., Jankovic, T., Savikin, K. and Menkovic, N., Radioprotective properties of the phytochemically characterized extracts of *Crataegus monogyna*, *Cornus mas* and *Gentianella austriaca* on human lymphocytes *in vitro*. *Planta Med.*, 2007, **73**, 1169–1175.
15. Tutin, T. G., *Gentiana* L. In *Flora Europea* (eds Tutin, T. G. *et al.*), Cambridge University Press, Cambridge, 1972, vol. 3, pp. 59–67.
16. Krstić, D., Janković, T., Aljančić, I., Šavikin-Fodulović, K., Menković, N. and Milosavljević, S., Phytochemical investigation of *Gentiana dinarica*. *Biochem. Syst. Ecol.*, 2004, **32**, 937–941.
17. Brahmachari, G., Mondal, S., Gangopadhyay, A., Gorai, D., Mukhopadhyay, B., Saha, S. and Brahmachari, A. K., *Swertia* (Gentianaceae): Chemical and pharmacological aspects. *Chem. Biodivers.*, 2004, **1**, 1627–1651.
18. Menkovic, N. *et al.*, Xanthones from *Swertia punctata*. *Phytochemistry*, 2002, **61**, 415–420.
19. Basnet, P., Kadota, S., Shimizu, M. and Namba, T., Bellidifolin: A potent hypoglycemic agent in streptozotocin (STZ)-induced diabetic rats from *Swertia japonica*. *Planta Med.*, 1994, **60**, 507–511.
20. Law on Health Care, Official Gazette of the Republic of Serbia, Parliament of the Republic of Serbia., 2005, vol. 107, pp. 112–161.
21. Arora, R. *et al.*, Radioprotection by plant products: Present status and future prospects. *Phytother. Res.*, 2005, **19**, 1–22.
22. Fenech, M., The cytokinesis blocks micronucleus technique: A detailed description on the method and its application to genotoxicity studies in human population. *Mutat. Res.*, 1993, **285**, 35–44.
23. Surrales, J., Xamena, N., Creus, A. and Marco, R., The suitability of the micronucleus assay in human lymphocytes as a new biomarker of excision repair. *Mutat. Res.*, 1995, **342**, 43–59.
24. Aruoma, O. L., Halliwell, B., Laughton, M. J., Quinland, G. J. and Gutteridge, J. M. C., The mechanism of initiation of lipid peroxidation. Evidence against a requirement for an iron(II)–iron(III) complex. *Biochem. J.*, 1989, **258**, 617–620.
25. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J., Protein measurement with the Folin phenol reagent. *J. Biol. Chem.*, 1951, **193**, 265–275.
26. Crompton, N. E. and Ozsahin, M., A versatile and rapid radiosensitivity assay of peripheral blood leukocytes based on DNA and surface-marker assessment of cytotoxicity. *Radiat. Res.*, 1997, **147**, 55–60.
27. Tiwari, A. K., Imbalance in antioxidant defence and human diseases: Multiple approach of natural antioxidants therapy. *Curr. Sci.*, 2001, **81**, 1179–1187.
28. Halliwell, B., Antioxidant defense mechanisms: From the beginning to the end (of the beginning). *Free Radic. Res.*, 1999, **31**, 261–272.
29. Toyokuni, S., Reactive oxigen species-induced molecular damage and its application in pathology. *Pathol. Int.*, 1999, **49**, 91–102.
30. Jagetia, G. C. and Venkatesha, V. A., Effect of mangiferin on radiation-induced micronucleus formation in cultured human peripheral blood lymphocytes. *Environ. Mol. Mutagen.*, 2005, **46**, 12–21.
31. Miura, Y. H., Tomita, I., Watanabe, T., Hirayama, T. and Fukui, S., Active oxygens generation by flavonoids. *Biol. Pharm. Bull.*, 1998, **21**, 93–96.
32. Hou, Z., Lambert, J. D., Chin, K. V. and Yang, C. S., Effects of tea polyphenols on signal transduction pathways related to cancer chemoprevention. *Mutat. Res.*, 2004, **555**, 3–19.
33. Nifli, A. P., Kampa, M., Alexaki, V. I., Notas, G. and Castanas, E., Polyphenol interaction with the T47D human breast cancer cell line. *J. Dairy Res.*, 2005, **72**, 44–50.
34. Watanabe, N., Hirayama, R. and Kubota, N., The chemopreventive flavonoid apigenin confers radiosensitizing effect in human tumor cells grown as monolayers and spheroids. *J. Radiat. Res.*, 2007, **48**, 45–50.

35. Sarkar, A., Sreenivasan, Y., Ramesh, G. T. and Manna, S. K., Beta-D-glucoside suppresses TNF-induced activation of nuclear transcription factor kappaB but potentiates apoptosis. *J. Biol. Chem.*, 2004, **279**, 33768–33781.
36. Peng, Z. G., Luo, J., Xia, L. H., Chen, Y. and Song, S. J., CML cell line K562 cell apoptosis induced by mangiferin. *Zhongguo Shi Yan Xue Ye Xue Za Zhi*, 2004, **12**, 590–594.
37. Elliot, A. J., Scheiber, S. A., Thomas, C. and Pardini, R. S., Inhibition of glutathione reductase by flavonoids. *Biochem. Pharmacol.*, 1992, **44**, 1603–1608.
38. Pinto, M. M. M., Sousa, M. E. and Nascimento, M. S. J., Xanthone derivatives: New insights in biological activities. *Curr. Med. Chem.*, 2005, **12**, 2517–2538.
39. Muruganandan, S., Gupta, S., Kataria, M., Lal, J. and Gupta, P. K., Mangiferin protects the streptozotocin-induced oxidative damage to cardiac and renal tissues in rats. *Toxicology*, 2002, **176**, 165–173.
40. Yen, G. C., Duh, P. D., Tsai, H. L. and Huang, S. L., Pro-oxidative properties of flavonoids in human lymphocytes. *Biosci. Biotechnol. Biochem.*, 2003, **67**, 1215–1222.
41. McConkey, D. J., Chow, S. C., Orrenius, S. and Jondal, M., NK cell-induced cytotoxicity is dependent on a Ca^{2+} increase in the target. *FASEB J.*, 1990, **4**, 2661–2664.
42. Juntti-Berggren, L. *et al.*, Increased activity of L-type Ca^{2+} channels exposed to serum from patients with type I diabetes. *Science*, 1993, **261**, 86–90.
43. Lotem, J. and Sachs, L., Different mechanisms for suppression of apoptosis by cytokines and calcium mobilizing compounds. *Proc. Natl. Acad. Sci. USA*, 1998, **95**, 4601–4606.
44. Coleman, C. N. *et al.*, Molecular and cellular biology of moderate-dose (1–10 Gy) radiation and potential mechanisms of radiation protection. *Radiat. Res.*, 2003, **159**, 812–834.
45. Ni, Y. B., Zhao, B., Hou, J. W. and Xin, W. J., Preventive effect of *Ginkgo biloba* extract on apoptosis in rat cerebellar neuronal cells induced by hydroxyl radicals. *Neurosci. Lett.*, 1996, **214**, 115–118.
46. Kapiszewska, M., Soltys, E., Visioli, F., Cierniak, A. and Zajac, G., The protective ability of the Mediterranean plant extracts against the oxidative DNA damage. The role of the radical oxygen species and the polyphenol content. *J. Physiol. Pharmacol.*, 2005, **56**, 183–197.
47. Kang, J. J., Cheng, Y. W., Ko, F. N., Kuo, M. L., Lin, C. N. and Teng, C. M., Induction of calcium release from sarcoplasmic reticulum of skeletal muscle by xanthone and norathyriol. *Br. J. Pharmacol.*, 1996, **118**, 1736–1742.

ACKNOWLEDGEMENTS. We thank Institute for Medicinal Plant Research 'Dr Josif Pancic', Belgrade for providing the plant material, and the Serbian Ministry of Science and Environmental Protection, Project No. 143046 for financial support.

Received 14 December 2007; revised accepted 27 August 2008