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Protective Activity against Ionizing Radiation of Antioxidative Plants Indigenous to Korea

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Abstract – We have screened the cytoprotective effect on γ-ray radiation induced oxidative stress from forty one Korean plant extracts. *Carpinus laxiflora* (caulis), *Quercus salicina* (caulis), and *Castanopsis cuspidata* (caulis) were found to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and intracellular reactive oxygen species (ROS). As a result, extracts of three plants reduced cell death of Chinese hamster lung fibroblast (V79-4) cells induced by H_2O_2 treatment. In addition, these extracts protected cell death of V79-4 cells damaged by γ-ray radiation. In addition, these extracts scavenged ROS generated by radiation. Taken together, the results suggest that *Carpinus laxiflora*, *Quercus salicina*, and *Castanopsis cuspidata* protect V79-4 cells against oxidative damage by radiation through scavenging ROS.
Keywords – γ-ray radiation, oxidative stress, reactive oxygen species

Introduction

The potential application of radiation protective chemicals in the event of planned exposure or radiation accidents has been investigated from the beginning of the nuclear era (Weiss and Simic, 1988). It has also been considered possible that radiation therapy for cancer patients could be improved by use of radiation protectors to protect normal tissue. Early investigators attempted to use radiation protectors to help elucidate the mechanism of interaction of radiation on molecules of biological importance. It was suggested that both radiation injury and oxygen poisoning occur through the formation of ROS (Gerschman *et al.*, 1954). Sulfhydryl agents such as cysteine, glutathione, β-mercaptoethylamine (cysteamine), and other antioxidants shown to protect mice against the lethal effects of radiation could also increase survival of mice exposed to high oxygen tension. Increased understandings of the interrelationship between oxygen effects and the radiation exposure lead to a rational application of naturally occurring antioxidants (Weiss and Landauer, 2000).

In the present study, we screened the antioxidative effect of plant extracts, and the active extract was investigated for the protective effect against γ-ray radiation.

Experimental

Plant material and its extract – The plant materials were purchased or obtained from Korea Research Institute of Bioscience and Biotechnology (KRIBB) and Jeju-do Agricultural Research & Extension Service, respectively. Voucher specimens were deposited in the KRIBB and Jeju-do Agricultural Research & Extension Service.

Reagents – 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and 2',7'-dichlorodihydrofluorescein diacetate (DCF-DA) were purchased from Sigma Chemical Company, St. Louis, MO, USA.

Cell culture – It is reported that lung is an organ sensitive to oxidative stress. Oxidative stress induced gene expression profiles were investigated using microarray in fibroblast and Hela cell lines. Many of genes in fibroblast other than Hela cells were induced by oxidative stress (Pryor *et al.*, 1998; Murray *et al.*, 2004). To study the effect of plant extracts on oxidative stress, we used Chinese hamster lung fibroblasts (V79-4 cells). The V79-

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4 cells from the American type culture collection, were maintained at 37 °C in an incubator with a humidified atmosphere of 5% CO_2 and cultured in Dulbecco's modified Eagle's medium containing 10% heat-inactivated fetal calf serum, streptomycin (100 µg/ml) and penicillin (100 units/ml).

Irradiation – Cells were exposed to γ-ray from a ^{60}Co γ-ray source (MDS Nordion C-188 standard source, located in Cheju National University, Jeju, Korea).

DPPH radical scavenging activity – Various concentrations of plant extracts were added to a 1×10^{-4} M solution of DPPH in methanol, and the reaction mixture was shaken vigorously. After 1 h, the amount of residual DPPH was determined at 520 nm using a spectrophotometer (Lo *et al.*, 2004).

Intracellular reactive oxygen species measurement – The DCF-DA method was used to detect the intracellular ROS level (Rosenkranz *et al.*, 1992). DCF-DA diffuses into cells, where it is hydrolyzed by intracellular esterase to polar 2',7'-dichlorodihydrofluorescein. This non-fluorescent fluorescein analog gets trapped inside the cells and is oxidized by intracellular oxidants to a highly fluorescent, 2',7'-dichlorofluorescein. The V79-4 cells were seeded in a 96 well plate. Sixteen hours after plating, the cells were treated with plant extracts and 1 h later, 1 mM H_2O_2 or γ-ray radiation at 10 Gy was added to the plate. The cells were incubated for an additional 30 min at 37 °C. After addition of 25 µM of DCF-DA solution, the fluorescence of 2',7'-dichlorofluorescein was detected at 485 nm excitation and at 535 nm emission using a PerkinElmer LS-5B spectrofluorometer.

Cell viability – The effect of plant extracts on the viability of the V79-4 cells was determined using the [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium] bromide (MTT) assay, which is based on the reduction of a tetrazolium salt by mitochondrial dehydrogenase in the viable cells (Carmichael *et al.*, 1987). To determine the effect of plant extracts on the viability of V79-4 cells on H_2O_2 or γ-ray radiation, cells were seeded in a 96 well plate at 1×10^5 cells/ml. Sixteen hours after plating, cells were treated with 10 µg/ml of plant extracts for 1 h. Plates were treated 1 mM H_2O_2 or irradiated at 10 Gy and the plate was incubated at 37 °C for 24 h and the cell viability was measured using MTT test. Fifty ml of the MTT stock solution (2 mg/ml) was then added to each well to attain a total reaction volume of 200 µl. After incubating for 4 h, the plate was centrifuged at 800 × g for 5 min and the supernatants were aspirated. The formazan crystals in each well were dissolved in 150 µl dimethylsulfoxide (DMSO) and the A_{540} was read on a scanning multi-well spectrophotometer.

Statistical analysis – All the measurements were made

Table 1. The list of species used in these experiments

scientific name	family	used part
Carpinus tschonoskii	Betulaceae	caulis
Carpinus laxiflora	Betulaceae	caulis
Carpinus laxiflora	Betulaceae	leaves
Hedera rhombea	Araliaceae	caulis
Hedera rhombea	Araliaceae	fruits
Trachelospermum asiaticum var. intermedium	Apocynaceae	leaves
Quercus salicina Bl.	Fagaceae	caulis
Lonicera japonica	Caprifoliaceae	whole plant
Rosa multiflora	Rosaceae	whole plant
Castanopsis cuspidata var. Sieboldii Nakai	Fagaceae	caulis
Stephanandra incisa	Rosaceae	caulis
Sapium japonicum	Euphorbiaceae	leaves
Rubus crataegifolius	Rosaceae	leaves
Kalopanax pictus	Araliaceae	leaves
Ixeris stolonifera	Compositae	whole plant
Houttuynia cordata	Saururaceae	whole plant
Euphorbia upine Rafin.	Euphorbiaceae	caulis
Rubus coreanus	Rosaceae	leaves, caulis
Machilus japonica S. et Z.	Lauraceae	caulis
Clematis apiifolia	Ranunculaceae	leaves, caulis
Quercus glauca	Fagaceae	caulis
Prunella vulgaris var. lilacina	Labiatae	whole plant
Ixeris dentata	Compositae	whole plant
Ranunculus japonicus	Ranunculaceae	whole plant
Quercus acuta Thunb.	Fagaceae	caulis
Ligularia fischeri	Compositae	whole plant
Rubus oldhamii	Rosaceae	leaves
Erigeron annuus	Compositae	whole plant
Ajuga decumbens	Labiatae	whole plant
Sasa quelpaertensis Nakai	Gramineae	roots
Sasa quelpaertensis Nakai	Gramineae	caulis
Sasa quelpaertensis Nakai	Gramineae	leaves
Acanthopanax koreanum Nakai	Araliaceae	roots
Prunus buergeriana	Rosaceae	leaves
Senecio nemorensis	Compositae	whole plant
Viburnum furcatum	Caprifoliaceae	caulis
Viburnum furcatum	Caprifoliaceae	leaves
Daphniphyllum macropodum	Euphorbiaceae	leaves
Scilla scilloides	Liliaceae	whole plant
Maackia fauriei	Leguminosae	caulis
Lotus corniculatus var. japonicus	Leguminosae	whole plant

Table 2. Effect of Korean plant extracts on scavenging DPPH

scientific name	concentration (μg/ml)		
	0.1	1	10
Carpinus tschonoskii	4.8 ± 1.3	12.0 ± 2.3	60.6 ± 1.6
Carpinus laxiflora (Bark)	0	4.6 ± 1.6	53.4 ± 0.5[a]
Carpinus laxiflora (Leaves)	4.4 ± 2.3	12.4 ± 5.2	54.8 ± 2.3
Hedera rhombea (Caulis)	3.9 ± 2.2	4.3 ± 1.1	21.2 ± 1.3
Hedera rhombea (Fruits)	2.8 ± 1.3	10.4 ± 1.1	15.3 ± 1.2
Trachelospermum asiaticum var. intermedium	0	4.5 ± 2.1	23.8 ± 2.2
Quercus salicina Bl.	0	4.8 ± 2.6	54.5 ± 2.7[a]
Lonicera japonica	0	5.9 ± 2.6	40.4 ± 2.3
Rosa multiflora	5.0 ± 2.1	5.4 ± 1.6	24.0 ± 1.9
Castanopsis cuspidata var. Sieboldii Nakai	0	2.5 ± 0.8	52.3 ± 2.6[a]
Stephanandra incisa	1.3 ± 2.1	4.5 ± 1.4	41.8 ± 2.4
Sapium japonicum	0	6.1 ± 1.1	44.1 ± 2.6
Rubus crataegifolius	6.2 ± 2.6	11.7 ± 2.7	25.2 ± 3.3
Kalopanax pictus	2.5 ± 1.3	2.1 ± 1.8	17.7 ± 3.4
Ixeris stolonifera	0	0	3.6 ± 1.2
Houttuynia cordata	3.2 ± 1.6	8.0 ± 1.5	23.0 ± 1.4
Euphorbia upine Rafin.	0	2.1 ± 0.8	45.9 ± 1.4
Rubus coreanus	0	0.7 ± 0.3	26.4 ± 2.8
Machilus japonica S. et Z.	0	0.5 ± 0.2	38.1 ± 3.5
Clematis apiifolia	0	0	3.2 ± 1.7
Quercus glauca	0	4.2 ± 2.3	41.9 ± 3.3
Prunella vulgaris var. lilacina	0	0	23.3 ± 3.6
Ixeris dentata	4.0 ± 3.3	7.1 ± 2.3	24.9 ± 3.9
Ranunculus japonicus	0	0	4.2 ± 1.8
Quercus acuta Thunb.	0	7.0 ± 1.7	42.7 ± 2.5
Ligularia fischeri	2.6 ± 1.2	7.3 ± 2.2	14.9 ± 2.3
Rubus oldhamii	0	0	2.9 ± 1.6
Erigeron annuus	4.2 ± 1.8	5.7 ± 1.3	13.1 ± 2.1
Ajuga decumbens	0	0	0
Sasa quelpaertensis Nakai (Roots)	0	0	8.5 ± 1.3
Sasa quelpaertensis Nakai (Caulis)	0	0	3.6 ± 1.1
Sasa quelpaertensis Nakai (Leaves)	0	0	0
Acanthopanax koreanum Nakai	0	0	0
Prunus buergeriana	0	0	3.4 ± 1.2
Senecio nemorensis	0	0	2.5 ± 1.4
Viburnum furcatum (Bark)	0	0.9 ± 0.4	1.3 ± 0.3
Viburnum furcatum (Leaves)	0	0	4.4 ± 1.7
Daphniphyllum macropodum	1.9 ± 0.5	2.9 ± 1.3	4.4 ± 1.3
Scilla scilloides	0	0	0
Maackia fauriei	4.9 ± 1.1	6.6 ± 1.3	17.7 ± 2.4
Lotus corniculatus var. japonicus	0	0	1.5 ± 1.3

[a]Significantly different from control ($p < 0.05$).

Table 3. Effect of Korean plant extracts on scavenging intracellular ROS induced by H_2O_2

scientific name	concentration (μg/ml)		
	0.1	1	10
Carpinus tschonoskii	34.8 ± 1.3	51.7 ± 1.6	71.5 ± 2.5
Carpinus laxiflora (Bark)	6.7 ± 1.6	50.6 ± 2.3	77.9 ± 0.2[a]
Carpinus laxiflora (Leaves)	30.9 ± 1.6	58.0 ± 2.3	74.5 ± 2.3
Hedera rhombea (Caulis)	36.0 ± 1.3	49.3 ± 2.3	78.6 ± 0.3
Hedera rhombea (Fruits)	18.1 ± 1.5	31.1 ± 1.7	48.5 ± 2.1
Trachelospermum asiaticum var. intermedium	23.3 ± 1.6	36.2 ± 1.1	76.3 ± 0.5
Quercus salicina Bl.	9.4 ± 1.6	41.4 ± 1.3	75.6 ± 0.8[a]
Lonicera japonica	41.8 ± 1.1	57.9 ± 1.6	74.1 ± 1.8
Rosa multiflora	46.5 ± 2.1	52.8 ± 1.7	74.0 ± 1.3
Castanopsis cuspidata var. Sieboldii Nakai	21.7 ± 1.1	49.9 ± 1.3	73.4 ± 1.6[a]
Stephanandra incisa	40.8 ± 2.6	45.1 ± 1.6	60.2 ± 1.7
Sapium japonicum	39.5 ± 1.3	53.0 ± 2.3	71.7 ± 2.2
Rubus crataegifolius	31.4 ± 2.3	41.9 ± 1.1	68.4 ± 1.6
Kalopanax pictus	39.6 ± 1.6	42.1 ± 1.3	68.3 ± 2.3
Ixeris stolonifera	41.2 ± 1.6	53.0 ± 2.3	68.3 ± 1.1
Houttuynia cordata	0	8.6 ± 1.3	68.3 ± 2.3
Euphorbia upine Rafin.	9.2 ± 1.1	41.1 ± 1.7	68.0 ± 1.3
Rubus coreanus	29.6 ± 1.6	43.6 ± 1.3	66.1 ± 1.1
Machilus japonica S. et Z.	19.6 ± 1.2	25.5 ± 1.1	64.7 ± 1.6
Clematis apiifolia	28.3 ± 1.7	41.2 ± 1.3	64.5 ± 2.3
Quercus glauca	5.9 ± 1.3	21.1 ± 2.3	64.5 ± 1.6
Prunella vulgaris var. lilacina	19.9 ± 1.6	28.0 ± 2.5	63.2 ± 1.3
Ixeris dentata	11.8 ± 2.7	22.1 ± 1.6	61.0 ± 1.3
Ranunculus japonicus	54.1 ± 1.7	53.6 ± 1.7	60.3 ± 2.5
Quercus acuta Thunb.	0	24.9 ± 1.3	60.0 ± 1.7
Ligularia fischeri	9.2 ± 1.1	25.8 ± 1.3	56.7 ± 1.6
Rubus oldhamii	28.4 ± 2.2	35.5 ± 1.7	46.5 ± 2.5
Erigeron annuus	20.6 ± 1.6	31.6 ± 2.1	44.3 ± 2.8
Ajuga decumbens	20.2 ± 1.7	22.8 ± 1.5	43.0 ± 2.1
Sasa quelpaertensis Nakai (Roots)	3.9 ± 1.5	7.4 ± 2.4	40.9 ± 3.2
Sasa quelpaertensis Nakai (Caulis)	0	30.6 ± 1.7	30.3 ± 1.5
Sasa quelpaertensis Nakai (Leaves)	0	9.2 ± 1.2	55.3 ± 1.5
Acanthopanax koreanum Nakai	0	0	38.9 ± 2.7
Prunus buergeriana	19.3 ± 0.7	27.3 ± 1.7	35.9 ± 2.8
Senecio nemorensis	16.9 ± 2.1	20.9 ± 1.7	34.6 ± 2.1
Viburnum furcatum (Bark)	0	8.2 ± 1.5	31.9 ± 0.9
Viburnum furcatum (Leaves)	0.9 ± 0.5	24.2 ± 1.7	26.6 ± 1.4
Daphniphyllum macropodum	2.5 ± 1.5	14.1 ± 1.7	28.3 ± 2.1
Scilla scilloides	0	2.7 ± 1.1	23.6 ± 2.1
Maackia fauriei	14.5 ± 1.6	32.3 ± 1.7	57.0 ± 1.5
Lotus corniculatus var. japonicus	0	0	14.4 ± 2.3

[a]significantly different from control ($p < 0.05$).

in triplicate and all values were represented as means ± S.E. The results were subjected to an analysis of the variance (ANOVA) using the Tukey test to analyze the difference. $p < 0.05$ were considered significantly.

Results and Discussion

A large number of plants contain antioxidant phytochemicals reported to be radiation protective in various model systems. Antioxidants interfere with the initial stage of apoptosis by ROS (Salganik, 2001), as well as later membrane lipid peroxidation, which is characteristic of radiation induced apoptosis (McClain *et al.,* 1995). From tested forty one Korean plant extracts (Table 1), *Carpinus laxiflora* (caulis), *Quercus salicina* (caulis), and *Castanopsis cuspidata* (caulis) were found to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical, showing at 10 μg/ml 53%, 55%, and 52%, respectively (Table 2) and intracellular ROS, showing at 10 μg/ml 78%, 76%, and 74%, respectively (Table 3). As a result, extracts of three plants reduced cell death of V79-4 cells induced by H_2O_2 treatment, showing the cell viability of 99%, 94%, and 98%, respectively, compared to cell viability of 90% in H_2O_2 treated cells (Fig. 1). These extracts protected cell death of V79-4 cells damaged by γ-ray radiation, showing the cell viability of 77%, 78%, and 84%, respectively, compared to cell viability of 70% in 10 Gy radiated cells (Fig. 2) and scavenged ROS generated by radiation, showing the percentage of intracellular ROS generation of 81%, 84%, and 86%, respectively, compared to 100% in 10 Gy radiated cells (Fig. 3). Genus *Carpinus* consists of 40 species and distributed in the temperate area of the northern hemisphere. Only 5 species of *C. laxiflora*, *C. tschonoskii*, *C. cordata*, *C. turczaninowi*, and *C. coreana* grow in Korean peninsula (Lee *et al.*, 1989). And there has been a report on the isolation of flavonoids from Genus *Carpinus*, which contains flavonols myricetin, kaempferol and quercetin, and the flavones apigenin and luteolin (Chang *et al.*, 2004). These naturally occurring flavonoids are widely distributed in plant kingdom and their antioxidant properties are well studied. Therefore, the antioxidant activity of *C. laxiflora,* which belongs to

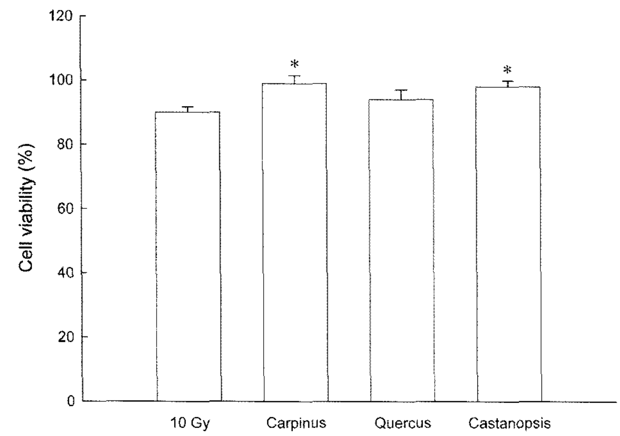

Fig. 1. Protective effect of Korean plant extracts upon H_2O_2 induced oxidative damage of V79-4 cells. The viability of V79-4 cells upon H_2O_2 was determined by MTT assay The measurements were made in triplicate and values are expressed as means ± S.E. *significantly different from control ($p < 0.05$).

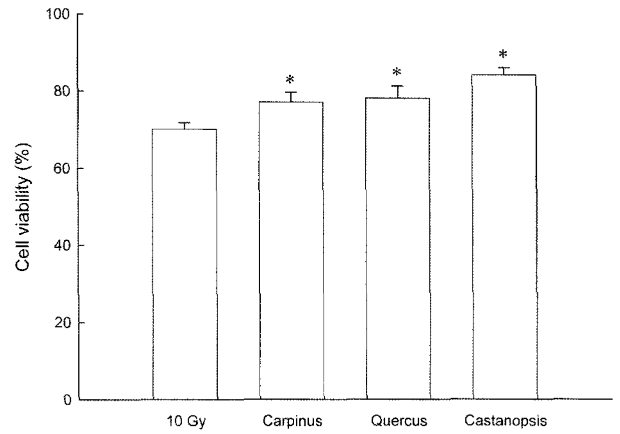

Fig. 2. Protective effect of Korean plant extracts upon γ-ray radiation induced oxidative damage of V79-4 cells The viability of V79-4 cells upon radiation was determined by MTT assay. The measurements were made in triplicate and values are expressed as means ± S.E. *significantly different from control ($p < 0.05$).

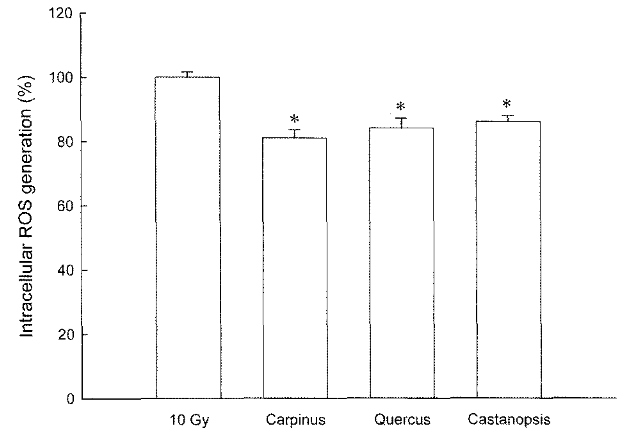

Fig. 3. Effect of Korean plant extracts on scavenging intracellular ROS generated by radiation. The intracellular ROS was detected by DCF-DA method The measurements were made in triplicate and values are expressed as means ± S.E. *significantly different from control ($p < 0.05$).

Genus *Carpinus,* might be related with the flavonoids, and the chemical constituents are remained for further research. From the Genus *Quercus*, monoglycoside of flavonols, kaempferol 3-*O*-D-glucopyranoside, quercetin 3-*O*-D-glucopyranoside, kaempferol 3-*O*-(6"-*trans-p*-coumaroyl)-D-glucopyranoside, kaempferol 3-*O*-(2",6"-di-*trans-p*-coumaroyl)-D-glucopyranoside, kaempferol 3-*O*-(2",4"-di-acetyl-3"-*cis-p*-coumaroyl-6"-*trans-p*-coumaroyl)-D-glucopyranoside and tannins were isolated (Meng *et al*., 2001; Vivas *et al.,* 2004). These natural polyphenols have an ideal and intrinsic structure of capturing of free radicals and electron delocalization, causing higher antioxidant activity than known antioxidants, such as vitamins A and E (Sokmen *et al*., 2005). The antioxidant activity of *Quercus salicina,* which belongs to the genus *Quercus,* might be related with polyphenols. In addition, phenolic compounds were reported from the genus *Castanopsis* (Chen *et al.,* 1993). These polyphenols also might be responsible for the antioxidant activity of *Castanopsis cuspidata,* which belongs to the genus *Castanopsis*. The radiation protective activity shown by these extracts have been attributed by their reducing ROS, oxidative stress. Crude extracts of plants like *Asparagus racemosus*, *Hippophae rhamnoides* and *Podophyllum hexandrum* have been reported to provide radioprotection owing to their antioxidant effects (Gupta *et al.,* 2004). Also, naturally occurring antioxidant compounds such as flavonoids, polyphenols, and vitamin E offer protection against the deleterious effects of ionizing radiation owing to their antioxidant effects (Maurya *et al*., 2004). Quercetin, apigenin, luteolin, nepitrin, scutellarein, rutin and naringin, well-known flavonoids have been reported to provide radioprotection due to their antioxidant effects (Gupta *et al.,* 2004; Agarwal and Nagaratnam, 1981; Rithidech *et al*., 2005; Shimoi *et al*., 1996). Chemical constituents accounting for antioxidant activity should be investigated and for further study on our tested plant extracts. Taken together, the results suggest that *Carpinus laxiflora, Quercus salicina*, and *Castanopsis cuspidata* protect V79-4 cells against oxidative damage by radiation through scavenging ROS.

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