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▣ 論 文 ▣

Radioprotective Effect of Extracts from Plants Indigenous to Korea

Kyoung Ah Kang[1] · Rui Zhang[1] · Sungwook Chae[1] · Mei Jing Piao[1] · Taekyun Shin[2] · Nam Ho Lee[3] · Jae Woo Park[4] and Jin Won Hyun[1*]

[1]Department of Biochemistry, College of Medicine and Applied Radiological Science Research Institute, Cheju National University, Jeju-si 690-756, Korea
[2]Department of Veterinary Medicine, Cheju National University, Jeju-si 690-756, Korea
[3]Department of Chemistry, College of Natural Sciences, Cheju National University, Jeju-si 690-756, Korea
[4]Department of Nuclear and Energy Engineering, Cheju National University, Jeju-si 690-756, Korea
*e-mail: jinwonh@cheju.ac.kr Tel:+82-064-754-3838 Fax:+82-064-726-4152

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Abstract - We have screened the cytoprotective effect on γ-ray radiation induced oxidative stress from eighteen Korean plant extracts. *Quercus salicina*, *Clerodendron trichotomum*, *Lamium amplexicaule*, *Lozoste lancifolia* and *Malus baccata* were found to scavenge 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical and intracellular reactive oxygen species (ROS). As a result, extracts of these 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 *Quercus salicina*, *Clerodendron trichotomum*, *Lamium amplexicaule*, *Lozoste lancifolia* and *Malus baccata* protect V79-4 cells against oxidative damage by radiation through scavenging ROS.
key words : γ-ray radiation, oxidative stress, reactive oxygen species

INTRODUCTION

The severity of radiation effects depends on whether the energy of radiation is absorbed by tissue molecules or the surrounding water. The animal body consists of 70% water; therefore, to a great extent the biological effects are mainly mediated through the action of radiation on water.

The radiolysis of water generates free radicals ($HO^{\cdot}$, $H^{\cdot}$, H_2O_2 etc)[1] which are capable of inducing lipid peroxidation in biological membranes. The effects of free radicals on human are considered to contribute to various diseases[2] and aging[3].

The potential application of radioprotective chemicals in the event of planned exposure or radiation accidents has been investigated from the beginning of the nuclear era[4]. The expanding role of radiotherapy in cancer treatment along with the potential threat of nuclear or radiological terrorism creates new imperatives for developing safe and effective agents for prophylaxis and treatment of ionizing radiation-induced normal tissue damage[5-7]. By definition, radioprotectors are chemical compounds that have the ability to reduce the biological effects of ionizing radiation on normal tissues, including lethality, mutagenicity and carcinogenicity[8,9] and have applications in clinical oncology, space travel, radiation site clean-up, radiological terrorism and military

scenarios[10]. An ideal radioprotector is relatively non-toxic to normal cells, easy to administer and does not degrade performance nor compromise the therapeutic effects of radiation treatment for cancer patients[11,12].

Many radioprotective compounds have been developed over the years, a majority of them designed to reduce the levels of radiation-induced free radicals within the cell[7,9,13-15].

In recent years, many phytochemicals are known to be antioxidants, that may help to protect humans from damage-induced by radiation exposure. It is, therefore, reasonable to expect that plants may contain certain compounds that can protect against radiation-induced ROS-mediated damage. Most of these plant extracts and phytochemicals are non-toxic and inhibit radiation-induced lipid peroxidation in model animal systems[16-18].

It is suggested that both radiation injury and oxygen poisoning occur through the formation of ROS[19]. 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[20].

In present study, we screened the antioxidative effect of plant extracts and in addition, from the selected antioxidative plant extracts, it was investigated whether it may show protective effect against γ-ray radiation.

MATERIALS AND METHODS

Plant material and its extract - The plant extracts were obtained from Dr. Nam Ho Lee (Cheju National University, Jeju, Korea).

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 - The V79-4 cells from the American type culture collection, were maintained at 37 ℃ 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 - Ten μg /ml 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[21].

Intracellular ROS measurement - The DCF-DA method was used to detect the intracellular ROS level[22]. 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 ℃. 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 Perkin Elmer LS-5B spectrofluorometer.

Cellviability - 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[23]. 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 ℃ for 24 h and the cell viability was measured using MTT test. Fifty μl 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 and the A_{540} was read on a scanning multi-well spectrophotometer.

Statistical analysis - All the measurements were made in triplicate and all values were represented as means ± standard error(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 significant.

RESULTS AND DISCUSSION

A large number of plants contain antioxidant phytochemicals reported to be radioprotective in various model systems. Antioxidants interfere with the initial stage of apoptosis by ROS[24], as well as later membrane lipid peroxidation, which is characteristic of radiation-induced apoptosis [25]. From tested eighteen Korean plant extracts (Table 1), *Quercus salicina*, *Picrasma quassioides* (leaves), *Quercus gilva*, *Lozoste lancifolia*, *Viburnum awabuki*, *Chamaecyparis obtusa* and *Malus baccata* were found to scavenge DPPH radical, showing at 10 μg/ml 20%, 23%, 26%, 24%, 27%, 23% and 23%, respectively (Table 2) and *Quercus salicina*, *Picrasma quassioides* (leaves), *Clerodendron trichotomum*, *Lamium amplexicaule* (leaves, twig),

Table 1. The list of plants used in these experiments.

Scientific name	Famliy	Used part
Quercus salicina	Fagaceae	Leaves
Picrasma quassioides	Simaroubaceae	Leaves
Picrasma quassioides	Simaroubaceae	Twig
Cayratia japonica	Vitaceae	Twig
Clerodendron trichotomum	Verbenaceae	Leaves
Lamium amplexicaule	Labiatae	Leaves
Lamium amplexicaule	Labiatae	Twig
Sonchus oleraceus	Compositae	Leaves
Quercus gilva	Fagaceae	Leaves
Lozoste lancifolia	Lauraceae	Leaves
Ilex integra	Aquifoliaceae	Leaves
Viburnum awabuki	Caprifoliaceae	Leaves
Anthriscus sylvestris Hoffm.	Umbelliferae	Twig
Chamaecyparis obtusa	Cupressaceae	Leaves
Sambucus sieboldiana var. pendula	Caprifoliaceae	Leaves
Malus baccata	Rosaceae	Leaves
Ilex rotunda Thunb.	Aquifoliaceae	Leaves
Corydalis incisa Pers.	Fumariaceae	Leaves

Table 2. Effect of plant extracts on scavenging DPPH.

Scientific name	10 μg/ml
Quercus salicina	20.2 ±0.6%*
Picrasma quassioides (leaves)	22.9 ±1.2%*
Picrasma quassioides (twig)	3.5 ± 0.4%
Cayratia japonica	2.5 ± 0.1%
Clerodendron trichotomum	14.6 ± 0.9%
Lamium amplexicaule (leaves)	2 ± 0.1%
Lamium amplexicaule (twig)	0%
Sonchus oleraceus	8.2 ± 1.1%
Quercus gilva	25.8 ±1.1%*
Lozoste lancifolia	23.9 ±0.7%*
Ilex integra	2.6 ± 0.1%
Viburnum awabuki	27 ± 0.4%*
Anthriscus sylvestris Hoffm.	0 %
Chamaecyparis obtusa	22.8 ±0.6%*
Sambucus sieboldiana var. pendula	4.5 ± 0.3%
Malus baccata	22.6 ±0.1%*
Ilex rotunda Thunb.	4.6 ± 0.1%
Corydalis incisa Pers.	0 %

The amount of DPPH radicals was determined spectrophotometrically. *Significantly different from control ($p<0.05$).

Quercus gilva, *Lozoste lancifolia*, *Viburnum awabuki*, *Anthriscus sylvestris Hoffm.*, *Chamaecyparis obtusa* and *Malus baccata* were found to scavenge intracellular ROS, showing at 10 μg/ml 68%, 60%, 71%, 76%, 69%, 66%, 69%, 72%, 70%, 79% and 64%, respectively (Table 3). Among these plants extracts we chose six extracts as *Quercus salicina*, *Clerodendron trichotomum*, *Lamium amplexicaule* (leaves, twig), *Lozoste lancifolia*, and *Malus baccata*. As a result, extracts of six extracts reduced cell death of V79-4 cells induced by H_2O_2 treatment, showing the cell viability of 61%, 64%, 60%, 47%, 51% and 58%, respectively, compared to cell viability of 41% in H_2O_2 treated cells (Table 4). These extracts protected cell death of V79-4 cells damaged by γ-ray radiation, showing the cell viability of 63%, 57%, 58%, 60%, 62% and 55% respectively, compared to cell viability of 48% in 10 Gy radiated cells (Table 5) and scavenged ROS generated by radiation, showing the percentage of intracellular ROS scavenging of 56%, 58%, 56%, 29%, 61% and 67%, respectively, compared to 0 % in 10 Gy radiated cells (Table 6). These naturally occurring flavonoids are widely distributed in plant kingdom and their antioxidant properties are well studied.

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″

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

Scientific name	10 μg/ml
Quercus salicina	68.4 ± 2.1%*
Picrasma quassioides (leaves)	60.2 ± 1.5%*
Picrasma quassioides (twig)	26.5 ± 1.2%
Cayratia japonica	30.7 ± 0.6%
Clerodendron trichotomum	70.9 ± 0.4%*
Lamium amplexicaule (leaves)	76 ± 1.8%*
Lamium amplexicaule (twig)	68.9 ± 1.6%*
Sonchus oleraceus	44.5 ± 1.1%
Quercus gilva	66.3 ± 1.4%*
Lozoste lancifolia	69.1 ± 0.7%*
Ilex integra	63.1 ± 0.7%
Viburnum awabuki	72.1 ± 1.7%*
Anthriscus sylvestris Hoffm.	70.4 ± 0.6%*
Chamaecyparis obtusa	78.6 ± 0.7%*
Sambucus sieboldiana var. pendula	35.3 ± 1.7%
Malus baccata	64.1 ± 1.3%*
Ilex rotunda Thunb.	53.8 ± 3.1%
Corydalis incisa Pers.	51.9 ± 1.7%

The intracellular ROS was detected by DCF-DA method. *Significantly different from control ($p<0.05$).

-di-acetyl-3″-*cis*-*p*-coumaroyl-6″-*trans*-*p*-coumaroyl)-d-glucopyranoside and tannins were isolated[26,27]. 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[28]. The antioxidant activity of *Q. salicina,* and *Q. gilva* which belongs to the genus *Quercus,* might be related with polyphenols. The antioxidant properties of *Clerodendron trichotomum* have been characterized. Jionoside D, one of major phenylpropanoid glycosides isolated from *C. trichotomum,* increased the intracellular ROS and DPPH radical scavenging activities and then enhanced the viability of V79-4 cells exposed to H_2O_2[29]. Phenylpropanoid glycosides and flavonoids were isolated from the genus *Lamium* [30] are potential sources of natural antioxidants, and phenylpropanoids are widely distributed in edible plants and foodstuffs derived from plants. The antioxidant activity of phenolic compounds is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors, and singlet-oxygen quenchers[31], supporting the antioxidant activity of *L. amplexicaule.* There has been no report on the chemical constituents of the genus *Lozoste* thus far, which support further investigation of phytochemical constituents related with antioxidant activity. Antioxidant activity of α-tocopherol from the genus *Malus* was studied. It is composed of a phytyl chain and a chromanon ring may be incorporated into the biological membrane, thus contributing to their physical

Table 4. Protective effect of plant extracts upon H_2O_2 induced oxidative damage of V79-4 cells.

Scientific name	10 μg/ml
H_2O_2	41.3 ± 0.5%
Quercus salicina	61.0 ± 1.1%*
Clerodendron trichotomum	64.3 ± 0.5%*
Lamium amplexicaule (leaves)	60.3 ± 1.9%*
Lamium amplexicaule (twig)	47.3 ± 1.5%
Lozoste lancifolia	51.2 ± 2.1%*
Malus baccata	57.5 ± 1.4%*

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 mean ± S.E. *Significantly different from control ($p<0.05$).

Table 5. Protective effect of plant extracts upon γ-ray radiation induced oxidative damage of V79-4 cells.

Scientific name	10 μg/ml
Radiation	48.3 ± 1.1%
Quercus salicina	63.4 ± 1.9%*
Clerodendron trichotomum	57.1 ± 2.1%*
Lamium amplexicaule (leaves)	58.3 ± 0.7%*
Lamium amplexicaule (twig)	59.8 ± 3.1%*
Lozoste lancifolia	61.8 ± 1.1%*
Malus baccata	54.6 ± 0.6%

The viability of V79-4 cells upon γ-ray radiation at day 2 was determined by MTT assay. The measurements were made in triplicate and values are expressed as mean ± S.E. *Significantly different from control ($p<0.05$).

Table 6. Effect of plant extracts on scavenging intracellular ROS generated by radiation.

Scientific name	10 μg/ml
Radiation	0 %
Quercus salicina	55.7 ± 2.5%*
Clerodendron trichotomum	58.0 ± 1.9%*
Lamium amplexicaule (leaves)	55.9 ± 1.5%*
Lamium amplexicaule (twig)	28.6 ± 3.1%*
Lozoste lancifolia	60.5 ± 1.1%*
Malus baccata	67.0 ± 2.5%*

The intracellular ROS at day 2 was detected by DCF-DA method. The measurements were made in triplicate and values are expressed as mean ± S.E. *Significantly different from control ($p<0.05$).

stability, which can be a function of interaction with ROS, preventing lipid peroxidation and subsequent membrane disorganization[32,33]. Chemical profiles responsible for antioxidant activity of *M. baccata* other than α-tocopherol are remained for further study.

Chemical constituents accounting for antioxidant activity should be investigated and for further study on our tested plant extracts. Taken together, the results suggest that *Quercus salicina*, *Clerodendron trichotomum*, *Lamium amplexicaule*, *Lozoste lancifolia* and *Malus baccata* protect V79-4 cells against oxidative damage by radiation through scavenging ROS.

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