Food Sci. Biotechnol. 26(1): 279-285 (2017)
DOI 10.1007/s10068-017-0037-y

Food Science
and
Biotechnology

Dietary hydroxycinnamates prevent oxidative damages to liver, spleen, and bone marrow cells in irradiation-exposed mice

Sung-Ho Kook[1,2], Sa-Ra Cheon[2], Jae-Hwan Kim[3], Ki-Choon Choi[4], Min-Kook Kim[1], and Jeong-Chae Lee[1,2,*]

[1]*Cluster for Craniofacial Development & Regeneration Research, Institute of Oral Biosciences and School of Dentistry, Chonbuk National University, Jeonju, Jeonbuk 54896, Korea*
[2]*Department of Bioactive Material Sciences and Research Center of Bioactives Materials, Chonbuk National University, Jeonju, Jeonbuk 54896, Korea*
[3]*Chonnam National University Dental Hospital, Kwangju 61186, Korea*
[4]*Grassland and Forages Research Center, National Institute of Animal Science, Cheonan, Chungnam 31002, Korea*

Received June 11, 2016
Revised December 16, 2016
Accepted December 21, 2016
Published online February 28, 2017

*Corresponding Author
Tel: +82-63-270-4049
Fax: +82-63-270-4004
E-mail: leejc88@jbnu.ac.kr

pISSN 1226-7708
eISSN 2092-6456

Abstract Dietary hydroxycinnamates are considered as attractive materials for radioprotection. This study explores whether hydroxycinnamates protect against γ-radiation-induced cellular damages and hematopoietic stem cell senescence. C57BL/6 mice were orally administered with each of caffeic acid, *p*-coumaric acid, and ferulic acid (20 mg/kg body weight) once per three days for five times before exposure to total body radiation (5 Gy). Irradiation increased the activities of alanine amino transaminase and aspartate aminotransferase in blood serum but decreased the anti-oxidant defense enzyme activities in the liver and spleen tissues. Oral administration of the compounds almost completely prevented irradiation-mediated changes in these enzyme activities. The hydroxycinnamates also inhibited the irradiation-mediated increases in the mitochondrial superoxide anions of $Lin^{-}Sca\text{-}1^{+}c\text{-}Kit^{+}$ (LSK) cells and $CD150^{+}CD48^{-}$ LSK cells in the bone marrow. These results suggest that dietary hydroxycinnamates protect against irradiation-mediated oxidative damages of tissues and bone marrow progenitor cells.

Keywords: total body irradiation, reactive oxygen species, bone marrow, radioprotection, phenolic compounds

Introduction

Radiotherapy is a general application to treat cancers after surgery. However, the amounts of irradiation that can be used to treat malignant tumors are often limited because of cytotoxicity to normal tissues and organs (1). In some cases, therapeutic irradiation induces irrecoverable damages to intact tissues and leads to radionecrotic injuries (2,3).

Many investigators have attempted to understand the mechanisms involved in irradiation-mediated cellular damages. It has been demonstrated that exposing cells to ionizing radiation induces oxidative stress via excessive generation of reactive oxygen species (ROS) (1). Irradiation itself interacts with other atoms or molecules within cells, particularly water, and then produces ROS that directly cause oxidative damage to cells and tissues (4,5). Moreover, exposure to total body irradiation causes a dysfunction in bone marrow (BM)-derived hematopoietic stem cells (HSCs), in which the capacities of HSCs to self-renew and repopulate are reduced (6,7). These impairments of HSCs are considered to be related to an increase in ROS production and senescence rather than a direct induction of apoptotic cell death (8,9). Accordingly, it is suggested that total body irradiation causes residual BM damages by inducing senescence of HSCs with increased ROS generation.

Naturally occurring phenolic compounds have been shown to protect cells against radiation-induced damages (10). Specifically, hydroxycinnamates are a class of major phenolic compounds present in the plant kingdom and exert various biological, pharmacological, and medicinal abilities (11). For example, caffeic acid, which is found mostly in coffee, some fruits, and wines, shows anti-oxidative (12), anti-inflammatory (13), and cancer chemopreventive activities (14). Ferulic acid also exhibits a strong potential to scavenge free radicals as well as to protect irradiation-mediated DNA and cellular damages (15,16). In addition, ferulic acid ameliorates radiation-induced inflammation with great bioavailability and low toxicity in a murine model (17). Furthermore, *p*-coumaric acid is a common dietary compound that is able to suppress inflammatory responses *in vitro* and *in vivo* (18). All these findings indicate that dietary hydroxycinnamates such as caffeic acid, *p*-coumaric acid, and ferulic acid might act as a radioprotector because of their anti-oxidant and anti-inflammatory potentials.

This study explores whether these phenolic compounds protect against total-body-irradiation-induced oxidative damages and HSC senescence using an experimental model of an animal. To this purpose, we orally administered C57BL/6 mice with caffeic acid, ferulic acid, and *p*-coumaric acid prior to total body irradiation at a sub-lethal dose and then analyzed various biochemical and cellular parameters in the liver, spleen, blood serum, and BM.

Materials and Methods

Chemicals and laboratory wares Primary anti-bodies for the nuclear factor (erythroid-derived 2)-like 2 (Nrf2) (sc-722) and β-actin (sc-47778) were purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Phenolic acid compounds such as caffeic acid, *p*-coumaric acid, and ferulic acid were obtained from Sigma-Aldrich (St. Louis, MO, USA). Unless otherwise specified, all other chemicals and laboratory items were purchased from Sigma-Aldrich and Falcon Labware (Becton-Dickinson, Franklin Lakes, NJ, USA), respectively.

Animals Male C57BL/6 mice (six weeks old) were supplied by Orient Bio (Daejeon, Korea) and assigned randomly into five groups (*n*=10/group): control, irradiation, and three experimental groups. The mean body weights among the groups did not differ. Mice were housed under the general conditions: 22±1°C, 55±5% humidity, and 12-h light/dark auto-cycling during the experimental periods. All animal care and guidelines for use were strictly followed as per the recommendations from the Chonbuk National University Committee on Ethics in the Care and Use of Laboratory Animals (Approval number: CBU 2014-00055).

Phenolic acid treatment and total body irradiation Phenolic compounds were dissolved in 99% ethanol and mixed with distilled water prior to use. The final concentration of ethanol administered did not exceed 0.5%. Three experimental groups of mice were administered orally with 200 μL of solutions containing caffeic acid, ferulic acid, and coumaric acid at the final concentration of 20 mg/kg body weight once per three days for five times. The control and irradiation groups received only 200 μL of the solutions without phenolic compounds at the same times throughout the experiment. One day after the last administration, the irradiation and experimental groups were exposed to 5 Gy total body irradiation at a rate of 0.5 Gy/min using a soft M-150 WE (Softex, Tokyo, Japan) ^{60}Co source. Mice (*n*=5/group) were anesthetized with halothane two days after irradiation, and then the body and organ weights, anti-oxidant enzyme activity, and anti-oxidant-sensitive gene expression were determined. A part of the animal group (*n*=5/group) was sacrificed two months after the irradiation via CO_2 asphyxiation; then, the mitochondrial ROS levels in Lineage$^-$Sca-1$^+$c-Kit$^+$ (LSK) and CD150$^+$ CD48$^-$LSK (SLAM LSK) cells in the BM were measured. In the LSK cells, lacking mature lineage cells characterized by lineage markers were made up of a heterogeneous mix of multipotent/committed progenitors and more primitive stem cell populations isolated using SLAM family markers, i.e., CD150$^+$CD48$^-$LSK cells. Therefore, LSK cells are characterized by short-term repopulating capacity, whereas, SLAM LSK cells are referred to as HSCs with long-term repopulating and self-renewal potentials.

Preparation of blood and tissue samples After two days of irradiation, the peripheral blood of mice were isolated from the vena cava and collected into serum separation tubes (BD Bioscience, San Jose, CA, USA). Blood samples were kept at room temperature for 20 min and then centrifuged at 10,000×*g* for 10 min to obtain blood serum. The serum samples were used to determine the enzyme activities specific to alanine amino transaminase (ALT) and aspartate aminotransferase (AST). Liver and spleen tissues were obtained from mice after collecting peripheral blood. The tissue samples were stored at 80°C prior to the analyses of the biochemical parameters. The tissues were rinsed with phosphate buffered saline to remove red blood cells and clots and then dissected into two parts. A part of the dissected tissues was homogenized in 50 mM KH_2PO_4 solution for 5 min using a homogenizer (PRO Scientific Inc., Oxford, CT, USA) and then centrifuged at 14,000×*g* for 10 min. The supernatants were collected, and the activity of superoxide dismutase (SOD) was determined. Other dissected tissues (10 mg per sample) were homogenized in a 200-μL cold assay buffer provided by BioAssay Systems (Hayward, CA, USA) and then centrifuged at 14,000×*g* for 10 min. The supernatants were collected to determine the activities of glutathione peroxidase (GPx) and catalase (CAT).

Determination of enzyme activities All enzyme activity assays were performed according to the manufacturer's instructions. In brief, AST (2.6.1.1) and ALT (EC 2.6.1.2) activities in the serum samples were measured using an automated chemical analyzer (ADVIA 1650; Bayer, Leverkusen, Germany). SOD (EC 1.15.1.1) activity in the liver and spleen tissues was measured using an assay kit provided by Cayman Chemical (No. 706002; Ann Arbor, MI, USA). The activities of CAT (EC 1.11.1.6) and GPx (EC 1.11.1.9) in these tissues were determined using an EnzyChrom™ CAT assay kit (ECAT-100; BioAssay Systems) and GPx assay kit (EGPX-100; Bioassay System), respectively.

Western blot analysis Whole protein lysates were prepared from the liver and spleen tissues, and protein samples (20 μg/sample) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis in 8–12% gels and electroblotted onto polyvinylidene fluoride membranes. The blots were washed, blocked, and incubated with a primary anti-body specific to Nrf2. The anti-body for â-actin was used as the internal control. The membranes were incubated with horseradish peroxidase-conjugated anti-IgG for 1 h and then treated with enhanced chemiluminescence (GE Healthcare, Buckinghamshire, UK) followed by exposure to an X-ray film

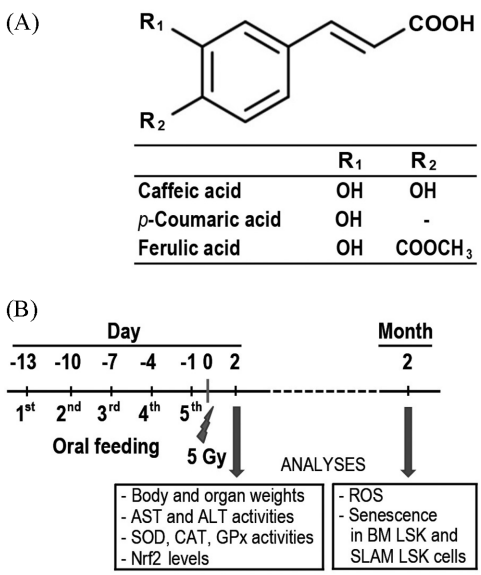
(Eastman-Kodak, Rochester, NY, USA). The intensities of the immunoreactive bands were quantified using ImageJ densitometry software provided by National Institutes of Health (Bethesda, MD, USA).

MitoSox™ Red Flow staining This study determined the effects of phenolic compounds on the production of mitochondrial superoxide anions in the BM cells of irradiated mice. Initially, BM cells were collected from mice after two months of irradiation and then stained with various lineage markers specific to LSK and SLAM LSK cells. In this study, PE-Cy7-conjugated anti-CD3, anti-CD4, anti-CD8, anti-CD45R, anti-CD11b, anti-Gr-1, and anti-TER-119 (BD Bioscience); FITC- or PE-conjugated anti-Sca-1 (BD Bioscience); APC-conjugated anti-c-Kit (BD Bioscience); PerCP/Cy5.5-conjugated anti-CD150 (eBioscience, San Diego, CA, USA); and APC-Cy7-conjugated anti-CD48 (BD Bioscience) were used as five different colors of anti-bodies to assess LSK and SLAM LSK cells in the BM. Thereafter, the BM cells were stained with PE-conjugated MitoSox™ Red (Invitrogen, Carlsbad, CA, USA), which reacts with mitochondrial superoxide anions. The frequency of the BM cells was investigated via flow cytometry (BD Bioscience) followed by cell sorting (BD Calibur), and a FlowJo software program was used to sequentially gate the cell populations. In this study, the c-Kit and Sca-1 were used as cell-surface markers to phenotypically identify LSK cells (Sca-1$^+$/c-Kit$^+$ cells), and SLAM LSK cells (CD150$^+$/CD48$^-$ cells) were gated from the LSK cells.

Statistical analysis All data are presented as the mean±standard error. One-way analysis of variance (ANOVA) was applied to determine significant differences among groups using the Statistical Package for the Social Science 12.0 program. Scheffe's test was used to determine the differences among groups when one-way ANOVA was significant. A value of $p<0.05$ was considered as statistically significant.

Results and Discussion

Oral supplementation with phenolic compounds inhibits irradiation-mediated increases of AST and ALT activities in the blood serum Figure 1 represents the chemical structures of the phenolic compounds used in this study (Fig. 1A) and the experimental design (Fig. 1B). In this study, mice were exposed to 5 Gy of total body γ-irradiation at a sub-lethal dose, which all mice survived for more than two months. This was because total body irradiation with a high dose (10 Gy) led to the death of mice at a median of five days on account of acute radiation toxicity to the epithelium of the small intestine (data not shown). The weight of the spleen, but not of the whole body and liver, in the mice exposed to irradiation was significantly lower ($p<0.05$) than that in the non-irradiated mice (Table 1). These results are in good agreement with previous findings, which show that sub-lethal irradiation causes an acute and abnormal decrease in the weight of the spleen (19).



	R_1	R_2
Caffeic acid	OH	OH
p-Coumaric acid	OH	-
Ferulic acid	OH	$COOCH_3$

Fig. 1. Chemical structures of the phenolic compounds (A) and the experimental design (B)

Table 1. Effects of irradiation on the weights of the body, spleen, and liver of mice supplemented with and without phenolic acids[1)]

Groups	Body weight (g)	Tissue weight (mg)	
		Spleen	Liver
Control	20.9±1.0	58.6±15.8	877.6±135.5
IR[2)]	20.1±0.6	36.3±0.9*	876.8±131.4
IR+CA	19.2±1.3	38.2±6.0	865.6±108.6
IR+CO	19.9±0.4	36.7±5.6	811.8±90.3
IR+FA	20.4±0.8	39.2±4.7	856.6±20.6

[1)]The experimental mice groups were orally administered with each of the phenolic acids at the final dose of 20 mg/kg body weight for five times in the interval of two days prior to sub-lethal irradiation exposure (5 Gy). The weights of the body (n=10), liver (n=5), and spleen (n=5) of the mice were measured two days after irradiation. *$p<0.05$ compared with the sham group.

[2)]IR, irradiation; CA, caffeic acid; CO, *p*-coumaric acid; and FA, ferulic acid.

Table 2. Inhibitory effects of dietary hydroxycinnamates on the irradiation-mediated increases in AST and ALP activities in blood serum

Groups	Activity (U/l)	
	AST	ALP
Control	182.2±16.7[1)]	36.8±9.8
IR	346.3±101.5*	42.6±4.4*
IR+CA	161.8±52.1[#]	37.4±4.6[#]
IR+CO	170.4±62.9[#]	37.1±6.1[#]
IR+FA	197.2±59.4[#]	38.1±3.4[#]

[1)]The activities of AST and ALT in the liver and spleen were determined two days after irradiation (n=5). *$p<0.05$ compared with the control group. [#]$p<0.05$ compared with the irradiation group.

We next determined the activities of liver-specific enzyme markers AST and ALT in the blood serum two days after irradiation (Table 2). Irradiation increased the activity of AST in the serum at a significant level ($p<0.05$) compared with the controls. Administration of phenolic

compounds reduced the irradiation-induced increase in AST activity up to the level of the control group, in which caffeic acid was the most efficient compound. Similarly, the activity of ALP in the blood serum was higher in irradiation-exposed mice than in the control ones, whereas oral treatment with each of the phenolic compounds significantly inhibited (p<0.05) the enzyme activity.

AST and ALT are liver-specific enzyme markers. The levels of these markers released into the circulating system are correlated with the levels present in the blood serum. Thus, it is commonly accepted that an increase in these markers is closely related to cellular damage of the liver. Serum ALT level also increases after hepatic injuries including cellular membrane damage and bleb formation (20). Overall, our current findings suggest that sub-lethal irradiation exposure induces liver damages with increased releases of AST and ALT, regardless of changes in the weight of the liver. Our results also support the notion that dietary phenolic compounds may ameliorate oxidative liver injury induced by irradiation.

Oral administration with phenolic compounds restores irradiation-induced reduction of anti-oxidant enzyme activities Total body irradiation significantly reduced (p<0.001) the SOD activity in the liver, and this reduction was inhibited by supplementation with phenolic compounds (Fig. 2A). Mice treated with *p*-coumaric acid or ferulic acid showed an SOD activity similar to that of the untreated control group. Total body irradiation also reduced the SOD activity in the spleen, whereas only the mice group administered with ferulic acid significantly prevented (p<0.05) the irradiation-mediated decrease in SOD activity (Fig. 2B). When the CAT activity was determined in the liver and spleen, the irradiation-mediated reduction in the activity and its restoration by phenolic compounds was observed (Fig. 2C and 2D). Similarly, the irradiated mice group showed a significantly low GPx activity both in the liver (p<0.001) and spleen (p<0.05) compared with the controls (Fig. 2E and 2F). Oral treatment with *p*-coumaric acid or ferulic acid but not with caffeic acid completely inhibited the irradiation-mediated reduction of the GPx activity in the liver. Specifically, the GPx activity in the liver of mice supplemented with ferulic acid was approximately 2.2 times higher than that in the control group (Fig. 2E). Furthermore, the experimental groups administered with caffeic acid or ferulic acid but not with *p*-coumaric acid exhibited almost complete restoration of the GPx activity in the spleen, similar to that observed in the control group (Fig. 2F).

Natural anti-oxidants can provide protection against irradiation-mediated injury by decreasing the levels of cellular ROS (21). Primary anti-oxidant defense systems mainly comprising glutathione, SOD, CAT, and GPx also exist. These systems convert active oxygen molecules into non-toxic substances and/or directly remove reactive oxidants. SOD converts superoxide anions into a non-toxic agent H_2O_2; the CAT and GPx present in H_2O_2 convert it into water and oxygen. This indicates that inactivation of these enzymes causes excessive generation of ROS, thereby leading to oxidative stress.

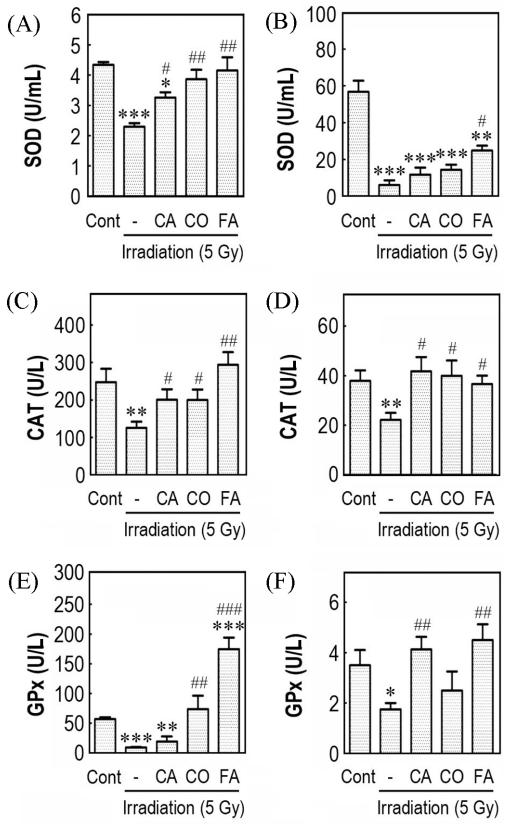


Fig. 2. Increases in the anti-oxidant enzyme activities in the liver and spleen of irradiated mice after oral supplementation with phenolic compounds. The activities of SOD (A, B), CAT (C, D), and GPx (E, F) in the liver (A, C, E) and spleen (B, D, F) were determined two days after irradiation (n=5/group). *p<0.05, **p<0.01, and ***p<0.001 compared with the control group. $^{\#}p$<0.05, $^{\#\#}p$<0.01, and $^{\#\#\#}p$<0.001 compared with the irradiation group. CA, caffeic acid; CO, *p*-coumaric acid; and FA, ferulic acid.

Consequently, the current findings highlight that the radioprotective effects of phenolic compounds are at least partially associated with their abilities to increase anti-oxidant enzyme activities.

Phenolic compounds reduce the irradiation-mediated induction of Nrf2 protein in the liver and spleen As the induction of Nrf2 is sensitively affected by oxidative stress, this study measured Nrf2 protein levels in the liver and spleen tissues. The induction of Nrf2 protein in the liver and spleen tissues of irradiated mice was greater than that in the tissues of the non-irradiated ones (Fig. 3A and 3B). This increase in liver-Nrf2 level in irradiated mice diminished significantly (p<0.05) upon the administration of caffeic acid or ferulic acid but not *p*-coumaric acid. In contrast, all experimental groups administered with each of the phenolic acids significantly inhibited the irradiation-mediated increase in the Nrf2 protein levels in the spleen. Among the phenolic compounds, ferulic acid showed the most effective potential to decrease the induction of Nrf2 in the spleen.

Nrf2 is a transcription factor that efficiently regulates the gene

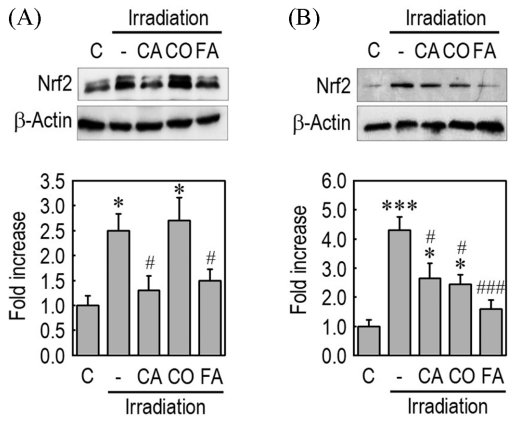


Fig. 3. Effects of the phenolic compounds on the induction of Nrf2 protein in the liver and spleen of irradiated mice. The levels of Nrf2 protein in the liver (A) and spleen (B) were determined two days after irradiation using the Western blot analysis. Lower panels represent the relative intensities specific to Nrf2 after normalizing the bands to the relative intensity of β-actin (*n*=5/group).

expression of anti-oxidant and anti-inflammatory enzymes (22). In general, oxidative stress induces translocation of Nrf2 into the nucleus, in which Nrf2 stimulates transcriptional activation of redox-sensitive target genes such as CAT and SOD. It has also been reported that the induction of Nrf2 protects against oxidative-stress-induced DNA damages (23). Therefore, it is hypothesized that irradiation-mediated accumulation of intracellular ROS activates the Nrf2 signal, and this activation positively controls cellular homeostasis and redox balance. However, contradictory findings showing that the Nrf2 signal can play dual roles, i.e., Nrf2 activation in response to a mild oxidative stress exerts protective roles whereas its activation under a prolonged and persistent oxidative stress predominantly mediates cell death (8), have also been reported. This study revealed that exposure to sub-lethal irradiation increases the levels of Nrf2 in the liver and spleen and that this induction is prevented by caffeic acid, *p*-coumaric acid, or ferulic acid. This may suggest that total body irradiation at sub-lethal doses cause oxidative stress in the organs and activates the induction of Nrf2, which negatively affects the primary anti-oxidant defense systems. However, it is important to consider that phenolic compounds are capable of stimulating different Nrf2 expression depending on the chemical structures and that the inhibition of Nrf2 induction is not associated directly with the radioprotection provided by the compounds. Therefore, it is likely that the chemical structure of phenolic compounds differently affects their biological properties, such as anti-oxidant potential, bioavailability, and intracellular interactions with receptors and/or enzymes (24). It has been suggested that the redox potential of phenolic anti-oxidants is the most important chemical property for activating the induction of Nrf2 and anti-oxidant enzymes (23). It is also considered that the number of hydroxyl groups and their location in a chemical structure affect the redox capacity of phenolic anti-oxidants (23,25). Further experiments to clarify the exact roles of the Nrf2 signal under oxidative stress caused by total body irradiation are required. In addition, more detailed experiments to verify the relationship between the chemical structure of phenolic compounds and their biological activities will be needed in the future.

Supplementation with phenolic compounds inhibits mitochondrial ROS generation in progenitor cells located in the BM of irradiation-exposed mice Total body irradiation increased the levels of mitochondrial superoxide anions in LSK and SLAM LSK cells present in the BM to approximately twice of those in the control group (Fig. 4). Supplementation with caffeic acid but not with *p*-coumaric acid or ferulic acid significantly diminished ($p<0.05$) the irradiation-mediated increase of mitochondrial ROS in LSK cells (Fig. 4B and 4C). However, all phenolic compounds were effective in decreasing superoxide anion levels that had increased in the SLAM LSK cells of irradiated mice. Irradiation also increased the SA-β-gal activity in both LSK and SLAM LSK cells up to approximately thrice of that of the control group, and this increase was attenuated by treatment with each of the phenolic compounds (data not shown).

The maintenance of tissue homeostasis and repair is closely related to the potentials of HSCs. This study revealed that total body irradiation increases the ROS levels in BM-derived LSK and SLAM LSK cells. Specifically, phenolic compounds exerted radioprotective effects by more effectively suppressing the accumulation of ROS in the BM SLAM LSK cells than in LSK cells. These findings suggest that dietary hydroxycinnamates inhibit irradiation-mediated ROS accumulation in the HSCs present in the BM, thereby protecting oxidative damages and aging of the HSCs (9).

In addition to the risk of radiation exposure from industrial applications, therapeutic radiation can cause irrecoverable damages to intact soft and hard tissues (2,26). Total body irradiation even at low doses can cause a remarkable reduction in the volume and mineral density of trabecular bone (27). As the oxidative stress is the predominant mediator of irradiation-induced cellular damages, an anti-oxidant-based application is a useful approach to relieve irradiation-mediated degenerative disorders. It has been reported that supplementation with dietary anti-oxidants such as α-tocopherol succinate, flavonoids, and phenolic acids exerts radioprotective effects in mice (28-30). Hydroxycinnamic acid derivatives such as chlorogenic and quinic acids also protect against X-ray-induced DNA damages in human lymphocytes (31). In accordance with these findings, the present study demonstrates that caffeic acid, coumaric acid, and ferulic acid are beneficial dietary hydroxycinnamates capable of protecting BM cells against irradiation-mediated oxidative damages.

In summary, this study highlights that total body irradiation at a sub-lethal dose increases the AST and ALP levels in the blood serum of mice but decreases the SOD, CAT, and GPx activities in the liver and spleen. These irradiation-mediated changes were blocked by supplementation with either caffeic acid, *p*-coumaric acid, or ferulic

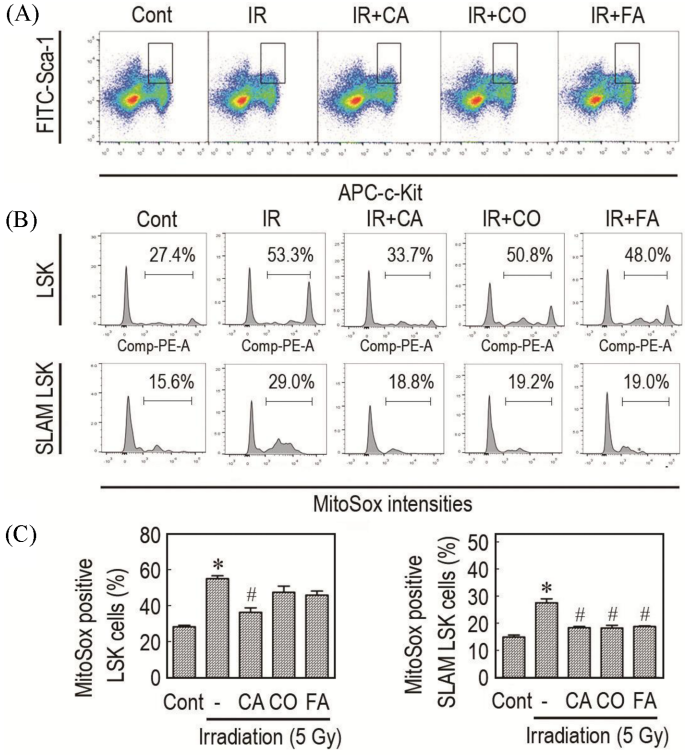


Fig. 4. Reduction in the mitochondrial ROS levels that increased in the BM-derived LSK and/or SLAM LSK cells of irradiated mice using phenolic compounds. (A) LSK cell population in the BM was gated using a FlowJo software after two months of irradiation. (B) The levels of mitochondrial superoxide anions in the BM LSK and SLAM LSK cells were measured via multi-color flow cytometry after two months of irradiation via fixation and permeabilization using the MitoSox™ Red reagent. Panels A and B show a representative result from three different samples. (C) The percentage of MitoSox positive cells in the BM LSK and SLAM LSK cells was calculated (n=3/group). *p<0.05 compared with the control group. $^{\#}p$<0.05 compared with the irradiation group.

acid. Oral treatment with these phenolic compounds also attenuated the irradiation-stimulated increase of Nrf2 induction in the liver and spleen. Furthermore, the current findings suggest that dietary hydroxycinnamates are capable of inhibiting irradiation-mediated oxidative damages to progenitor cells in the BM.

Acknowledgments This paper was supported by the research funds for newly appointed professors at Chonbuk National University in 2015. This work was also supported by a grant from the RDA, Ministry of Agriculture and Forestry, Republic of Korea (Grant No. PJ010903032015).

Disclosure The authors declare no conflict of interest.

References

1. Weiss JF. Pharmacologic approaches to protection against radiation-induced lethality and other damage. Environ. Health Persp. 105(Suppl 6): 1473-1478 (1997)
2. Schultze-Mosgau S, Lehner B, Rödel F, Wehrhan F, Amann K, Kopp J, Thorwarth M, Nkenke E, Grabenbauer G. Expression of bone morphogenetic protein 2/4, transforming growth factor-beta1, and bone matrix protein expression in healing area between vascular tibia grafts and irradiated bone-experimental model of osteonecrosis. Int. J. Radiat. Oncol. 61: 1189-1196 (2005)
3. Kang KA, Zhang R, Lee KH, Chea S, Kim BJ, Kwak YS, Park JW, Lee NH, Hyun JW. Protective effect of triphlorethol-A from Ecklonia cava against ionizing radiation *in vitro*. J. Radiat. Res. 47: 61-68 (2006)
4. Kovacs E, Keresztes A. Effect of gamma and UV-B/C radiation on plant cells. Micron 33: 199-210 (2002)
5. Arora R, Gupta D, Chawla R, Sagar R, Sharma A, Kumar R, Prasad J, Singh S, Samanta N, Sharma RK. Radioprotection by plant products: Present status and future prospects. Phytother. Res. 19: 1-22 (2005)
6. Chua HL, Plett PA, Sampson CH, Joshi M, Tabbey R, Katz BP, MacVittie TJ, Orschell CM. Long-term hematopoietic stem cell damage in a murine model of the hematopoietic syndrome of the acute radiation syndrome. Health Phys. 103: 356-366 (2012)
7. Chang J, Feng W, Wang Y, Luo Y, Allen AR, Koturbash I, Turner J, Stewart B, Raber J, Hauer-Jensen M, Zhou D, Shao L. Total-body proton irradiation causes long-term damage to hematopoietic stem cells in mice. J. Radiat. Res. 183: 240-248 (2015)
8. Wang Y, Schulte BA, LaRue AC, Ogawa M, Zhou D. Total body irradiation selectively induces murine hematopoietic stem cell senescence. Blood 107: 356-366 (2006)
9. Shao L, Feng W, Li H, Gardner D, Luo Y, Wang Y, Liu L, Meng A, Sharpless NE, Zhou D. Total body irradiation causes long-term mouse BM injury via induction of HSC premature senescence in anlnka- and Arf-independent manner. Blood 123: 3105-3115 (2014)
10. Zhang R, Kang KA, Kang SS, Park JW, Hyun JW. Morin (2',3,4',5,7-pentahydroxyflabone) protected cells against radiation-induced oxidative stress. Basic Clin. Pharmacol. 108: 63-72 (2011)
11. Mathew S, Abraham TE. Bioconversions of ferulic acid, a hydroxycinnamic acid. Crit. Rev. Microbiol. 32: 115-125 (2006)
12. Masuda T, Ellsworth PC, Mesquita B, Leu J, Tanida S, Van de Veerodonk E. Placing the face in context: Cultural difference in the perception of facial emotion. J. Pers. Soc. Psychol. 94: 365-381 (2008)
13. Song IH, Poddubnyy DA, Rudwaleit M, Sieper J. Benefits and risks of ankylosing spondylitis treatment with nonsteroidal anti-inflammatory drugs. Arthritis Rheum. 58: 929-938 (2008)
14. Hudson EA, Dinh PA, Kokubun T, Simmonds MS, Gescher A. Characterization

of potentially chemopreventive phenols in extracts of brown rice that inhibit the growth of human breast and colon cancer cells. Cancer Epidem. Biomar. 9: 1167-1170 (2000)
15. Ma ZC, Hong Q, Wang YG, Tan HL, Xiao CR, Liang QD, Gao Y. Ferulic acid protects lymphocytes from radiation-predisposed oxidative stress through extracellular regulated kinase. Int. J. Radiat. Biol. 87: 130-140 (2011)
16. Piazzon A, Vrhovsek U, Masuero D, Mattivi F, Mandoi F, Nardini M. Antioxidant activity of phenolic acids and their metabolites: Synthesis and antioxidant properties of the sulfate derivatives of ferulic and caffeic acids and of the acyl glucuronide of ferulic acid. J. Agr. Food Chem. 90: 12312-12323 (2012)
17. Das U, Manna K, Sinha M, Datta S, Da DK, Chakraborty A, Ghosh M, Saha KD, Dey S. Role of ferulic acid in the amelioration of ionizing radiation induced inflammation: A murine model. PLoS ONE 9: e97599 (2014)
18. Islam MS, Nagasaka R, Ohara K, Hosoya T, Ozaki H, Ushio H, Hori M. Biological abilities of rice bran-derived antioxidant phytochemicals for medical therapy. Curr. Top. Med. Chem. 11: 1847-1853 (2011)
19. Baliga MS, Haniadka R, Pereira MM, Thilakchand KR, Rao S, Arora R. Radioprotective effects of *Zingiber officinale* Roscoe (ginger): Past, present and future. Food Funct. 3: 714-723 (2012)
20. Nagiev ER, Karpovich GA. Activity of alanine- and aspartate-aminotransferases of organs of albino rats subjected to total body gamma-radiation and physical exercise. Radiats. Biol. Radioecol. 34: 639-644 (1994)
21. Hosseinimehr SJ, Azadbakht M, Mousavi SM, Mahmoudzadeh A, Akhlaghpoor S. Radioprotective effects of hawthorn fruit extract against gamma irradiation in mouse bone marrow cells. J. Radiat. Res. 48: 63-68 (2007)
22. Kim SB, Pandita RK, Eskiocak U, Ly P, Kaisani A, Kumar R, Cornelius C, Wright WE, Pandita TK, Shay JW. Targeting of Nrf2 induces DNA damage signaling and protects colonic epithelial cells from ionizing radiation. P. Natl. Acad. Sci. USA 109: E2949-E2955 (2012)
23. Kim KA, Kook SH, Song JH, Lee JC. A phenolic acid phenethyl urea derivative protects against irradiation-induced osteoblast damage by modulating intracellular redox state. J. Cell. Biochem. 115: 1877-1887 (2014)
24. Scalbert A, Williamson G. Dietary intake and bioavailability of polyphenols. J. Nutr. 130: 2073S-2085S (2000)
25. Kim SS, Son YO, Chun JC, Kim SE, Chung GH, Hwang KJ, Lee JC. Antioxidant property of an active compound purified from the leaves of paraquat-tolerant *Rehmannia glutonosa*. Redox Rep. 10: 311-318 (2005)
26. King AD, Griffith JF, Abrigo JM, Leung SF, Yau FK, Tse GM, Ahuja AT. Osteoradionecrosis of the upper cervical spine: MR imaging following radiotherapy for nasopharyngeal carcinoma. Eur. J. Radiol. 73: 629-635 (2010)
27. Rana T, Schultz MA, Freeman ML, Biswas S. Loss of *Nrf2* accelerates ionizing radiation-induced bone loss by upregulating RANKL. Free Radical Bio. Med. 53: 2298-2307 (2012)
28. Singh PK, Wise SY, Ducey EJ, Fatanmi OO, Elliott TB, Singh VK. α-Tocopherol succinate protects mice against radiation-induced gastrointestinal injury. J. Radiat. Res. 177: 133-145 (2012)
29. Shimoi K, Masuda S, Shen B, Furugori M, Kinae N. Radioprotective effects of antioxidative plant flavonoids in mice. Mutat. Res. 350: 153-161 (1996)
30. Shanthakumar J, Karthikeyan A, Bandugula VR, Rajendra Prasad N. Ferulic acid, a dietary phenolic acid, modulates radiation effects in Swiss albino mice. Eur. J. Pharmacol. 691: 268-274 (2012)
31. Cinkilic N, Cetintas SK, Zorlu T, Vatan O, Yilmaz D, Cavas T, Tunc S, Ozkan L, Bilaloglu R. Radioprotection by two phenolic compounds: Chlorogenic and quinic acid, on X-ray induced DNA damage in human blood lymphocytes *in vitro*. Food Chem. Toxicol. 53: 359–363 (2013)