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Effects of *Ixeris dentata* Extracts on the Genotoxicity Induced by Gamma Irradiation in Rats

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Abstract - *Ixeris dentata* is a typical oriental herb. It is a widely distributed perennial in Korea, Japan and China, which belongs to the Compositae Family. The whole plant of *I. dentata* has been used for the treatment of pneumonia, contusion, tumor and hepatitis. It has also been used for the treatment of allergic diseases as a folk therapy in Korea. *I. dentata* is known to have aliphatics, triterpenoids and sesquiterpene glycosides in its composition. The present study was designed to explore the protective effects of water- and ethanol-extracts from *I. dentata* on irradiated rodents. For oral administration (twice per day), the extractive powder of *I. dentata* and the positive control (ascorbic acid) were dissolved at a concentration of 0.5 and 250 mg ml^{-1} in saline, respectively. Thirty days after irradiation, the ratio of the weight of the testis to the body weight was lower than 50% in the radiation groups than the control group. The ALP concentrations in the group treated with the water-extracts of the leaf were $79.68 \pm 1.39\%$ ($p<0.05$) of those of the radiation control. Both of the SGOT and SGPT in the group treated with the ethanol-extract of the root were 72.68 ± 0.95 and 77.87 ± 5.74 ($p<0.05$) of those of the radiation control, respectively. The levels of DNA damage induced by gamma radiation decreased in the experimental group to which the extracts of *I. dentata* were administered before irradiation. In conclusion, these results indicate that the extracts of *I. dentata* have an excellent ability to reduce the radicals and they have a protective effect on DNA breakage caused by radiation.

Key words : ionizing radiation, genotoxicity, antioxidant, radioprotection, rat

INTRODUCTION

Radiation therapy for cancer patients has been improved by the use of radioprotectors to protect the normal tissues or to elevate the efficacy with chemotherapy. The identification of radiation-protecting agents with no-side effects is an important goal for radiation oncologists and basic radiation biologists. Recently, several herbal preparations and phytochemicals have been reported to have a radioprotective action via *in vitro* and *in vivo* studies. Therefore this study is aimed at investigating the possible radioprotective effect of endemic plants on irradiation-induced damage by the comet assay.

Ixeris dentata is a typical oriental herb. It is a perennial of the Compositae Family, which is widely distributed in Korea, Japan and China. The flowering time is from May to July and the flowers are lingulate and yellow. This plant grows to a height of about 25-50 cm. The plant has been used for the treatment of pneumonia, contusion, tumors and hepatitis. It has also been used for the treatment of allergic diseases as a folk therapy in Korea. According to recent research, *I. dentata* has been proved

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to have a hypocholesterolaemic effect and an antioxidant activity, as well. *I. dentata* is known to have aliphatics, triterpenoids and sesquiterpene glycosides in its composition (Kim and Lee 1988; Chung *et al.* 2002). Studies on the roots revealed the effects of an antioxidant, antimutagenicity and an anticancer (Kim *et al.* 2002). The methanol extract was most effective in the anticancer activity, inhibiting the growth of MG-63 cells by more than ninety percent (Kim 1995). Also, the effect of *I. dentata* on the cardiovascular system in hyperlipidemic rats was examined (Lim and Lee 1997).

The sensitivity of cells to ionizing radiation depends to a great extent on their capacity for repairing DNA double-strand breaks (dsb). It has been demonstrated for fibroblast strains derived from patients or healthy persons that cells exhibit substantial differences in their dsb-repair capacity and that the number of residual dsb was correlated with the cellular radiosensitivity (Bramer *et al.* 2004). Ionizing radiation is a well-known carcinogen due to the resulting oxidative damage, and the molecule most often reported to be damaged by this physical agent is DNA. About 60-70% of cellular DNA damage produced by ionizing radiation is caused by · OH, formed from the radiolysis of water. Oxidative stress occurs when there is an excessive free radical production and/or low antioxidant defense, and results in the chemical alterations of biomolecules leading to structural and functional modifications. The generation of the reactive oxygen metabolites plays an important role in the pathogenesis of irradiation-induced tissue injury. An extensive literature review implicates cellular DNA as the primary target for the biological and lethal effects of ionizing radiation. Besides DNA, lipids and proteins are also attacked by free radicals (Sener *et al.* 2003).

The single cell gel electrophoresis assay (comet assay) was developed by Ostling and Johansen in 1984. The assay was later improved by applying a weak electric field to pull the broken DNA strands from the nucleus after a lysis of the cells in a neutral nucleus after a lysis of the cells in a neutral detergent solution. As the electric current was passed through the gel, DNA loops with double-stranded breaks would unwind from the tightly bound nucleus and be pulled into a tail behind the nuclear head. DNA was then stained with propidium iodide and the cells were viewed using an epifluorescent microscope (Browden *et al.* 2003).

The blood assays included the analyses on ALP (alkaline phosphatase), SGOT (serum glutamine oxaloacetic transaminase), and SGPT (serum glutamate pyruvate transaminase). ALP exists in various organs and is the enzyme that accepts monophosphatester. Especially, it exists at a high concentration in the kidney (proximal tubule cells), small intestines, osteoblast, placenta, liver (canalicular liver), and mammary gland. It takes part in the phosphoric acid transport to a membrane. The rise of activated ALP is observed when in brain trouble, biliary tract disease, pregnancy, and cancer (EI-Demerdash 2001; Kumar *et al.* 2003). Transaminase is the catalyst enzyme of the amino group that changes amino acid to alpha-keto acid. SGOT and SGPT among the transaminases are of great importance in a blood test. SGOT exists at a high concentration in heart muscles, liver, and the brain. On the whole, the concentration of SGPT is lower than that of SGOT. The concentration of SGPT is highest in liver among the organs in a living body. The rise of activated SGOT and SGPT is an important index of acute hepatitis, biliary tract disease and myocardial infarction (Fukuda *et al.* 2004; Hsiao *et al.* 2003).

MATERIALS AND METHODS

1. Preparation of the extracts of plant

The powders of *Ixeris dentata* leaf and root were kindly provided by the ChunGil Biotec Co. Each powder (100 g of each) was separately suspended in distilled water and EtOH at the rate of 1 g of powder per 10 mL of solvent. The water extract was made by stirring the powder in the water bath at 100°C for 3 h, followed by a centrifugation for 15 min at 12,000 rpm. The EtOH extract was made by stirring the powder at 24°C for 3 h, followed by a centrifugation for 15 min at 12,000 × g. This process was done in triplicate. The supernatant was reduced by evaporation under reduced pressure at 60°C. After the concentration step, the extracts were lyophilized and the lyophilized powder was stored at −80°C until use.

2. Measurements of the radical scavenging activity

The use of 1, 1-diphenylpicrylhydrazyl (DPPH) as a reagent for screening the antioxidant activity of small molecules has been reported. DPPH is a stable free radical and is often used to evaluate the antioxidant activity of several natural compounds. Antioxidants, on interaction with DPPH, transfer electrons or hydrogen atoms to DPPH, and thus neutralize its free-radical character. DPPH shows a strong absorption at 517 nm.

The DPPH (0.01 mM) in methanol was mixed well with the diluted extracts solution in DMSO and kept in the dark for 30 min. The absorbance at 517 nm was monitored in the presence of different concentrations of the extracts. Black group is also measured to determine the absorbance of DPPH before interacting with the extracts. The antioxidant activity was expressed as the IC_{50} (concentration in μg mL^{-1} required to inhibit a DPPH radical formation by 50%) determined from the dose curve (Xiong *et al.* 1996). The inhibitory percentage of DPPH was calculated according to the following equation:

Scavenging effect;

$$\% = [1 - \text{absorbance}_{\text{sample}}/\text{absorbance}_{\text{control}}] \times 100$$

3. Animal treatments and irradiation

The animal was purchased from the Daehan Biolink (Chungbuk, Korea) Fisher 344 rats (male, 10-week-old) were used throughout the investigations. All the animals were maintained under the following conditions; temperature (23°C) and lighting (12 hr light : 12 hr dark) and allowed free access to food and water *ad libitum*.

For oral administration (twice per day), the extractive powders of the *I. dentata* leaf and root were dissolved at a concentration of 0.5 mg mL^{-1} in saline, respectively. And the positive control (ascorbic acid) was dissolved at a concentration of 250 mg mL^{-1} in saline. After a treatment of 5 days, the irradiation groups were exposed to gamma-rays from a ^{60}Co source with a total dose of 6.5 Gy, and a dose rate of 12.8 Gy hr^{-1} (Kim *et al.* 1999).

4. Measurements of the amounts of enzymes in blood

Thirty days after irradiation, the animals were sacrificed by cervical dislocation. The levels of some serum enzymes were checked in the experimental animals. The reaction of SGOT (serum glutamine oxaloacetic transaminase) and SGPT (serum glutamate pyruvate transaminase) were measured with ALT reagents (Bayer, USA). ALP (alkaline phosphatase) was assayed by means of an ADVIA 1650 reaction of the kinetic UV method.

5. Single Cell Gel Electrophoresis Assay

Mean while, 100 μL of the whole blood was then added to 1% of 100 μL of a low melting point agarose (LMA). A second layer of 200 μL of the sample mixture was poured onto precoated slides (1% regular melting agarose) and allowed to solidify for 20 min at 4°C. A third layer of 200 μL of the LMA was poured onto the slides and allowed to gel for 20 min at 4°C. The slides were placed in a prepared cold lysing solution (2.5 M NaCl, 100 mM EDTA, 10 mM Tris (pH 10), 1% sodium sarcosinate with 1% Triton X-100, and 10% DMSO added just before use) for 1 h at 4°C in darkness. Lysis removes the cell contents except for nuclear material. DNA remains highly supercoiled in the presence of a small amount of nonhistone protein but when placed in alkali, it commences to unwind from its sites of a strand breakage (Yildiz *et al.* 2002). After lysis, slides were then placed in an electrophoresis buffer (300 mM NaOH and 1 mM, EDTA, pH 13) for 15 min to allow for the unwinding of the DNA to occur. Electrophoresis was conducted for 40 min at 26 V and 300 mA. Slides were then drained, placed on a tray and washed slowly with three changes of 15 min each of a neutralization buffer (400 mM Tris-HCl pH 7.5). Slides were stained with ethidium bromide (20 μL mL^{-1}). Nuclei of 50 lymphocytes per slide per sample were examined at random at a 200-fold magnification using an epi-fluorescence microscope with a green filter. For quantification we used a CCD camera and Komet ver. 4.0 image analysis (Kinetic Imaging Ltd, Liverpool, UK). Fifty images were randomly selected from each sample and the comet tail DNA was measured. Three parallel tests with aliquots of the same sample of cells were performed for a total of 150 cells. The olive tail moment and tail extent moment were analyzed. The mean value of the tail moment in a particular sample was taken as an index of the DNA damage in this sample. The

olive tail moment and tail extent moment were calculated according to the following equation:

Olive Tail Moment = (Tail mean-Head mean) × Tail % DNA / 100

Tail Extent Moment = Tail Length × Tail % DNA /100

6. Statistical analysis

Statistical analysis was performed by Students *t* test for a simple comparison of the two groups using Sigma Plot software (Jandel Scientific, Germany). They are expressed as mean ± SEM. $p < 0.05$ was considered significant.

RESULTS AND DISCUSSIONS

1. Radical scavenging activities of the plant extracts

The antioxidant assay of the water- and ethanol-extracts from the *I. dentata* leaf and root was carried out using the free radical 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) scavenging method. The comparisons were made on the basis of the IC_{50} value which indicated the amount required for a 50% reduction of DPPH. The results of the DPPH free radical scavenging assay were that the IC_{50} of the ascorbic acid and glutathione were 0.0074 and 0.0152, respectively ($p < 0.05$). The IC_{50} of the water- and ethanol-extracts of the *I. dentata* leaf and root were 0.0378, 0.0232, 0.6070 and 0.2425 μg mL^{-1} as presented in Fig. 1, respectively ($p < 0.05$). Between the experimental groups, the extracts from the leaf had an excellent activity compared with that of the *I. dentata* root. The groups of ethanol extracts resulted in a higher scavenging activity than the water extracts. Especially, the antioxidant activity of the ethanol-extract of the leaf was similar to the radical scavenging activity of glutathione. This result has revealed that we will be able to obtain a natural antioxidant better than the established antioxidants if the extracts of *I. dentata* are purified.

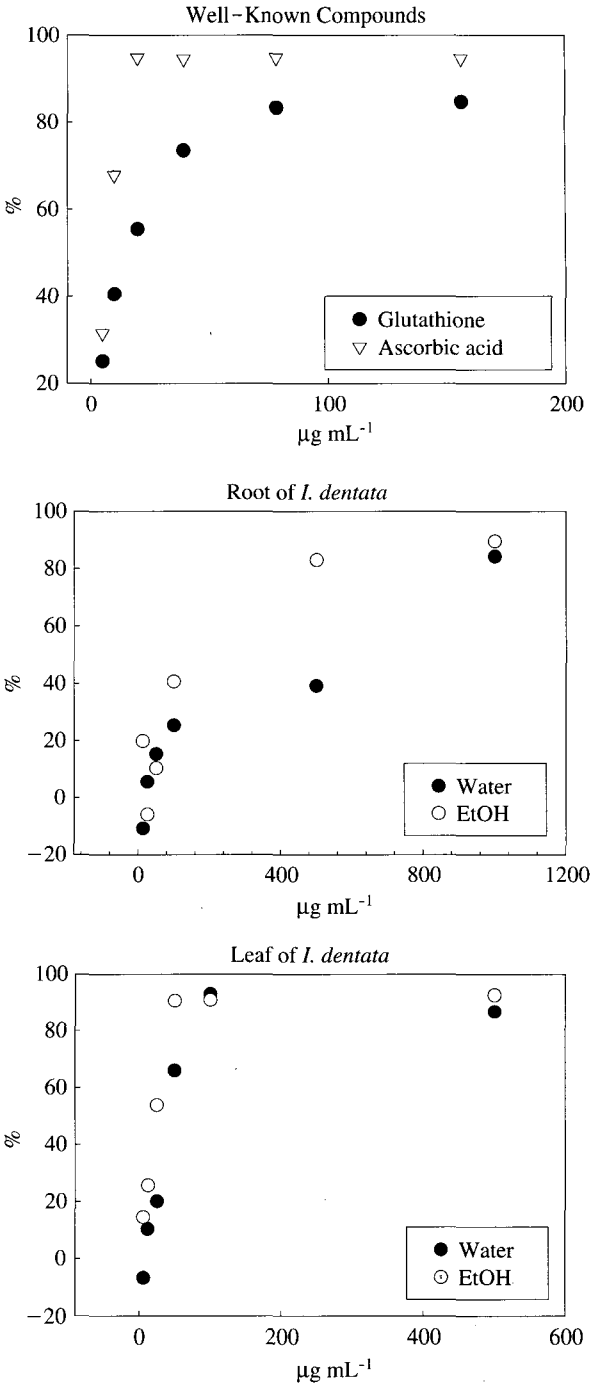

Fig. 1. DPPH free radical scavenging activity of plant extracts and the well-known antioxidants. Results are expressed as percentage decrement of absorbance at 517 nm.

2. Changes of the body weights and organ indices in the experimental groups

Thirty days after irradiation, the body and organ weights were measured. The change of the body weight during the experimental period is represented in Fig. 4. The rate of increase in the body weight of the irradiation groups showed a lower rate than those of the others. Interestingly, the groups of ascorbic acid and the

Table 1. The organ indices[π] (liver, testis, kidney and spleen) of the experimental group 4 weeks after whole-body irradiation

		Liver	Testis	Kidney	Spleen
	CON	4.8±0.04	0.54±0.002	0.43±0.020	0.27±0.009
	CON+RAD	5.0±0.11	0.27±0.019	0.43±0.009	0.27±0.021
	AsA+RAD	4.6±0.06	0.26±0.035	0.47±0.024	0.43±0.145
Leaf Portion	W+RAD	4.4±0.10	0.33±0.003	0.42±0.004	0.27±0.011
	E+RAD	4.7±0.02	0.28±0.003	0.46±0.000	0.27±0.011
Root Portion	W+RAD	4.5±0.15	0.29±0.012	0.46±0.010	0.26±0.011
	E+RAD	4.5±0.28	0.24±0.009	0.47±0.011	0.31±0.012

[π], Values (%) of organ indices are calculated from the equation of (organ weight/body weight of each animal) × 100. Abbreviations: CON, the control group; RAD, the whole-body irradiated group; AsA, the ascorbic acid treated group; W, water extracts of the plant; and E, ethanol extracts of the plant.

Table 2. Relative value of the level of some enzymes in the serum of the experimental groups (%)

		ALP	SGOT	SGPT
	CON	100.00±2.25	100.00±8.79	100.00±6.56
	CON+RAD	91.76±2.14	122.33±25.42	99.18±0.66
	AsA+RAD	93.48±0.00	60.81±2.85*	73.77±3.28*
Leaf portion	W+RAD	79.68±1.39*	94.06±1.90*	77.87±0.82*
	E+RAD	82.67±1.82	98.57±4.99	86.07±4.10
Root portion	W+RAD	81.60±1.82	89.31±1.35*	81.97±6.56
	E+RAD	80.00±4.28	72.68±0.95*	77.87±5.74*

Abbreviations: ALP, alkaline phosphatase; SGOT, serum glutamic oxaloacetic transaminase; and SGPT, serum glutamate pyruvate transaminase. Asterisk indicates the significant difference from the level of the irradiated control group.

ethanol extracts of the root were shown to have a difference at more than 60 g of the change of the body weight between the radiation groups and control groups. But there was no significant difference between the treatment groups and the control. The ratio of the weight of the testis to the body weight was lower more than 50% of the radiation groups than that of the control groups. But the ratio of the organ weight to the body weight of the others showed no significant difference as in Table 1.

3. Measurements of enzymes in blood after irradiation

The blood assays were analyzed for ALP, SGOT and SGPT. Table 2 indicates the percent age amount of each enzyme in the blood compared with the value of the control group. There was no significant difference in the values of the ALP in the irradiated control group and the control group. The ALP concentrations of all the treated groups showed a lower value than the control group. The concentrations of ALP in the treatments of the water extracts of the leaves showed the lowest amount by 79.68±1.39% ($p<0.05$).

The SGOT concentrations of the irradiation control group and the control group were 122.33±25.42 and 100.00±8.79%, respectively ($p<0.05$). It indicates that an ionizing radiation caused cell damage by irradiation. In the case of the ascorbic acid group, the value of the SGOT concentration was 60.81±2.85%. It was shown that the cell damage by irradiation was decreased by over a half. The values of the water- and ethanol extracts of the leaf and root group were 94.06±1.90, 98.57 ±4.99, 89.31±12.35 and 72.68±0.95%, respectively ($p<0.05$). Each extract of *I. dentata* showed a higher value than the well-known antioxidants, ascorbic acid. In the case of SGPT, the value of the irradiation control, ascorbic acid, water- and ethanol extract of the leaf and the root group was 99.18±0.66, 73.77±3.28, 77.87± 0.82, 86.07±4.10, 81.97±6.56 and 77.87±5.74%, respectively ($p<0.05$). Both the SGOT and SGPT of each extract of *I. dentata* showed lower values than the negative control group. The experimental results have revealed that the extracts of *I. dentata* have an excellent ability to reduce the radicals and thus might act as radioprotectors.

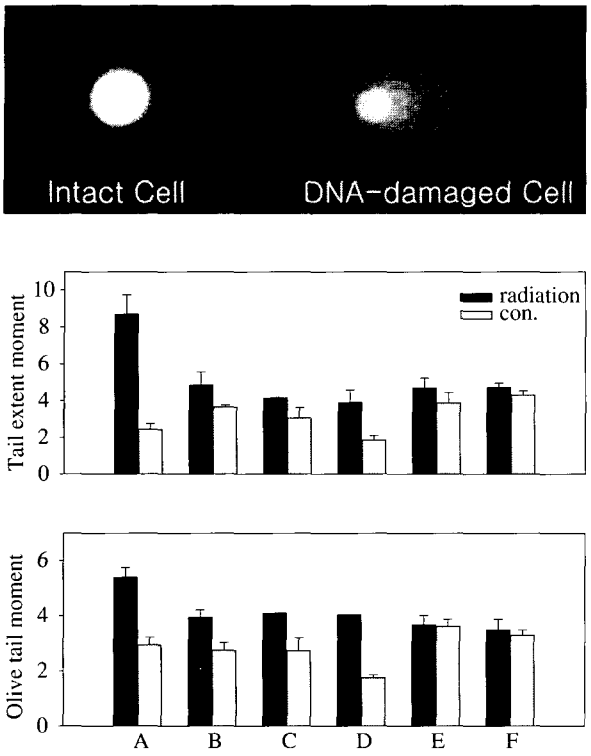

Fig. 2. The *in vivo* genotoxicity assays of the extracts of *I. dentata* and the detection of the anti-mutagenic effects against irradiation using the comet assay of rat lymphocytes; olive tail moment = (tail mean-head mean) * tail % DNA/100; tail extent moment = tail length * tail % DNA/100. A, vehicle; B, ascorbic acid; C, water extracts of leaves; D, ethanol extracts of leaves; E, water extracts of roots; F, ethanol extracts of roots.

4. The protective effects on DNA damages in single cells

The comet assays in the present study were accomplished by 10-week-old F344 rats. Because the repair efficacy of DNA damage in adult showed a lower efficacy rather than the repair activity in pubertal, we used adult rats to identify the obvious difference between the experimental groups (Vorobtsova *et al.* 2001). The genotoxicity in the irradiation group showed that DNA breakage had occurred when compared with the control groups as shown in Fig. 2. Increases in the tail extent moment and olive tail moment in the irradiation group are measures of the increase in the relative amount of DNA in the tail when compared with a portion of the head. There was no significant difference in the values of the tail moments in all the treated groups and the control group, except for the irradiation group in Fig. 3. The tail extent moment and olive tail moment of the irradiated control group increased remarkably in comparison to those of the others. The tail extent moment and olive tail moment of the control group were 2.45±0.29 and 2.94±0.28, respectively ($p<0.05$). The values of the irradiation group were 8.69±1.07 and 5.39±0.36, respectively ($p<0.05$). It is shown that DNA damage by an irradiation *in vivo* was twice as much as the control. The values of the ascorbic acid treated irradiated group, were 4.85±0.72 and 3.94±0.27, respectively ($p<0.05$). In the group of the irradiation after treatment of the water- and ethanol-extracts of the leaves and root part, the tail extent moment and olive tail moment were 4.14±0.04, 4.09±0.00, 3.90±0.69, 4.03±0.00, 4.69±0.54, 3.67±0.34, 4.72±0.24 and 3.48±0.39, respectively ($p<0.05$). The tail moment of the each extract of *I. dentata* showed lower values than the values of the well-known antioxidants, ascorbic acid. These experimental results have revealed that the extracts of *I. dentata* have a protection effect for DNA damage by irradiation.

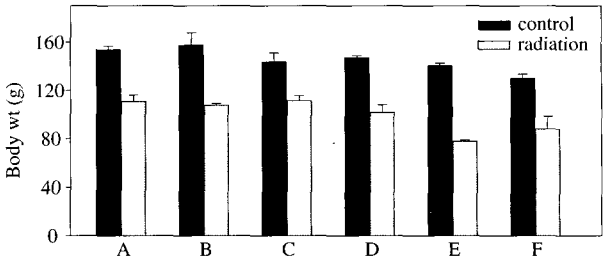

Fig. 3. The effect of the extracts of *I. dentata* and chemical compound on the relative body weight gain of a rat. Changes in the body weight of the experimental group 4 weeks after whole-body irradiation.

CONCLUSIONS

The present study was designed to explore the protective effects of water- and ethanol-extracts of *I. dentata* in a typical oriental herb on radiation damage. In the DPPH assay, the antioxidant activity of the ethanol-extract of the leaves was similar to the scavenging activity of glutathione, an excellent antioxidant. The results of the tail moment in the SCGE assay showed

that both the extracts of *I. dentata* had a protective ability better than ascorbic acid for the DNA breakages caused by ionizing radiation. Thirty days after irradiation, Treatments of the water extracts of the leaves showed a much lower value in the ALP concentrations by $79.68 \pm 1.39\%$ ($p < 0.05$). Also the treatments of the ethanol extract of the *I. dentata* roots affected the levels of the SGOT and SGPT and they had much lower values by 72.68 ± 0.95 and $77.87 \pm 5.74\%$ ($p < 0.05$), respectively. These data suggest that the extracts of *I. dentata* have an excellent ability to reduce the radicals and thus they will protect genetic materials from damage by whole-body irradiation.

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REFERENCES

Bowden RD, MR Buckwalter, JF McBride, DA Johnson, BK Murray and KL O'Neill. 2003. Tail profile: a more accurate system for analyzing DNA damage using the Comet assay. Mutat. Res. 537:1-9.

Bramer I, C Herskind, O Haase, HP Rodemann and E Dikomey. 2004. Induction and repair of radiation-induced DNA double-strand breaks in human fibroblasts are not affected by terminal differentiation. DNA Repair. 3:113-120.

Chung HS, HJ Jeong, MJ Han, ST Park, KK Seong, SH Baek, DM Jeong, MJ Kim and HM Kim. 2002. Nitric oxide and tumor necrosis factor-α production by *Ixeris dentata* in mouse peritoneal macrophages. J. Ethnopharmacology. 82:217-222.

El-Demerdash, FM. 2001. Effects of selenium and mercury on the enzymatic activities and lipid peroxidation in brain, liver, and blood of rats. J. Environ. Sci. Health B. 36:489-499.

Fukuda S, S Tsuchikira and H Iida. 2004. Age-related changes in blood pressure, hematological values, concentrations of serum biochemical constituents and weights of organs in the SHR/Izm, SHRSP/Izm and WKY/Izm. Exp. Anim. 53:67-72.

Grover P, BS Banu, KD Devi and S Begum. 2001. In vivo genotoxic effects of mercuric chloride in rat peripheral blood leucocytes using comet assay. Toxicology 167: 191-197.

Hsiao G, MY Shen, KH Lin, MH Lan, LY Wu, DS Chou, CH Lin, CH Su and JR Sheu. 2003. Antioxidative and hepatoprotective effects of Antrodia camphorata extract. J. Agric. Food Chem. 51:3302-3308.

Kim JK, CJ Lee, KW Song, BR Do and YD Yoon. 1999. γ-Radiation accelerates ovarian follicular atresia in immature mice. In Vivo 13:21-24.

Kim MK and MS Lee. 1988. Volatile flavor components of *Ixeris Dentata* and *Amaranthus mangostanus*. J. Korea Soc. Agri. Chem. Biotech. 31:394-399.

Kim MJ, JS Kim, DM Jeong, SS Ham and CY Yu. 2002. Effect of antioxidant, antimutagenicity and anticancer of root extract from *Ixeris dentata* Nakai. Korean J. Medicinal Crop Sci. 10:222-229.

Kim SH. 1995. Inhibitory effects of *Ixeris dentata* on the mutagenicity of aflatoxin B1, *N-methy-N'-nitro-N-nitrosoguanidine* and the growth of MG-63 human osteosarcoma cell. J. Korea Soc. Food Sci and Nutr. 24: 304-305.

Kumar O, K Sugendran and R Vijayaraghavan. 2003. Oxidative stress associated hepatic and renal toxicity induced by ricin in mice. Toxicon. 41:333-338.

Lim SS and JH Lee. 1997. Effect of *Aster scaber* and *Ixeris dentata* on contractility and vasodilation of cardiovascula and endothelial cell in hyperlidemic rat. J. Korean Soc. Food Sci. Nutr. 26:300-307.

Vijayalaxmi RJ, TS Reiter, Herman and ML Meltz. 1998. Melatonin reduces gamma radiation-induced primary DNA damage in human blood lymphocytes. Mutat. Res. 397:203-208.

Vorobtsova I, A Semenov, N Timofeyeva, A Kanayeva and I Zvereva. 2001. An investigation of the age-dependency of chromosome abnormalities in human populations exposed to low-dose ionizing radiation. Mech. Age. Devel. 122:1373-1382.

Xiong Q, S Kadota, T Tadata and T Namba. 1996. Antioxidative effects of phenylethanoids from Cistanche deserticola. Biol. Pharm. Bull. 19: 1580-1585.

Yildiz D, T Akçay, Z Alademir and H Ilkova. 2002 Assessment of DNA base oxidation and glutathione level in patients with type 2 diabetes. Mutat. Res. 505:75-81.

Sener G, N Jahovic, O Tosun, BM Atasoy and BÇ Yeğen. 2003. Melatonin ameliorates ionizing radiation-induced oxidative organ damage in rat. Life Sci. 74:563-572.

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