Radioprotective Effects of Korean Endemic Plants as Assessed by *in vivo* and *in vitro* Assays

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1. Introduction

Numerous antioxidants such as phosphorothioates, bioactive lipids, and immunomodulators have proven to have radioprotective effects [1]. In that sense, naturally occurring antioxidants are a class of radioprotectors. The extract from plants such as garlic, ginseng, *Aloe*, podophyllum, *Ocimum*, *Spirulina* and certain herbal drugs have been found to have radioprotective effects in mammals [2]. Plant products appear to have an advantage over the synthetic compounds in terms of low or no toxicity at the effective dose with minimum or no side effects.

In this study, three species of Korean endemic plants were chosen as experimental candidates, based on the previous study [3]. The aims of this study were to evaluate the antioxidant effect of water- or ethanol-extracts of Korean endemic plants *in vivo* and *in vitro* systems and to apply them to protection of living organisms against irradiation.

2. Methods and Results

2.1Preparation of the Extracts from Endemic Plants

Three species of Korean endemic plants, *Salicornia herbacea* (SH), *Aster scaber* (AS), *and Ixeris dentata* (ID) were collected in the non-polluted areas in June. The dried whole-body or different part of each plant was roughly ground and extracted with distilled water or ethanol. The extracts were finally lyophilized to obtain a form of stock powder. The processes for the extract preparation can be found in the literature [3]. Distinctly, extracts of SH before lyophilization were dialyzed for desalination using the dialysis tube (M.W. 12,400) for 48 hours at room temperature.

2.2 Cell Culture and Assay

Cell viability was determined by the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay, measures the metabolic reduction of MTT to formazan by mitochondrial dehydrogenases in live cells [4]. The free radical scavenging ability of the extracts was assessed by the method of DPPH [5].

The standard Comet assay for the detection of DNA strand breaks is carried out under alkaline conditions [6].

2.3 Animals and Treatments

Prepubetal male Fisher 344 rats (3 weeks old with body weight 82 ± 5 g) were maintained under controlled conditions of temperature (23°C) and light (14 hour light: 10 hour dark) in an animal house and were provided standard rats feed and water *ad libitium*.

Extracts treated groups were administrated with 0.5 mg/ml in saline by *po* administration, daily twice, during five days before irradiation. A positive control was treated ascorbic acid solubilized in saline by orally administration. Irradiated groups were exposed to γ-irradiation from a ^{60}Co source with a total dose of 6.5 Gy, and a dose rate of 12.8 Gy/hr [7]. All the rats were euthanized immediately, six hours or one month after irradiation. Right upon death, blood was collected from the heart.

3. Results and Discussions

3.1 Activity of Radical Scavenging

As a result of the DPPH assay (Table 1), the value of 50% reduction on BHT-DPPH reaction was 6.68 μg/ml. The ethanol-extracts of SH and leaves of ID showed an effective scavenging activity, 6.99 and 3.48 times, respectively higher than that of well-known commercial antioxidants except for BHT in our experimental system. Water extracts of AS and leaves of ID exhibited the activity of 3.78 and 5.72 times compared with the scavenging value of BHT. In result, the free radical scavenging activity of both extracts of leaves of ID was superior to another extracts.

Table 1. Scavenging effects of well-known antioxidants and the extracts of the endemic plants on DPPH radicals

Materials	50 % Reduction (mg/ml)	Materials	50 % Reduction (mg/ml)
BHT*	0.0056754	Glutathione	0.0152332
Ascorbic acid	0.0073641	Tocopherol Acetate	6.466632
Water extracts		*Ethanol extracts*	
S. *herbacea*	0.1195314	S. *herbacea*	0.0435161
A. *scaber*	0.0250903	A. *scaber*	0.0881057
I. *dentata* – Leaves	0.0378501	I. *dentata* – Leaves	0.023204
I. *dentata* – Stems	0.0642178	I. *dentata* – Stems	0.0835375
I. *dentata* – Roots	0.5070908	I. *dentata* – Roots	0.242483

*, butylated hydroxytuluene.
A parenthesized value showed irradiated groups.
The bar represents the mean ± SEM of 5 rats.

3.2 Body and Organ weights in Irradiated Rats

No detectable amelioration in body weights of experimental administrations was found in rats irradiated with 6.5 Gy of gamma rays. The testis is known to be sensitive to ionizing radiation. The value of ascorbic acid used as a positive control showed 82% of the irradiated control. This result was due to reduction of body weights. In the experimental groups, both extract of SH and water extract of leaves and roots of ID resulted in the higher values than that of the irradiated control. The extract from leaves of ID resulted in a significant increase in the body and organ weights.

3.3 Changes of Hematological Components

Treatment of ethanol extracts of SH resulted in the control level of leukocyte counts, whereas the irradiated control showed a decrease by 60% from that of the control. The water extracts of ID leaves lead to about 30% decrease from that of the control. However, the counts of experimental groups except above two groups were in the similar range to the values of the irradiated control. Treatment of the ethanol extracts of SH was more effective on inhibition of radiation-induced impairment than treatment of ascorbic acid..

3.4 Single Cell Gel Electrophoresis Assay

The comet assay was done immediately after whole-body irradiation of 6.5 Gy. Damage was induced by gamma irradiation as shown in Figure 2. Ascorbate- and the extracts-treated groups showed bout 25% decrease in the radiation-induced DNA damage. Difference between the extracts- and ascorbic acid treated groups was not significant..

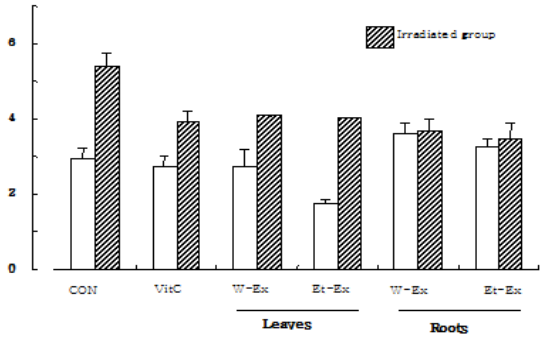

Fig. 1. Protective effect of the extracts of ID in whole blood immediately after irradiation. Radiation-induced DNA damage was measured by the Comet assay. Each column represents the mean of Olive Tail Moment ± SD.

Plant materials have long been used as traditional medicines for the treatment of a wide variety of ailments and diseases. Also, extracts of plants are known to contain many different components that can act as antioxidants. These actions make plant materials of particular importance in the diet since it has been demonstrated that consumption of the antioxidant compounds in vegetables protects living organisms from oxidative damage.

The obtained results showed that ethanol extracts of SH, water extracts of AS, and both extracts of ID possess free radical scavenging activity. Although an individual component or the most effective fraction has not been identified, the extracts of the endemic plants tested in this study show the strong antioxidant scavenging activity and protection against irradiation-induced oxidative stress.

4. Conclusions

The findings of this study indicated that water and ethanol extracts of the chosen endemic plants can have a promising role of protecting normal tissues surrounding tumor against accidental or therapeutic irradiation.

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