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Radioprotective Effects of Hairy Roots of Ginseng

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1. Introduction

Panax ginseng is an important medicinal plant in Korea, which has broad efficacious effects against hypertension, diabetes, nociception and cancer. And it improves weakness [1]. The native ginseng is a slow growing plant taking 5-7 years from seed planting to mature root harvesting, during which time much care is needed since its growth is susceptible to many environmental factors such as soil, shade, climate, pathogens and pests. Nowadays, a wild ginseng has become extremely scarce and the ginseng supply depends almost exclusively on field cultivation, which is a time-consuming and labor-intensive process. To meet the demand for the plant in the international market, a bioreactor technology is a useful tool for production of root biomass on a large scale. Therefore, suspension culture of ginseng roots in bioreactors is viewed as a primary alternative method for large-scale production and recently our laboratory has developed a protocol for the *in vitro* culture of *P. ginseng* [2]. 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 bio-molecules causing structural and functional modifications. The generation of the reactive oxygen metabolites plays an important role in the pathogenesis of the 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 [3]. The purpose of this study, aimed at investigating the possible radioprotective effect of the hairy roots of *P. ginseng* on irradiation-induced damage by the comet assay.

2. Methods and Results

2.1 Extracts of plants

The extract of the hairy roots of *P. ginseng* was kindly provided by the *Chungbuk National University*.

2.2 MTT Cell Viability Assay

B16 melanoma cells were obtained from ATCC. B16 melanoma cells were cultured in RPMI-1640 medium supplemented with 10% heat-inactivated fetal bovine serum at 37℃ in 5% CO_2, 95% O_2 in a humidified cell incubator. We treated cells with the extract of hairy roots of *P. ginseng*. After treatments of the extracts, the cells were cultured in CO_2 incubator for 2 hr prior to irradiation. The irradiated groups were exposed to γ-radiation from a ^{60}Co source with a total dose of 6.5 Gy, and a dose rate of 76.8 Gy/hr [4]. The cell viability was assessed by MTT (3-[4,5-dimethylthiazol - 2yl] - 2,5 - diphenyl - tetrazolium bromide; Sigma) assay after irradiation. The absorbance at 570 nm was monitored in presence of different cell viability of treatments.

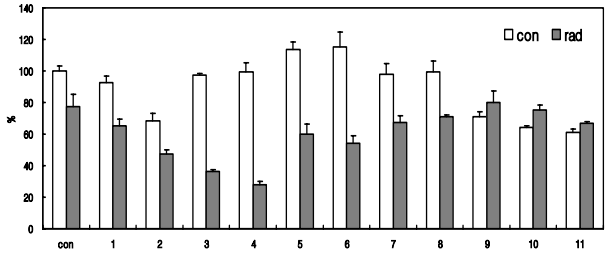

Figure 1. The viability of the irradiated B16 cells.

The cell viability of the control and iraradiation group were 100±3.28 and 77.3±7.99, respectively (Figure 1). The cell viability of the control groups of 5 and 6 lines were 113.8±4.88 and 115.2±9.77, respectively. The cell viability of the radiation groups of 9 and 10 lines were 80.1±7.39 and 75.3±3.13, respectively.

2.3 Single Cell Gel Electrophoresis Assay

After irradiation, 100㎕ of the cells were then added to 1% of 100㎕ of a low melting point agarose. A second layer of 200㎕ of the sample mixture was poured out to the precoated slides. A third layer of 200㎕ of the LMA was poured out to the slides. The slides were placed in a prepared cold lysing solution for 1 h at 4℃ in darkness. After lysis, the slides were then placed in an alkaline buffer for 5 min to allow for the unwinding of the DNA to occur. Electrophoresis was conducted for 20 min at 26 V and 300 mA. The slides were then drained, placed on a tray and washed slowly with three changes of 15 min each of a neutralization buffer. The slides were stained with ethidium bromide. Nuclei of 50 lymphocytes per slide per sample were scored at random under an epi-fluorescence microscope

[5].

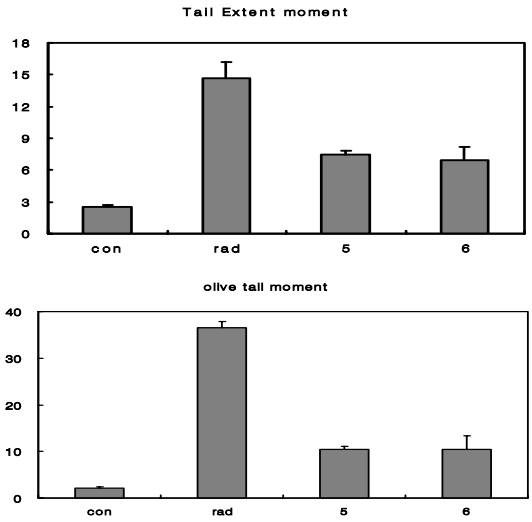

Figure 2. The *in vitro* genotoxicity assays of B16 cells after irradiation and treatment with the extract of hairy roots of *P. ginseng* with the comet assay. Extent tail moment = tail length × tail % DNA /100, tail extent moment = tail length * tail % DNA/100.

In the *in vitro* comet assays for the genotoxicity in the B16 melanoma cells, more DNA breakage was observed in the irradiation group than in the extract-treated group. The tail extent moment of the irradiation groups of control and 5 and 6 lines were 14.66±1.56, 7.48±0.31 and 6.89±1.30, respectively (Figure 2). The olive tail moment of the irradiation groups of control and 5 and 6 lines were 36.51±1.38, 10.47±0.63 and 10.50±3.94, respectively. The group treated with the extracts of 5, 6 line of hairy roots of *P. ginseng* exhibited lower damage than that of the control. These experimental results have revealed that the extracts of hairy roots of *P. ginseng* have an excellent ability to reduce radicals and thus can act as radioprotectors

3. Conclusion

Panax ginseng has been used for more than 2,000 years as a general tonic in the traditional medicine. The cell viability was assessed by the MTT assay after irradiation. The genotoxicity test in the B16 melanoma cells with the comet assay indicated that the extracts of hairy roots of *P. ginseng* have an excellent ability to reduce radicals and thus can act as radioprotector.

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