Radioprotective Effect of Extract from *Ealeocarpus sylvestris*

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Abstract

We have investigated the cytoprotective effect on γ-ray radiation induced oxidative stress from *Ealeocarpus sylvestris* extract. As a result, the extract scavenged reactive oxidative stress (ROS) generated by radiation. However, the extract did not protect cell death of V79-4 cells damaged by γ-ray radiation. Taken together, *Ealeocarpus sylvestris* extract did not protect V79-4 cells against oxidative damage by radiation through scavenging ROS.

keywords: γ-ray radiation, oxidative stress, reactive oxygen species

Introduction

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, which 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 reactive oxygen species (ROS)-mediated damage. It is suggested that both radiation injury and oxygen poisoning occur through the formation of ROS [16]. 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 [17].

In present study, we investigated whether *Ealeocarpus sylvestris* extract may show protective effect against γ-ray radiation.

Materials and Methods

Ealeocarpus sylvestris extract - The extract were obtained from Dr. Nam Ho Lee (Cheju National University, Jeju, Korea).

Reagents - 2',7'-Dichlorodihydrofluorescein diacetate (DCF-DA) were purchased from Sigma Chemical Company (St. Louis, MO, USA). MTT ([3-(4,5-dimethylthiazol-2-yl) - 2,5-diphenyltetrazolium] bromide) were purchased from Sigma Chemical Company (St. Louis, MO, USA).

Cell culture - It is reported that lung is an organ sensitive to oxidative stress. To study the effect of plant extracts on oxidative stress, we used Chinese hamster lung fibroblasts (V79-4 cells). 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 ug/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).

Intracellular reactive oxygen species measurement - The DCF-DA method was used to detect the intracellular ROS level [18]. 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 the extract and 1 h later, γ-ray radiation at 10 Gy was added to the plate. The cells were incubated for an additional 48 h at 37℃. After addition of 25 uM of DCF-DA solution, the fluorescence of 2',7'-dichlorofluorescein was detected at 485 nm excitation and at 535 nm emission using a PerkinElmer LS-5B spectrofluorometer.

Cell viability - The effect of the extract 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 [19]. To determine the effect of the extract on the viability of V79-4 cells on 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 ug/ml of the extract for 1 h. Plates were irradiated at 10 Gy and the plate was incubated at 37 ℃ for 48 h, the cell viability was measured using MTT test. Fifty ul of the MTT stock solution (2 mg/ml) was then added to each well to attain a total reaction volume of 200 ul. After incubating for 4 h, the plate was centrifuged at 800 ug for 5 min and the supernatants were aspirated. The formazan crystals in each well were dissolved in 150 ul dimethylsulfoxide (DMSO) 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.

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 [20], as well as later membrane lipid peroxidation, which is characteristic of radiation-induced apoptosis [21]. The extract decreased ROS generation by γ-ray radiation, showing the ROS generation of 75%, 76%, 80% and 85% respectively, compared to the ROS generation of 106%, 121%, 122% and 132% in 5 Gy, 10 Gy, 15 Gy and 20 Gy radiated cells (Figure 1). However, the extract did not protect cell death of V79-4

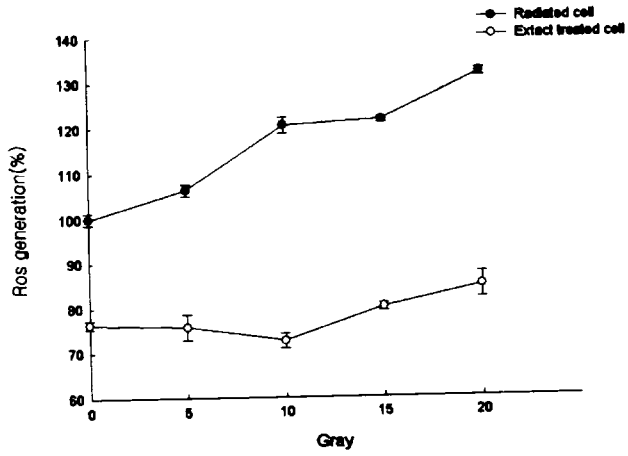

Figure 1. Effect of *Ealeocarpus sylvestris* extract on generation intracellular ROS induced by radiation. The intracellular ROS was detected by DCF-DA method.

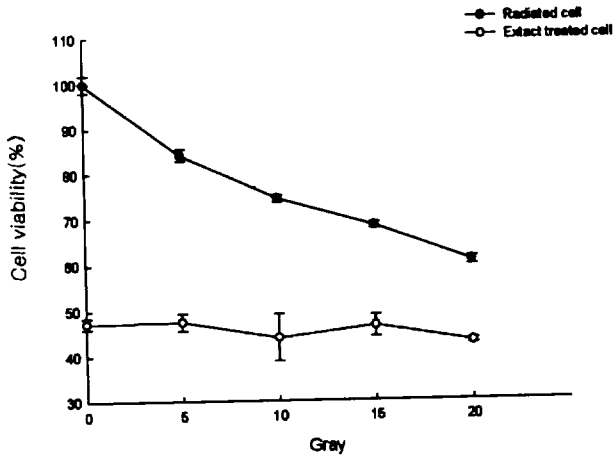

Figure 2. Protective effect of *Ealeocarpus sylvestris* extract upon γ-ray radiation induced oxidative damage of V79-4 cells. The viability of V79-4 cells upon γ-ray radiation at day 2 was determined by MTT assay.

cells damaged by γ-ray radiation, showing the cell viability of 48%, 46%, 44% and 42% respectively, compared to cell viability of 84%, 74%, 68% and 60% in 5 Gy, 10 Gy, 15 Gy and 20 Gy radiated cells (Figure 2). 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 [22], supporting the antioxidant activity of *Ealeocarpus sylvestris*.

Taken together, *Ealeocarpus sylvestris* extract did not protect V79-4 cells against oxidative damage by radiation through scavenging ROS.

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초 록

우리는 담팔수과 추출물로부터 γ-ray 방사선 조사로 유도된 산화적 손상에 세포보호 효과를 연구하였다.

결과적으로 추출물은 방사선에 의한 ROS생성을 감소 시켰지만, V79-4세포를 방사선조사로 인한 손상의 세포사로부터 보호작용을 나타내지는 않았다.

따라서 담팔수과 추출물은 ROS제거를 통한 방사선 조사에 의한 산화적 손상에 대해 보호작용을 나타내지 않는 것으로 추측된다.