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Brief Communication

Protective Effect of Aqueous Extract from *Erigeron annuus* Against Cell Death Induced by Free Radicals

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The extract of EA lacks studies showing its efficacy other than that it contains caffeic acid, an active compound that has antioxidant and neuroprotective effects on nerve cells. Therefore, in this study, we attempted to determine the effectiveness of EA extraction. In this study, we performed a DPPH assay to determine the antioxidant potential of EA. And then, the cytotoxic concentration of EA in HaCaT keratinocytes was determined, and the antioxidant effect was determined by measuring the malondialdehyde (MDA). The results of DPPH, a chemical antioxidant assay, clearly demonstrated the antioxidant capacity of EA extracted with distilled water. In addition, cell-based assays provide useful information on the protective effect of EA on oxidative stress-induced apoptosis.

Key Words: *Erigeron annuus*, Protective effect, 2,2-diphenyl-1-picrylhydrazyl (DPPH), Malondialdehyde (MDA), Free radicals

Erigeron annuus (EA) is exactly an annual plant. EA comes from the Greek words meaning that the plant blooms in late spring and forms fuzzy white seed heads while still producing new flowers. Only the leaves of EA are edible. You can use it anywhere you cook with vegetables. The extract of EA lacks studies showing its efficacy other than that it contains caffeic acid, an active compound that has antioxidant and neuroprotective effects on nerve cells. Therefore, in this study, we attempted to determine the effectiveness of EA extraction. There are many chemical methods that can be used to measure the activity of natural products. Among them, antioxidant activity is known to play an important role in many cellular activities, including signaling pathways, cell proliferation and differentiation (Wellen and Thompson, 2010). Excessive increases in reactive oxygen species (ROS) can lead to loss of cell function and eventually to apoptosis (Maynard et al., 2009; Circu and Aw, 2010; Ayala et al., 2014; Volpe et al., 2018). Antioxidant activity is based on free radical scavenging and competition with free radicals. Although limited, free radical scavenging assays have been widely used to determine the antioxidant capacity of natural products due to their ease and sensitivity (Olszowy and Dawidowicz, 2018). Moreover, Oxidative stress is assessed by detecting markers of lipid or protein oxidation. Lipid peroxidation is determined by the measurement of the final lipid peroxidation product, MDA. It is a good indicator of cellular antioxidant status (Szychta et al., 2014). Therefore, in this study, we performed a DPPH

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assay to determine the antioxidant potential of EA. And then, the cytotoxic concentration of EA in HaCaT keratinocytes was determined, and the antioxidant effect was determined by measuring the MDA.

Preparation of EA extracts. EA was purchased Daum International (Hanam, Korea). The EA was washed and dried at room temperature for about 1 week. The dried EA was ground into a fine powder in a blender. About 10 g of EA powder was extracted with distilled water at 60℃. for 15 hours. The extract was filtered with filter paper. The filtrate was concentrated under reduced pressure at 45℃ using a rotary evaporator. The concentrated extract was lyophilized and quantified. The stock solution of the extract was stored frozen at a concentration of 1 mg/mL and used for further experimental analysis.

2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. DPPH analysis was performed with modifications as previously described (Lee et al., 2017). Briefly, 100 μL of two-fold serial dilutions of extracts from various concentration ranges were placed in 96-well microplates. Then, 100 μL of 200 μM DPPH (Sigma, St. Louis, USA) in methanol was added to each sample. For blank samples, the extract and DPPH solutions were replaced with distilled water. Assay controls contained 100 μL ascorbic acid and 100 μL DPPH solution. After incubating the reaction mixture for 15 min at room temperature, absorbance was recorded at 515 nm.

Cell culture. HaCaT keratinocytes, a human keratinocyte cell line used in this study, were purchased from American Type Culture Collection (ATCC, VA, USA), and HaCaT cell lines were cultured in Dulbecco's modified Eagles medium (Gibco, Paisley, UK). A medium containing 10% fetal bovine serum (Gibco, Paisley, UK) and 1% penicillin (100 IU/mL)/streptomycin (100 μg/mL) (Gibco, Paisley, UK) was used. Culture conditions were maintained at 37℃ and 5% CO_2.

Cell viability assay. A methylthiazolyldiphenyl-tetrazolium bromide (MTT) colorimetric assay was performed to evaluate the cytotoxicity of the extract. Various concentrations of EA were exposed to HaCaT keratinocytes for 24 h. After removal of the medium, the cells were incubated with a 0.5 mg/mL MTT (Sigma, ST. Louis, USA) solution prepared in serum and phenol red-free culture medium for 4 hours. The resulting formazan crystals were dissolved in DMSO and absorbance was measured at 570 nm using a multi-plate reader.

MDA assay. The level of the lipid peroxidation product MDA in cell lysates was determined according to the instructions in a commercial assay kit (Cayman, MI, USA). MDA was analyzed using the thiobarbituric acid (TBA) assay, which is based on the release of color complexes resulting from the reaction of TBA with MDA. The level of the lipid peroxidation products were measured spectrophotometrically.

Statistical analysis. Data was expressed as mean ± standard error and compared by one-way analysis of variance. Significant differences between groups used Duncan's test. It was considered statistically significant when $P < 0.05$.

Extraction yield. In this study, distilled water was used as the extraction solvent. After removing the extraction solvent using a rotary evaporator, the extract was lyophilized and quantified. The yield was 20.0%.

DPPH radical scavenging activity. DPPH is a stable radical and is mainly used to determine the antioxidant potential of natural and synthetic compounds. First, the concentration of the substance that reduced the absorbance of the DPPH radical solution by 50% and the ascorbic acid standard was calculated. Then, the antioxidant capacity of the extract was shown graphically (Fig. 1). It was confirmed that the extract increased in a concentration-dependent manner compared to the ascorbic acid standard up to 800 g/mL.

Cytotoxic effects of EA extracts on HaCaT keratinocytes. We determined the effect of EA on the viability of HaCaT keratinocytes according to the MTT colorimetric assay method. EA affected the viability of HaCaT cells in a dose-

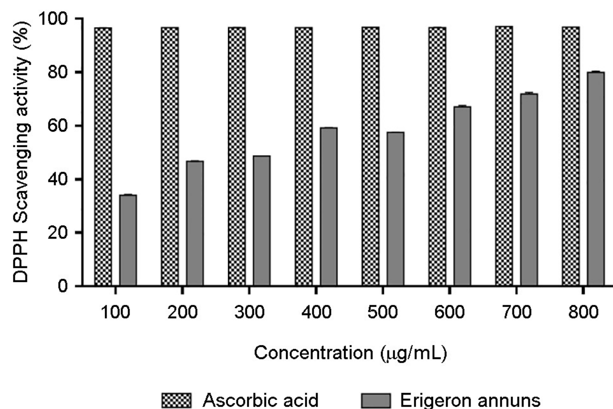

Fig. 1. DPPH radical scavenging activity.

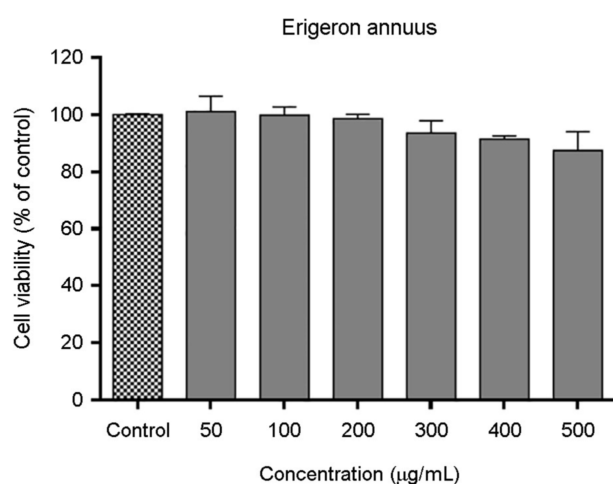

Fig. 2. Cytotoxic effects of EA extracts on HaCaT keratinocytes.

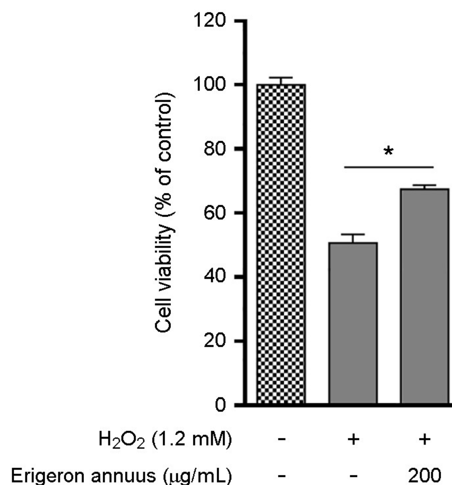

Fig. 3. Protective Effect of EA extracts against H_2O_2-induced HaCaT keratinocytes.

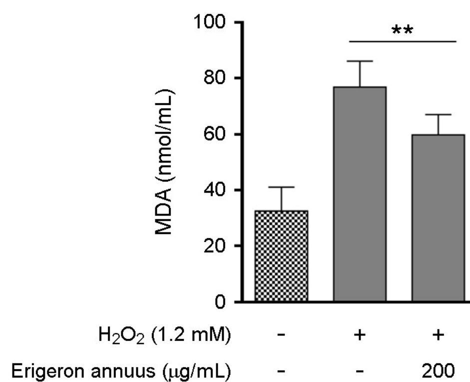

Fig. 4. Effect of EA extracts against H_2O_2-induced MDA depletion in HaCaT keratinocytes.

dependent manner. At concentrations up to 200 µg/mL, cell viability was greater than 90% (Fig. 2). Therefore, we limited the concentration of the extract to 200 µg/mL for further experimental analysis.

Protective Effect of EA extracts against H_2O_2-Induced HaCaT keratinocytes. EA extract shows cytoprotective effect on HaCaT keratinocytes against oxidative damage caused by H_2O_2. Exposure of HaCaT keratinocytes to 0.4 mM H_2O_2 for 2 h significantly reduced cell viability. Pretreatment of HaCaT keratinocytes with EA extract at a concentration of 200 µg/mL significantly reduced the cytotoxicity induced by H_2O_2 exposure (Fig. 3).

Effect of EA extracts against H_2O_2-induced MDA depletion in HaCaT keratinocytes. MDA is considered as one of the key molecules in cellular antioxidant defense mechanisms. Thus, the production of MDA mainly depends on the amount of H_2O_2 produced by cells under stress conditions. In this study, we confirmed that pretreatment of cells with EA significantly rescued the H_2O_2-induced the production of MDA depletion (Fig. 4).

Oxidative stress results from overproduction of ROS or failure of cellular endogenous antioxidant defense mechanisms (Ray et al., 2012). In this respect, dietary supplementation of exogenous antioxidants such as plant extracts is very important to maintain a balanced cellular antioxidant

status. In this study, we determined the antioxidant potential of EA using widely considered chemical radical scavenging assays such as DPPH. In addition, the antioxidant capacity of natural products is generally expressed as compared to the well-established standard antioxidant ascorbic acid. Furthermore, because even the same antioxidants have different effects in chemical and cell-based assays, we further investigated the antioxidant potential of EA extracts using a cell-based model. HaCaT keratinocytes, a human keratinocyte line, have a stable antioxidant defense system and are widely used to screen for beneficial effects of various antioxidants (Alía et al., 2006). The H_2O_2, a non-radical derivative of oxygen, is widely used as an exogenous oxidant to induce oxidative stress in cell-based antioxidant assays (Chen et al., 2007; Jiang et al., 2014; Han et al., 2017). Based on this background, in this study, the antioxidant efficacy of EA extract was confirmed by inducing oxidative stress in HaCaT keratinocytes using the H_2O_2.

In conclusion, the results of DPPH, a chemical antioxidant assay, clearly demonstrated the antioxidant capacity of EA extracted with distilled water. In addition, cell-based assays provide useful information on the protective effect of EA on oxidative stress-induced apoptosis.

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CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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