Cornus walteri leaf extract attenuates UVB-induced cell damage in Human dermal fibroblast

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Short Summary
The present study investigated the photo-protective properties of hydrolyzed Cornus walteri leaf extract (CWE) against ultraviolet B (UVB)-induced cell damage in human dermal fibroblast (HDFs). CWE reduced UVB-induced cellular damage in Hs68 cells and significantly inhibited intracellular ROS generation. Apoptosis and cyclobutane pyrimidine dimers (CPDs) formation were also inhibited by treatment with CWE for 12 h after UVB irradiation. Collectively, these results suggest that CWE could be a new potential candidate as photo-protective agent against UVB-induced cellular damage in HDFs.

Introduction
The skin is the largest organ of the human body and exposed constantly to potentially harmful compounds and radiation. UV light is composed of UVC (200-280 nm), UVB (280-315 nm), and UVA (315-400 nm). UV radiation depletes the cutaneous defense system and leads to the accumulation of DNA damages, excessive cell apoptosis, and skin aging. In particular, UVB radiation can penetrate the skin epidermis and papillary dermis. UVB interacts directly with DNA bases and modifies them and also induces DNA damage indirectly by inducing oxidative stress and free radicals [1].
In recent years, bioactive compounds from natural sources had been used as regulatory agents. Many investigators had been searching for the photo-protective properties from plants. The extracts of Punica granatum, Trapa Japonica, Crataegus monogyna, Bonnemaisonia hamifera, Ginkgo biloba, Vitis vinifera, Krameria triandra, and Pinus pinaster attenuates UV-induced cell damage in skin cells [2-6].
Cornus walteri (C. walteri) is a deciduous shrub that grows in valley areas of Asia,
particularly Korea and China. In Chinese folk medicine, its fruits and leaves have been used for treatment of inflammation of the skin or boils caused by lacquer poison, phenolic compounds from the stem bark of *C. walteri*. In Korean folk medicine, the leaves have been used as an antidiarrheal [7,8]. The presence of metabolites including gallic acid and flavonoids from previous investigations on *C. walteri* was confirmed. *C. walteri* has various therapeutic properties, including anti-hyperglycemic, anti-obesity effects, and anti-inflammation [9,10]. Notably, *C. walteri* extract also possesses antioxidative properties. However, very little research has been conducted regarding the protective effects of *C. walteri* extract against UVB radiation. Therefore, the aim of the present study was to examine the ability of *C. walteri* extract to protect human fibroblasts from UVB-induced cell damage.

**Methodology**

*Preparation of CWE*

*C. walteri* was purchased from GUmediherb (Seoul, Korea) that is the special herb shop in Korea. *C. walteri* was extracted twice with 75 % aqueous ethanol under reflux at 60–90°C for 4 h, filtered, and evaporated under reduced pressure. The 75 % aqueous ethanol extract was dissolved in 10 % aqueous ethanol and then was hydrolyzed by Novozyme® 33095 (Novozyme, Bagsvaerd, Denmark) at 50–60°C for 20 h and fractionated by ethyl acetate. The final residues, hydrolyzed Cornus walteri leaf extract (CWE), were freeze-dried and stored in a closed container until use.

*Cell culture*

The normal human fibroblast cell line, Hs68 cell was obtained from the American Type Culture Collection (ATCC, MD, USA). The cells were plated in 100 mm tissue culture dishes and grown in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin (all from GIBCO, NY, USA). CWE was dissolved in dimethyl sulfoxide (DMSO) and diluted with the medium to appropriate concentrations; the final volume of DMSO was adjusted to 0.1% (v/v).

*Cell viability assay*

Hs68 cells were seeded on a 24 well plate at a density of $1 \times 10^5$ cells per well and grown in a culture medium for 24 h. The cells were then rinsed twice with phosphate-buffered saline (PBS), and exposed 150 mJ/cm² UVB (CX-312, Vilber Lourmat, France) in PBS. After
irradiation, the cells were replenished with 1% serum medium including CWE and followed for up to 12 h. Cell viability was evaluated by MTT assay.

**Apoptosis assay**

Cellular DNA fragmentation was measured using the Cell Death Detection ELISA plus kit (Roche Molecular Biochemicals, Mannheim, Germany). Cells were treated with or without the indicated concentrations of CWE at 37°C for 12 h, the procedures were performed according to the manufacturer’s protocol, and spectrophotometric data were obtained using a SynergyHT microplate reader (Bio-Tek, VT, USA) at 405 nm.

**Cyclobutane pyrimidine dimer (CPD) quantification**

The extent of CPD formation was determined using an Oxiselect™ Cellular UV-Induced DNA Damage ELISA kit (Cell Biolabs, CA, USA), according to the manufacturer’s instructions.

**Detection of intracellular ROS**

ROS were measured in arbitrary units using 2,7-dichlorofluorescein diacetate (DCF-DA) dye. Cells were incubated in medium containing 10 μM DCF-DA for 30 min at 37 °C, and relative ROS units were determined using a fluorescence ELISA reader (excitation 485 nm, emission 530 nm). An aliquot of the cell suspension was lysed, the protein concentration was determined, and the results were expressed as arbitrary absorbance units/mg protein.

**Statistical analysis**

All values are expressed as the mean±S.E. Statistical significance was determined using a one-way analysis of variance (ANOVA) and Student’s t-test for paired data. A p-value of <0.05 was considered statistically significant. The calculations were performed using SPSS (SPSS, IL, USA).

**Results**

**Effect of CWE against UVB-induced Cytotoxicity**

We initially investigated the effect of CWE (1~50 μg/mL) on the UVB-induced cell death. Hs68 cells were treated with different concentrations of CWE (1~50 μg/mL) for 12 h after being exposed to UVB. The percentage of cells viability was assessed by using the MTT
assay at 12 h after UVB irradiation. CWE attenuated the UVB-induced cell death of Hs68 cells in a dose-dependent manner (Figure 1).

Figure 1. Protective effect of CWE against UVB-mediated cytotoxicity. Hs68 cells were treated with the indicated concentrations of CWE for 12 h at 37°C. Cell viability was measured by MTT assay. Values are mean±S.E. *p < 0.05 and *p < 0.01 compared to UVB untreated group.

Effect of CWE against UVB-mediated Apoptosis

Figure 2. Protective effect of CWE against UVB-induced apoptosis. Hs68 cells were treated with CWE after UVB irradiation. Cytoplasmic histone-associated DNA fragmentation was quantified. Values are mean±S.E. *p < 0.05 and *p < 0.01 compared to UVB untreated group.
A number of recent studies show that UVB light induces apoptosis in skin cells [11,12]. In keeping with this observation, intact nuclei were observed in control, whereas significant nuclear fragmentation was observed in UVB-irradiated cells. However, treating cells with CWE resulted in a reduced nuclear fragmentation compared to UVB-irradiated cells (Figure 2).

*Effect of CWE against UVB-induced CPD formation*

![Figure 2](image)

**Figure 3. Effect of CWE on UVB-induced CPD in Hs68 cells.** Hs68 cells were treated with CWE after UVB irradiation. CPD formation was measured by specific ELISA kit. Values are mean±S.E. *p < 0.05 and *p < 0.01 compared to UVB untreated group.

Then we have checked the effect of CWE on UVB-induced DNA damage in the form of CPDs formation. CPD represents major UVB-induced DNA damage, therefore, CPD formation was determined to evaluate the protective effect of CWE. CWE dose-dependently prevented the UVB-induced CPD formation (Figure 3).

*Scavenging Effect of CWE against Free Radicals*

We evaluated the ability of CWE to scavenge intracellular ROS. As shown in Figure 4, ROS levels were markedly higher in UVB-exposed Hs68 cells than in control cells; however, CWE (20–50 μg/mL) attenuated the UVB-induced ROS generation in Hs68 cells.
Figure 4. Cellular antioxidative activity of CWE in UVB-induced oxidative stress in Hs68 cells. Intracellular reactive oxygen species (ROS) levels generated by UV radiation were detected using a spectrofluorometer after DCF-DA staining. Values are mean±S.E. *p < 0.05 and *p < 0.01 compared to UVB untreated group.

Conclusions and Discussion
Skin is the largest organ of the body exposed constantly to potentially harmful compounds and radiation. UV radiation can induce biological responses, including the development of hyperplasia, erythema, photoaging, and skin cancer. First of all, the increase in UVB radiation that reaches the earth’s surface enhances the deleterious effects of this radiation on human health. Therefore, new strategies are needed to combat UV skin damage. The development of promising photo-protective agents requires continuous research throughout the development process. In addition, this study is the report describing the photo-prevention of CWE in HsS68 cells. These results demonstrate that CWE contains ROS scavenging ability and inhibits the apoptosis and CPD formation. The present findings demonstrate that CWE is endowed with good skin photo-protective properties and that this is likely due to the polyphenol content of CWE. Therefore, these results suggest that CWE may exhibit some potential to prevent photo-damage. Additional studies to better understand the photo-protective mechanism of CWE are in progress.

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References