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Efficacy of *Camellia japonica* fruit shell extract on hair loss

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Introduction

Hair loss is caused by various factors, and the impacts are often overlapped and intensified. Currently, mitigation of hair loss is being studied in proliferation of dermal papilla cells (DPCs), and inhibition of deleterious factors such as dihydrotestosterone (DHT) and oxidative stress on hair growth.

The leaves, seeds, and flowers of *Camellia japonica* (*C. japonica*) contain active compounds which exert antioxidant, antimicrobial, wound healing, anti-pollution, and anti-inflammatory activity [17, 18, 19]. *C. japonica* fruit shell is discarded after seed collection for obtaining oil, and its biological activity remains to be elucidated. In this study, the effectiveness of *C. japonica* fruit shell extract (CJFSE) on hair loss mitigation was investigated by measuring the proliferation of DPCs and the inhibitory effect of DHT and oxidative stress.

Materials & Methods

Cell culture
Human follicle dermal papilla cells (HFDPCs) were purchased from Promocell (Sickingenstr, Heidelberg, Germany) and cultured in Follicle dermal papilla cell growth medium mixed with Growth medium supplementMix (Promocell, Sickingenstr, Heidelberg, Germany) at 37 °C, 5% CO_2 incubator.

Preparation of Camellia japonica fruit shell extract
The fruit pericarps of *Camellia japonica* were collected at Siheung-ri, Seongsan-eup, Seogwipo-si, (Jeju Island, Korea). To prepare the EtOH extract, the pericarps (1 kg) were extracted with 70% (v/v) EtOH at 80 °C for 3 h. After removing the raw material, the extracted solution was concentrated using rotary vacuum evaporation (EYELA, Tokyo, Japan). The final extract was lyophilized (18.5 g, yield 1.85%), and utilized in this study.

Spheroid culture of HFDPCs
Spheroid culture was conducted by using Ultra-low attachment multiple well plate (ULA, Corning® Costar® , Corning, NY, USA). Cells were seeded on ULA at 4 × 10^4 per wells, centrifuged at 220 × g briefly, and cultured for 24 h in a 37 °C, 5% CO_2 incubator. Then cells were treated with CJFSE and cultured for 72 h. After images of the spheroids were taken by microscope (EVOS® FL, Fisher Scientific, Waltham, MA, USA), the size of spheroids was measured using the ImageJ system.

DHT induced senescence
HFDPCs were seeded on 24 well plate at 2 × 10^4 cells per well and incubated at 37 ℃, 5% CO_2 for 24 h. After cells were cultured with 200 μM DHT (Stanolone, Tokyo Chemical Industry, Tokyo, Japan) and CJFSE for 72 h, cell viability was analyzed by MTT assay.

H_2O_2 induced senescence
HFDPCs were seeded on 24 well plate at 2 × 10^4 cells per well and incubated at 37 ℃, 5% CO_2 for 24 h. After cells were pre-treated with 300 μM hydrogen peroxide (H_2O_2, SigmaAldrich) for 2 h and cultured with CJFSE for 72 h, cell viability was analyzed by MTT assay.

Results

CJFSE increased HFDPCs viability and proliferation

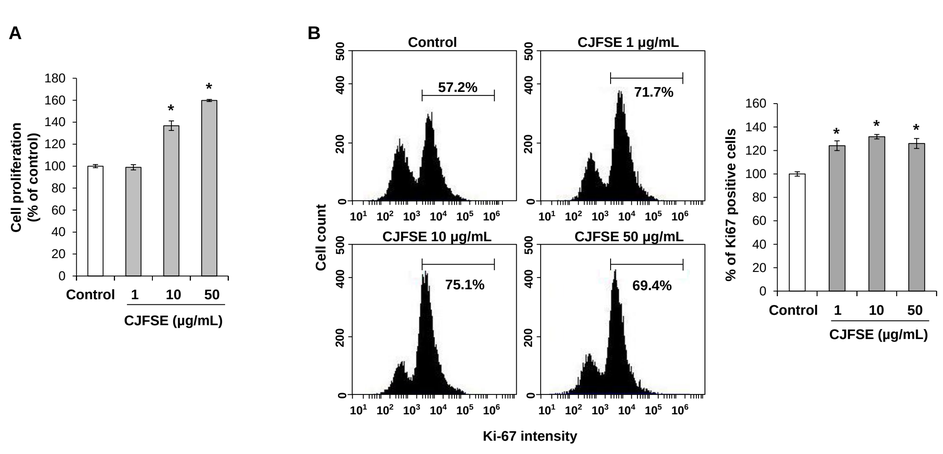

Figure 1. CJFSE increases the proliferation of HFDPCs. (A) HFDPCs were cultured with 1, 10, and 50 μg/mL CJFSE for 72 h, and cell viability was measured as MTT assay. (B) Cell proliferation was confirmed by Ki67 staining. All values were measured in triplicate and represent the mean ± SD of each experiment. Values were expressed as a percentage of the control group. *p < 0.05 vs. control group.

CJFSE augmented the spheroid size

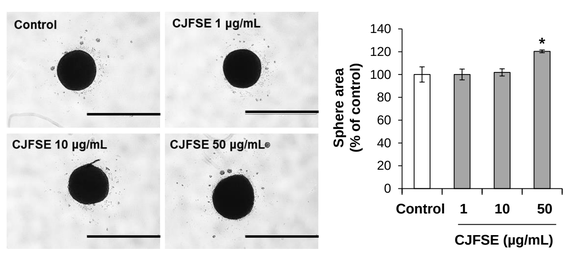

Figure 2. CJFSE enhanced the size of cellular spheroids formation. For the formation of spheroids, HFDPCs were seeded on ULA plates and cultured with CJFSE for 72 h. Spheroid size was analyzed by ImageJ program, and this experiment was conducted in triplicate. Scale bar is 1000 μm. *p < 0.05 vs. control group.

CJFSE induced the expression of VEGF and genes related to HFDPCs proliferation

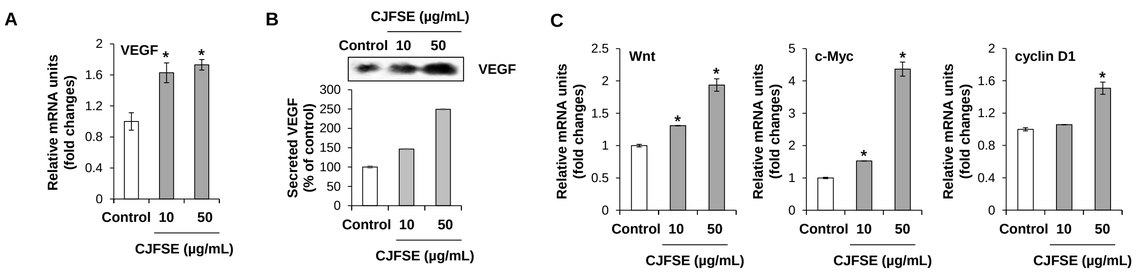

Figure 3. CJFSE increased VEGF secretion and the expression of Wnt signaling. (A, C) HFDPCs were treated with CJFSE for 72 h, and then measured the expression of mRNA level of VEGF, Wnt, c-Myc and cyclin D1. (B) Secreted VEGF protein was detected through western blot. All experiments were conducted in triplicate. *p < 0.05 vs. control group.

CJFSE inhibited the effect of DHT on HFDPCs

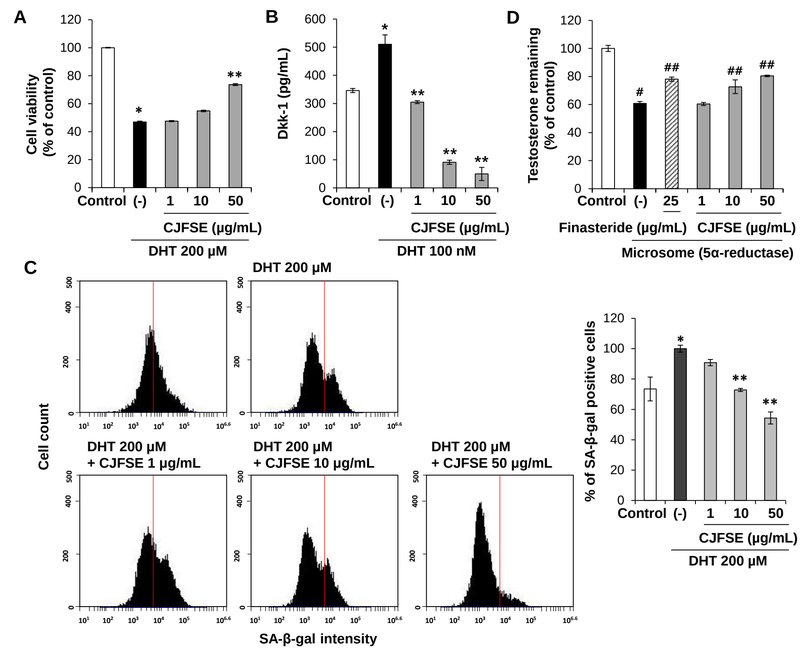

Figure 4. CJFSE ameliorated the effect of DHT on HFDPCs. (A) Cells were cultured in CJFSE and 200 μM DHT for 72 h, subsequently cell viability was checked by MTT assay. (B) 100 nM DHT was treated in HFDPCs with CJPSE for 72 h and the Dkk-1 secreted in the medium was measured as ELISA. (C) Cells cultured with CJFSE and 200 μM DHT for 72 h were stained with β–gal and β–gal activity was measured by FACS. (D) Inhibitory effect of 5α-reductase activity was confirmed by measuring the remained testosterone as HPLC. *p < 0.05 vs. control group; **p < 0.05 vs. DHT treated group; #p < 0.05 vs. control group; ##p < 0.05 vs. group with only microsome treated. All experiments were conducted in triplicate.

CJFSE inhibited the effect of oxidative stress on HFDPCs

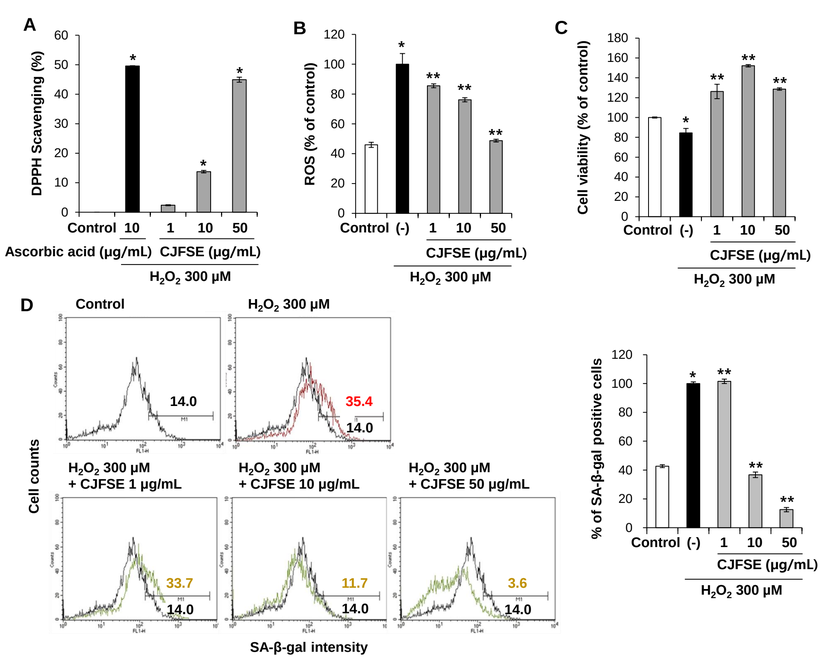

Figure 5. CJFSE mitigated the oxidative stress on HFDPCs. (A) The antioxidant capacity of CJFSE was evaluated by DPPH assay. Ascorbic acid was used as the positive control. (B) Cells were incubated with CJFSE for 72 h and 100 μM DCFDA was treated for 30 min. After cells were cultured with 300 μM H_2O_2 for 30 min, fluorescence was detected. (C) 300 μM H_2O_2 was pre-treated for 2 h and cells were cultured with CJFSE for 72 h, then cell viability was measured by MTT assay. (D) 300 μM H_2O_2 was pre-treated for 2 h and cells were cultured with CJFSE for 72 h. Cells were stained with β–gal and β–gal activity was measured by FACS. All experiments were conducted in triplicate. *p < 0.05 vs. control group; **p < 0.05 vs. H_2O_2 treated group.

Discussion

- It is known that Wnt-β-catenin, an important signaling pathway for the development of hair follicles and hair cycles, is involved in the interaction between DP and epithelial cells, and induces DPC proliferation [22]. CJFSE up regulated proliferation-related gene expression (Figure 3). In addition, the spheroid size of DPCs, which is considered the volume of the hair shaft, was increased by CJFSE (Figure 2).
- DHT, known as the main cause of androgenic alopecia, DPCs not only react more sensitively to related signals to express hair loss-related genes, but also adversely affect due to increased Dkk-1 [10, 11]. Dkk-1 as an inhibitor of Wnt inhibits HF development [23]. CJFSE redueced Dkk-1 secretion induced by DHT (Figure 4).
- Oxidative stress inhibits collagen synthesis and induces premature senescence in DPCs. CJFSE can relieve these effects by reducing ROS production in DPCs (Figure 5).

Conclusions

Taken together, CJFSE, a natural raw material that recycles discarded part, suggested the possibility that hair loss can be prevented through hair growth and the influence of DHT and oxidative stress can be reduced to alleviate hair loss in various ways.

Acknowledgements

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