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

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Abstract

Background: 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. *Camellia japonica* (*C. japonica*) fruit shell is discarded part and its biological activity remains to be elucidated. In this study, we investigated the capacity of *C. japonica* fruit shell extract (CJFSE) on hair loss mitigation.

Materials and Methods: MTT assay and spheroid culture were performed to observe proliferative effect of CJFSE on hair follicle dermal papilla cells (HFDPCs). The expression of VEGF and, Wnt, c-Myc and cyclin D1 were detected through western blot and quantitative RT-PCR respectively. The effects of CJFSE against DHT-induced hair-loss were confirmed by Dkk-1 expression, β-galactosidase (β-gal) assay and 5α-reductase activity assay. In addition, the impacts of CJFSE oxidative stress were confirmed through DPPH, β-gal activity and ROS production assay.

Results: CJFSE increased the proliferation, spheroid size of HFDPCs. The expression of VEGF, Wnt, c-Myc and cyclin D1 were upregulated by CJFSE. It also suppressed 5α-reductase activity and DHT-induced decrease in cell proliferation, Dkk-1 secretion and β-gal activity. Moreover, CJFSE showed DPPH scavenging activity and ameliorated hydrogen peroxide (H_2O_2)-induced ROS production and β-gal activity in HFDPCs.

Conclusion: In this study, CJFSE suggested the possibility of alleviating hair loss by promoting hair cell growth and suppressing the effects of DHT and oxidative stress on hair.

Keywords: *Camellia japonica* fruit shell; Hair loss; dermal papilla cells; dihydrotestosterone; oxidative stress

INTRODUCTION

Hair loss is not a fatal disease, but it causes psychological stress, including extreme emotional pain, which can lead to secondary diseases such as low self-esteem or interpersonal phobia [1]. Nutritional deficiency, hormonal imbalance, stress, infection and chemical irritants are associated with hair loss [2]. Currently, mitigation of hair loss is being studied in various targets, such as activation of dermal papilla cells (DPCs), reduction of dihydrotestosterone (DHT) production, attenuation of oxidative stress, and improvement of blood flow.

Hair develops in a hair follicle (HF) connected to sebaceous gland and arrector pili muscle [3]. Dermal papilla (DP), consisting of mesenchymal fibroblast located below the HF, plays a critical role in controlling hair growth by maintaining hair cycle [4]. The hair cycle consists of a growing phase (anagen), a regressing phase (catagen phase), and a restoring phase (telogen) [5]. The depletion of DPCs shows alopecia like symptoms in human through prolonged telogen and/or failure to re-enter anagen phase [6]. Cellular senescence of DPCs is also associated to hair loss, which cause HF atrophy, extension of the telogen phase, and shortening of the anagen phase [7]. Increased β-gal activity, decreased proliferation capacity, and morphological changes were observed in senescent DPCs [8]. DHT and oxidative stress, known as deleterious factors for hair growth, induce premature senescence of DPCs and promote HF regression by inhibiting the proliferation of keratinocytes around DPCs [9, 10]. DHT is closely related to androgenic alopecia, produced by the conversion of testosterone by 5α-reductase present in DPCs. It binds to the androgen receptor (AR) located in cytoplasm and translocates to the nucleus, then expresses an androgen sensitive gene, which eventually causes hair loss [11]. Oxidative stress causes damage to DNA, proteins, lipids, etc. in hair follicles, and reduces dermal extracellular matrix (ECM) [12]. Moreover, increased ROS affects the morphology, migration, and proliferation of DPCs [13].

Representative hair loss treatments approved by the FDA include minoxidil and finasteride. Minoxidil is an arteriolar vasodilator, which increases proliferation by regulating potassium channels and β-catenin, and increases VEGF mRNA expression [15]. Finasteride is known to inhibit the production of DHT, which causes hair loss by inhibiting 5α-reductase. However,

minoxidil or finasteride requires continuous prescription, which can cause various adverse events such as erythema of scalp, loss of libido, contact dermatitis, pain in the testis, and induction of gynecomastia [16]. Thus, there have been many attempts to develop safe hair growth agent by using natural product.

Camellia japonica (*C. japonica*) belongs to the *Camellia* genus in Ericales order and is an evergreen tree that grows naturally in Korea, Japan, and China. The leaves, seeds, and flowers of *C. japonica* contain active compounds such as polyphenol, vitamin E, saponin, triterpenoids, and various fatty acids which exert antioxidant, antimicrobial, wound healing, anti-pollution, anti-inflammatory, and tyrosinase inhibitory 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.

EXPERIMENTAL

1. 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 ℃, 5% CO_2 incubator.

2. 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 ℃ 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.

3. Cell viability assay

Cell viability was determined by 3-(4,5-dimethyl-2-thiazoyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT, Sigma-Aldrich, St. Louis, MO, USA) assay. HFDPCs were treated with

CJFSE or finasteride (Sigma-Aldrich) as positive control for 72 h solubilized in Dulbecco's modified Eagle's medium (DMEM, Hyclone, Logan, UT, USA) including 4 mM L-glutamine, 4500 mg/L glucose, sodium pyruvate supplemented with 1% penicillin and streptomycin (Gibco, Carlsbad, CA, USA), and 1% fetal bovine serum (FBS, Gibco, Carlsbad, CA, USA) at 37 ℃, 5% CO_2 incubator. After cells were incubated for 1 h 30 min at 37 ℃ with the MTT diluted 1:10 in culture media, the optical density of formazan dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich) was measured at 570 nm by using a spectrophotometer (Epoch, Bio-Tek Inc., Winooski, VT, USA).

4. Cell proliferation assay

Proliferation was analyzed using Ki67 (Ki-67 Monoclonal Antibody, FITC, eBioscience™, invitrogen, Carlsbad, CA, USA) staining. Seeded HFDPCs on 100 mm dish were cultured with CJFSE for 24 h. After cultured cells were washed using 1× phosphate-buffered saline (PBS), cells were suspended in 1× PBS and fixed by ice-cold 70% EtOH for 16 h at -20 ℃. EtOH was removed by spinning down and cells were incubated with Ki-67-FITC antibody for 1 h at room temperature (RT). Then cells were washed with 1× PBS twice and measured the fluorescent level using a FACS Calibur flow cytometer (BD Biosciences, San Jose, CA, USA) with a blue laser at 488 nm.

5. 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 ℃, 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 Image J system.

6. Quantitative real time PCR

Total RNA was purified using Trizol reagent (Thermo Fisher Scientific). To put it simply, cultured cells were treated with Trizol and collected in tubes. After chloroform (Sigma-Aldrich) was added, separated upper part was mixed with 100% isopropyl alcohol (DAEJUNG, Siheung, Korea). Then supernatant was removed, and RNA was washed with 70 % EtOH. Purified total RNA (1 μg) was synthesized into cDNA using amfiRivert cDNA Synthesis Plantinum Master Mix (GenDEPOT, Katy, TX, USA) through TaKaRa Thermal

Cycler Dice Touch (TP350, TAKARA BIO INC., Shiga, Japan), and gene expression was detected by 7500 Real Time PCR System (Thermo Fisher Scientific, MA, USA) using AMPIGENE® qPCR Green Mix Hi-ROX (Enzo Life Sciences, Formingdale, NY, USA). Primers for PCR were ordered from Bioneer Corp. (Daejeon, Korea). The sequences of human VEGF-A primers were: forward primer, 5'-TTGCCTTGCTGCTCTACCTCCA-3'; reverse primer, 5'-GATGGCAGTAGCTGCGCTGATA-3', human Wnt-1 primers were: forward primer, 5'-CTCTTCGGCAAGATCGTCAACC-3'; reverse primer, 5'-CGATGGAACCTTCTGAGCAGGA-3', human c-Myc primers were: forward primer, 5'-CCTGGTGCTCCATGAGGAGAC-3'; reverse primer, 5'-CAGACTCTGACCTTTTGCCAGG-3', human cyclin D1 primers were: forward primer, 5'-TCTACACCGACAACTCCATCCG-3'; reverse primer, 5'-TCTGGCATTTTGGAGAGGAAGTG-3', and human GAPDH primers were: forward primer, 5'-AGCCACATCGCTCAGACAC-3'; reverse primer, 5'-GCCCAATACGACCAAATCC-3'.

7. Western blot analysis

For secreted protein analysis, cells were seeded into a 100 mm dish and cultured with CJFSE for 72 h. Supernatants were collected and concentrated by using Vivaspin® 20 3 kDa (Cytiva, Marlborough, MA, US). Then proteins of the same volume was loaded and detected with VEGF antibody (1:500) (Santa Cruz Biotechnology, Inc., Dallas, TX, USA).

8. 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.

9. 5 alpha reductase activity assay

The pH 6.5, 40 mmol/L potassium phosphate (Sigma-Aldrich) solution was prepared containing 1 mmol/L dithiothreitol (Thermo Fisher Scientific, MA, USA), 100 μmol/L NADPH (Roche Holding AG, basel, switzerland), and 3.5 μmol/L testosterone (ChemFaces, Hubei, China) for 20 min at RT. After 20 μg rat liver microsome (Sigma-Aldrich) was added with or without CJFSE, incubated for 1 h at 37 ℃ to reaction. Reacted solution was lyophilized using lysophilizer (TF-10D, TEFIC BIOTECH, China) and dissolved in MeOH then filtered with 0.2-micro filter (Pall, NY, USA). Testosterone remained in sample was measured by HPLC (Waters 2695 Separation Module, Waters 2996 Photodiode Array

Detector, Waters, Milford, MA, USA). The reverse-phase HPLC analysis was performed on a C18 column (4.6 × 150 mm, 2.6 μm, Phenomenex, Torrance, CA, USA) at RT as optimal column temperature and UV detection of 245 nm (Testosterone). The isocratic mobile phase of 0.1 % trifluoroacetic acid in water : acetonitrile (50 : 50, v/v) was flowed at 1.0 mL/min through the column.

10. Enzyme-linked immunosorbent assay

Dickkopf-related protein 1 (Dkk-1) secretion level was measured by Dkk-1 ELISA (Enzo Life Sciences). Cells were cultured with 10 nM testosterone or 100 nM DHT (Stanolone, Tokyo Chemical Industry, Tokyo, Japan) treated with CJFSE for 72 h. Conditioned media was added to ELISA plate and sample was incubated at RT for 1 h and sequentially reacted with antibody and conjugate for 1 h and 30 min respectively. After substrate was added, absorbance was detected by a spectrophotometer at 450 nm (Epoch, Bio-Tek Inc.).

11. SA-β-gal staining

To detect senescence level, β-galactosidase assay was performed in DPCs. Harvested cells were stained with SPiDER-β-gal (Dojindo Laboratories, Kumamoto, Japan) diluted in PBS at RT for 30 min. Finally, the relative β-gal level was measured by a FACS Calibur flow cytometer (BD Biosciences).

12. DPPH radical scavenging assay

CJFSE or ascorbic acid was diluted in MeOH and mixed with 2,2-Diphenyl-1-picrylhydrazyl (DPPH, Sigma-Aldrich) dissolved in MeOH at the same ratio. Ascorbic acid was used as positive control. After activation for 30 min at RT, avoiding the light, DPPH remained in solution was detected at 517 nm by spectrophotometer (Epoch, Bio-Tek Inc.).

(% inhibition) = 〔1 - Absorbance of test material / Absorbance of control〕 × 100

13. 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, Sigma-Aldrich) for 2 h and cultured with CJFSE for 72 h, cell viability was analyzed by MTT assay.

14. Analysis of ROS production

HFDPCs were seeded on 24 well plate and incubated at 37 ℃, 5% CO_2 for 24 h. After cells were cultured in CJFSE dissolved media for 72 h, cells were treated with 100 μM 2',7'-Dichlorofluorescin diacetate (D6883) (DCFDA, Sigma-Aldrich) for 30 min. Then cells were incubated with 300 μM H_2O_2 for 30 min and cultured medium was removed. Cells were washed twice with PBS and Sodium hydroxide (S5881) (NaOH, Sigma-Aldrich) was added to lyse the cells. Cell lysate was transferred into black plate 96well (30496) (SPL Life Sciences, Pocheon, Korea) and the fluorescence was measured (Ex/Em = 485/535 nm).

15. Statistical analysis

All experiments were performed at least three times and analyzed as student's t-test, and the results were expressed as mean ± standard deviation (S.D). P-values less than 0.05 were considered statistically significant.

RESULTS

CJFSE increased HFDPCs viability and proliferation

The number of DPCs and activation of bulge stem cells by DPCs contribute to new hair growth by regulating the hair cycle. On the other hand, since a decrease in the number of DPCs is a major cause of hair loss, increasing DPC proliferation is important for the development and maintenance of hair follicles. To check the effect of CJFSE on viability and proliferation of HFDPCs, cells were treated with 1, 10, and 50 μg/mL of CJFSE for 72 h, and then MTT assay and Ki67 staining were performed. The cell viability of 10 and 50 μg/mL CJFSE-treated groups were increased up to 37% and 60% compared to CJFSE-none treated group, control, respectively ***(Figure 1A)*****.** Also, the ratio of Ki67 was increased in CJFSE treated groups than that of control ***(Figure 1B)***. These data show that CJFSE increases the viability and proliferation of HFDPCs.

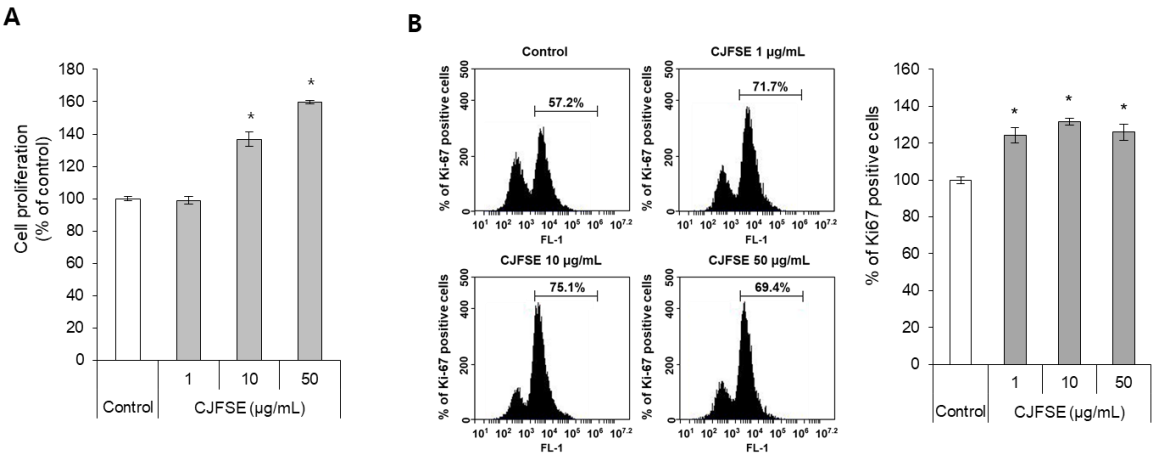

Figure 1. CJFSE increases the proliferation of HFDPCs. (A) HFDPCs were cultured with 1, 10, and 50 μg/mL CJFSE for 72 h, and cell viability was measured as MTT assay. (B) Cell proliferation was confirmed by Ki67 staining. All values were measured in triplicate and represent the mean ± SD of each experiment.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

CJFSE augmented the spheroid size

When the volume of the hair shaft decreases due to the depletion of the DPCs that determine the size of the DP, follicle miniaturization, a typical symptom of hair loss, is accompanied [5, 20]. Therefore spheroid culture was performed to determine whether the proliferation of HFDPCs by CJFSE was associated to the spheroid size. HFDPCs were cultured in ULA plate with CJFSE. The spheroid size was increased at 50 μg/mL CJFSE up to 20 % compared to control condition ***(Figure 2)***. This result suggests that CJFSE would increase or maintain the volume of the hair shaft by up regulating the proliferation of HFDPCs.

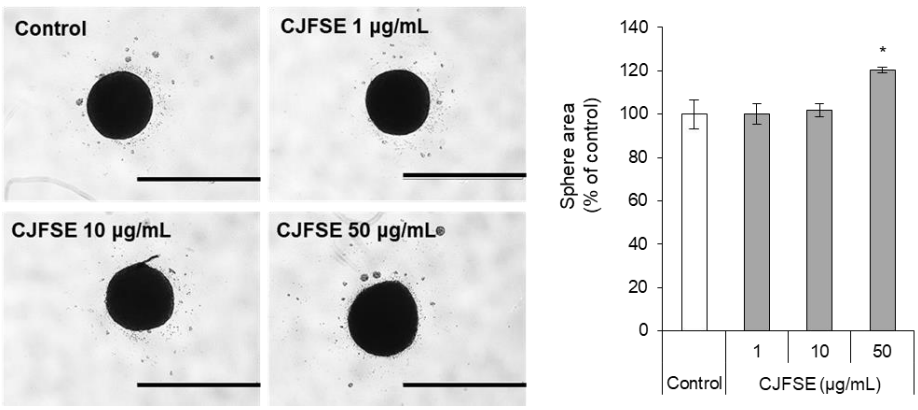

Figure 2. CJFSE enhanced the size of cellular spheroids formation. For the formation of spheroids, HFDPCs were seeded on ULA plates and cultured with CJFSE for 72 h. Spheroid size was analyzed by ImageJ program, and this experiment was conducted in triplicate. Scale bar is 1000 μm. *$p < 0.05$ vs. control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

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

HF is developed and maintained by interaction between the dermal tissue and the epidermal tissue that secrete various growth factors such as VEGF, TGFβ, β-FGF, and IGF. In particular, VEGF shows the highest expression rate in DPCs among follicular structures, and it is known that it controls the hair cycle by inducing the disappearance of blood vessels in catagen phase and vascularization in anagen phase [14, 21]. We confirmed that CJFSE increased the expression and production of VEGF ***(Figure 3A, 3B)***. Next, we checked several genes related to proliferation such as Wnt, c-Myc, and cyclin D1. In HFDPCs, Wnt-β-catenin pathway is considered as major signaling in hair growth. When β-catenin signaling is interrupted, the regeneration of HF does not occur normally due to the induction of the catagen and suppression of the anagen [22]. c-Myc and cyclin D1 are well known major targets for Wnt signaling. We observed that CJFSE increased the expression of Wnt, c-Myc and cyclin D1 gene level ***(Figure 3 C)***. These results suggest that CJFSE can be involved in the proliferation of HFDPCs and hair growth by up regulating VEGF secretion and Wnt signaling-related gene expression.

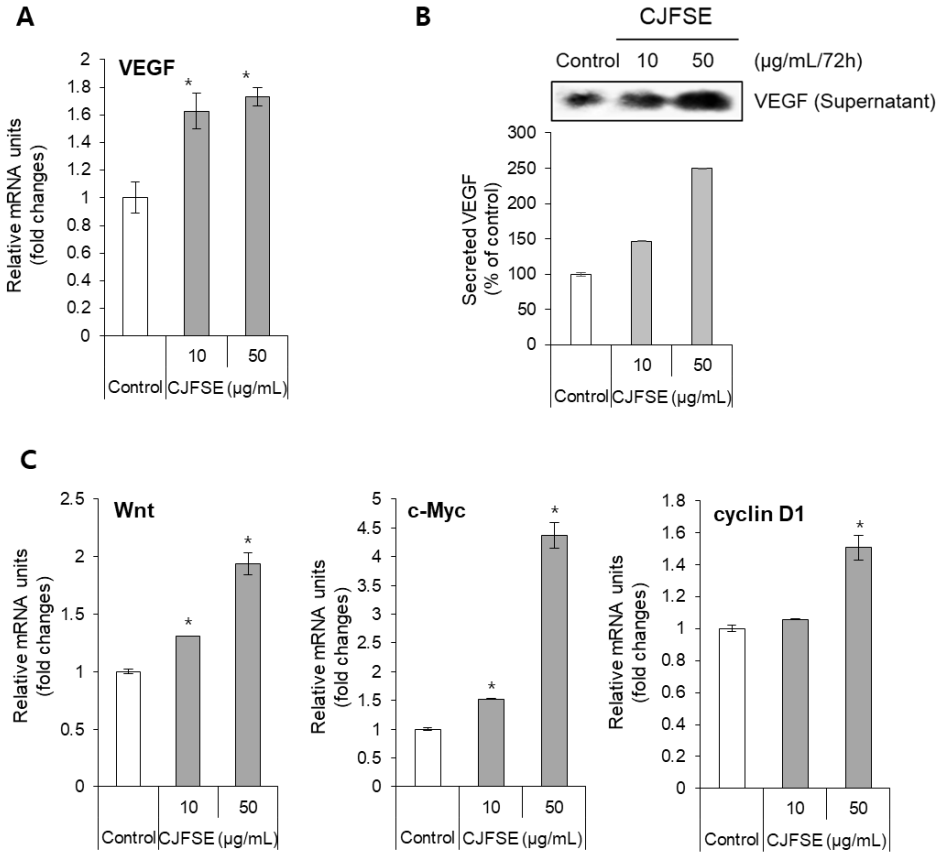

Figure 3. CJFSE increased VEGF secretion and the expression of Wnt signaling. (A, C) HFDPCs were treated with CJFSE for 72 h, and then measured the expression of mRNA level of VEGF, Wnt, c-Myc and cyclin D1. (B) Secreted VEGF protein was detected through western blot. All experiments were conducted in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

CJFSE inhibited the effect of DHT on HFDPCs

DHT binds to the AR receptors on the HFDPCs to disturb the hair cycle and causes hair follicle miniaturization like senescence condition. Thus, we observed whether CJFSE inhibited the effect of DHT on HFDPCs. First, we confirmed that DHT reduced HFDPCs viability was recovered by CJFSE ***(Figure 4A)***.

Dkk-1, a Wnt inhibitor, is induced by DHT, secreted Dkk-1 from DP is known to inhibit growth of ORS and induce epithelial cell death [23]. In order to confirm whether CJFSE could inhibit DHT effects on Wnt signaling, Dkk-1 ELISA assay was performed. As shown in ***Figure 4B***, CJFSE inhibited Dkk-1 secretion in a dose dependent manner compared to DHT treated group. These results suggest that CJFSE enhance HFDPCs proliferation by

suppressing DHT-induced Dkk-1 production. In addition, we checked whether the senescence induced by DHT could be suppressed by CJFSE by measuring SA-β-gal activity. CJFSE decreased the SA-β-gal activity compared to DHT-treated control ***(Figure 4C)***.

Finally, the effect of CJFSE on 5α-reductase activity was confirmed by using HPLC analysis. As a result, the 5α-reductase activity was inhibited by CJFSE ***(Figure 4D)***. Finasteride was used as positive control to inhibit 5α-reductase in microsome. Collectively, CJFSE could ameliorates hair loss by inhibiting the generation of DHT and improving the effect of DHT on hair cell proliferation and senescence.

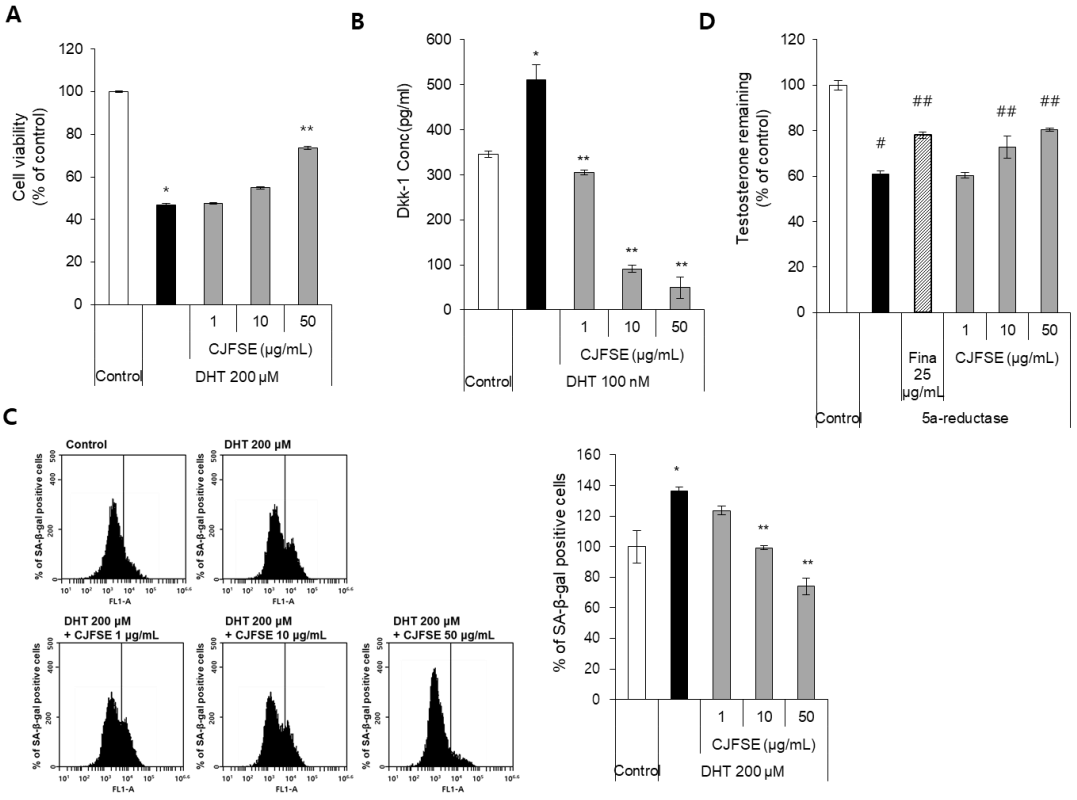

Figure 4. CJFSE ameliorated the effect of DHT on HFDPCs. (A) Cells were cultured in CJFSE and 200 μM DHT for 72 h, subsequently cell viability was checked by MTT assay. (B) 100 nM DHT was treated in HFDPCs with CJPSE for 72 h and the Dkk-1 secreted in the medium was measured as ELISA. (C) Cells cultured 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

Oxidative stress, generated from external chemical stimuli, UV or mitochondrial function, cause hair loss through cellular damage and senescence [13]. To check the free radical scavenging activity of CJFSE, DPPH assay was performed. As shown in ***Figure 5A***, radical scavenging activity was increased to 13 % and 44 % in the groups treated with 10 and 50 μg/mL CJFSE respectively. In addition, CJFSE inhibited intracellular ROS generation up to 50% at 50 μg/mL compared to H_2O_2 –treated group ***(Figure 5B)***. Next, to check whether CJFSE could recover cell proliferation decreased by oxidative stress, cell viability was observed. The cell viability was increased by CJFSE in a dose-dependent manner compared to H_2O_2 –treated group ***(Figure 5C)***. Finally, we verified that CJFSE could inhibit HFDPC senescence induced by H_2O_2. As a result, H_2O_2 increased β-gal activity, which was inhibited by CJFSE in a dose-dependent manner ***(Figure 5D)***. Based on these results, CJFSE can alleviate hair loss by suppressing oxidative stress.

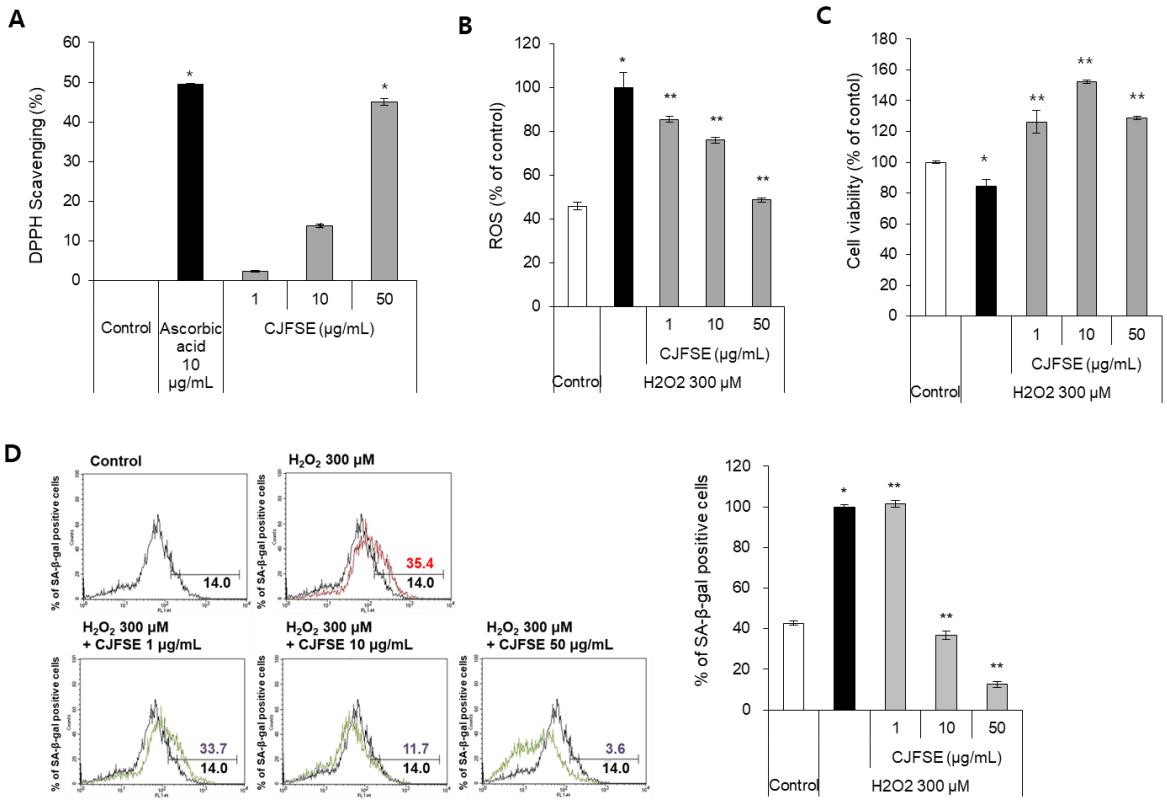

Figure 5. CJFSE mitigated the oxidative stress on HFDPCs. (A) The antioxidant capacity of CJFSE was evaluated by DPPH assay. Ascorbic acid was used as the positive control. (B)

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]. In this study, we showed that CJFSE increase HFDPC proliferation, induce VEGF secretion, and upregulate proliferation-related gene expression such as Wnt, c-Myc and cyclin D1 ***(Figure 1, 3)***. From these results suggest that CJFSE appears to increase the proliferation of HFDPCs and this seems to be related to VEGF and Wnt signaling. In addition, the spheroid size of HFDPCs, which is considered the volume of the hair shaft, was increased by CJFSE ***(Figure 2)***. Androgenic alopecia is known to proceed through miniaturization in which the hair shaft is reduced, and it is considered that the decreased in the size of the hair shaft is related to the reduction in the size of the dermal papilla [5, 20]. Therefore, it is expected that the increase in the size of the hair shaft associated to the proliferation of dermal papilla cells by CJFSE can be effectively applied to androgenetic alopecia.

DHT, known as the main cause of androgenic alopecia, has a relatively 5-6 times higher affinity for AR than testosterone, so DPCs not only react more sensitively to related signals to express hair loss-related genes, but also adversely affect surrounding keratinocytes due to increased Dkk-1 and IL-6 secretion [10, 11]. Dkk-1 as an inhibitor of Wnt acts on low density lipoprotein receptor-related protein (LRP) and reduces canonical Wnt signal activity to inhibit HF development or cause apoptosis of follicular keratinocytes [23]. In this regard, CJFSE alleviated the viability of HFDPCs reduced by DHT, inhibiting 5α-reductase activity converting testosterone to DHT, and reducing the secretion of Dkk-1 induced by DHT. In addition, DHT is known to induce prematurity by prolonging telogen at the same time as

anagen shortening, resulting in hair miniaturization [9], and it was confirmed that CJFSE mitigates senescence in HFDPCs induced by DHT ***(Figure 4)***.

ROS such as hydrogen peroxide and superoxide are produced through photochemical reactions of UV energy and cause oxidative stress in the skin [24]. ROS upregulates Cysteine rich protein 61 (CCN1) in the skin, which is observed in aging skin, and CCN1 elevated in dermal fibroblasts (DFs) increases the activity of MMP-1, inhibiting collagen synthesis and promoting collagen degradation [12]. In addition, there is a result that H_2O_2 can induce premature senescence in DPCs, and H_2O_2-induced premature senescent DPCs has lower aggregation ability than non-senescent DPCs, and the proliferation of co-cultured keratinocytes is also lower than that of keratinocytes cultured with non-senescent DPCs [7]. Therefore, as a result of confirming whether CJFSE can relieve oxidative stress in DPCs, it was confirmed that CJFSE showed DPPH scavenging activity and could suppress ROS production and the senescence of DPCs induced with H_2O_2 ***(Figure 5)***.

CONCLUSION

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.

Acknowledgments

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Conflict of Interest Statement

None

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