Original Article
Food Science and Technology

ISSN 0101-2061 (Print)
ISSN 1678-457X (Online)

DOI: https://doi.org/10.1590/fst.115921

Hair loss improvement effect of *Chrysanthemum zawadskii*, peppermint and *Glycyrrhiza glabra* herbal mixture in human follicle dermal papilla cell and C57BL/6 mice

Jae Young SHIN[1,2], Da Jeong SHIN[3], Hyun Ju KANG[3], Byoung Ok CHO[1,3], Ji Hyeon PARK[1], Seon Il JANG[1,4]*

Abstract

In today's society, hair is not only used to protect the body and maintain body temperature, but to play an aesthetic role. Therefore, interest in natural products that can improve hair loss is increasing. In this study, the hair loss improvement effect of a mixture of *Chrysanthemum zawadskii*, peppermint and *Glycyrrhiza glabra* (FHH-CZ) was investigated in Human follicle dermal papilla cell (HFDPC) and C57BL/6 mice. ELISA, Western blot, RT-PCR and animal experiments were performed. The results showed that FHH-CZ suppressed the expression of 5α-reductase type 2 and androgen receptor in testosterone-treated HFDPC. In addition, pretreatment with FHH-CZ reduced the Bax/Bcl2 expression ratio through inhibition of Dkk-1 expression in testosterone-treated HFDPC. In animal experiments, FHH-CZ promoted hair growth and increased the number of hair roots in C57BL/6 mice. Therefore, the results of this study suggested that FHH-CZ could be used as a natural material with few side effects to improve hair loss.

Keywords: *Chrysanthemum zawadskii*; peppermint; *Glycyrrhiza glabra*; Hair loss; herbal mixture.

Practical Application: FHH-CZ is a natural material with few side effects and can be used in the functional food industry to improve hair loss.

1 Introduction

Human scalp hair functions to absorb external shocks, protect from UV rays, and keep warm from cold. However, in modern society, hair acts not only as a functional role, but also as an important factor in determining appearance. Because of this, mental problems such as low self-esteem, depression, and anger are observed in alopecia patient (de Koning et al., 1990). Hair loss has a variety of causes, such as genetics, Androgenetic alopecia (AGA), fungal infections, trauma, radiation therapy, chemotherapy, and nutritional deficiencies (Marberger, 2006). AGA is a form of alopecia in which scalp hair follicles are gradually reduced under the influence of androgens, and accounts for 50% of male pattern hair loss and is affected by genetic factors (Marberger, 2006; Sinclair, 1998). It is known that testosterone is converted to dihydrotestosterone by 5α-reductase (5αR) in dermal papilla cells and binds to androgen receptor (AR) to cause hair loss (Hines et al., 1999). Therefore, pharmacological inhibition of 5αR could potentially be a key solution for AGA prevention.

Hair follicles have a life cycle of anagen, catagen, exogen and telogen. During catagen, the expression of Bax in the hair matrix is increased, which leads to apoptosis (Botchkareva et al., 2001). Therefore, the regulation of apoptosis in hair follicles is used as a strategy to improve the hair loss condition, and some methods have been proven to be effective in clinical practice. (Kiechle & Zhang, 2002; Paus & Cotsarelis, 1999). Various drugs are used to improve hair loss, but there are limitations in use because side effects have been reported. For example, the most commonly used hair growth drug, Minoxidil, has been reported to cause scalp irritation and allergic contact dermatitis (Rossi et al., 2012). Cardiovascular side effects were also reported when Minoxidil was used for the treatment of hair loss in children (Georgala et al., 2007). It has also been reported that topical application of diphenylcyclopropenone (DPCP) and anthralin, another alopecia treatment agent, caused side effects such as blister reaction and pruritus (Nasimi et al., 2019). On the other hand, herbal drugs, which have been used for centuries to treat alopecia, are in the spotlight because they have fewer side effects than synthetic drugs (Ayyanar & Ignacimuthu, 2005; Rathi et al., 2008).

Chrysanthemum zawadskii is a perennial plant belonging to the family Asteraceae and is native to all parts of Asia including Korea and Russia (Kim, 2017). According to a previous study, *C. zawadskii* was reported to have hair loss improvement effects, anti-inflammatory and antioxidant effects (Li et al., 2014). Peppermint (*Mentha piperita L.*) is a plant widely used as an herbal tea. Peppermint oil has been reported to promote hair growth, gastritis, flatulence, biliary disorders, enteritis and indigestion effects (McKay & Blumberg, 2006; Oh et al., 2014). Licorice (*Glycyrrhiza glabra*), the most commonly used herbal medicine, is used to reduce the bitterness of herbal medicines.

Received 01 Nov., 2021
Accepted 06 Dec., 2021
[1] *Institute of Health & Science, Jeonju University, Jeonju, Republic of Korea*
[2] *Department of Food Science and Technology, Jeonbuk National University, Jeonju-si, Jeollabuk-do, Republic of Korea*
[3] *Research Institute, Ato Q&A Co., LTD, Jeonju-si, Jeollabuk-do, Republic of Korea*
[4] *Department of Health Management, Jeonju University, Jeonju-si, Jeollabuk-do, Republic of Korea*
*Corresponding author: sonjjang@jj.ac.kr

It has also been reported to promote hair growth and have anti-inflammatory, antiviral, and immunomodulatory effects (Roy et al., 2013). Attempts to create a synergistic effect by combining various herbal drugs rather than using a single herbal drug for hair loss treatment are increasing (Pal et al., 2016). The purpose of this study was to investigate the hair growth promoting effect of a mixture of different herbs *C. zawadskii*, Peppermint and *G. glabra* in Human follicle dermal papilla cell (HFDPC) and C57BL/6 mice.

2 Materials and methods

2.1 Materials

Quanti-MAX™ WST-8 Cell Viability Assay Kit and WestGlow™ FEMTO Chemiluminescent substrate were obtained from BIOMAX (Seoul, Korea). Protease inhibitors was purchased from Sigma-Aldrich (St. Louis., MO, USA) Radio-immunoprecipitation assay buffer (RIPA buffer) and 5-AR antibodies were purchased from Thermo Fisher Scientific, Inc. (Carlsbad, CA, USA). Testosterone was purchased from TCI (Tokyo Japan). Bax, Bcl-2, Actin, Dkk-1 and AR antibodies were purchased from Santa Cruz Biotechnology (CA, USA). Human follicle dermal papilla cell (HFDPC) and cell growth medium were purchased from Promo cell (Heidelberg, DE, Deutschland).

2.2 Cell culture

HFDPC were cultured in the culture medium provided by the manufacturer. At 37 °C under 5% CO_2 in an incubator.

2.3 Cell viability

Cell viability was performed using Quanti-MAX™ WST-8. The HFDP cells (2×10^5 cells/mL) were seeded in 96-wells plates for 24 h and treated with or without FHH-CZ at various concentrations for 20 h. Then 10 μL of Quanti-MAX™ WST-8 reagent was added to the wells and incubated for 4 h. The absorbance of each well was then measured using a spectrophotometer (Tecan, Männedorf, Switzerland) at 450 nm. The absorbance of each well corresponded with the HFDPC viability and was calculated as the percentage of the control.

2.4 Protein extraction and western blot

HFDPC (2×10^5 cells/mL) were cultured in 60 mm cell culture dishes for 24 h, treated with CPG (0, 50, 100, 200 μg/mL), and incubated for 1 h in an incubator. After that, cells were stimulated with testosterone (100 μM) for 24 h. Whole proteins were then extracted from each sample using protease inhibitors treated-RIPA buffer. After quantification using Bradford protein analysis, proteins (30 μg) present in each sample were separated for 1 h at 100 V on 12% poly-acrylamide gels. After separation, proteins were transferred onto PVDF membranes for 1 h at 100 V. After blocking with 5% bovine serum albumin (BSA) for 1 h, membranes were washed three times with TBST solution (10 min each wash) and then incubated with each antibody (Dkk-1, Bax, Bcl-2, AR, 5αR and β-actin) at 4˚C overnight. Membranes were washed three times with TBST solution for 10 min and then incubated with mouse or rabbit HRP-conjugated secondary antibodies containing BSA for 2 h at RT. Subsequently, membranes were washed three times with TBST solution for 10 min and visualized with an imaging system (Alliance version 15.11; UVITEC) using EZ-western Lumi Pico Alpha chemiluminescent reagent. Band densities were analyzed using ImageJ (devel-oped by the National Institutes of Health) and converted into a graph.

2.5 RT-PCR analysis

HFDPC (2×10^5 cells/ml) were cultured in 60 mm cell culture dishes for 24 h, treated with CPG (0, 50, 100, 200 μg/mL), and incubated for 1 h in an incubator. After that, cells were stimulated with testosterone (100 μM) for 24 h. Total RNA was extracted and purified using GeneAll Ribospin II extraction kit (Seoul, Korea) according to the manufacturer's specifications. The concentration of the total RNA isolated and purified was determined by spectrophotometry. Five micrograms of each RNA sample were used to synthesize cDNA using ReverTra Ace qPCR RT master mix cDNA synthesis kit (Toyobo, Japan). Artificial PCR was performed using a Bioneer AccuPower PCR premix kit (Daejeon, Korea) following the manufacturer's protocol and with the primers (Bioneer) listed in Table 1**.** PCR amplification re-action conditions consisted of an initial denaturation step at 95 °C for 3 min, denaturation at 95°C for 30 s, annealing at 60 °C for 10 s, and extension phase at 72 °C for 30 s. The PCR cycle was of 40 rounds. The PCR products were separated on a 1.5% agarose gel and visual-ized by staining with GelRed nucleic acid stain. The gels were photo-graphed using BIO-RAD molecular imager.

2.6 Animals and treatment

Specific pathogen-free male C57BL/6 mice (six weeks old) weighing 18–20 g were obtained from Orient Bio Inc. (Gwangju, Korea). They were housed in a room with standard environmental conditions of temperature 22 ± 2 °C, humidity of 50-60% and a 12/12 h light-dark cycle. The mice were fed with a commercial standard laboratory diet and water ad libitum. The experimental procedures were performed in accordance with the Jeonju University Institutional Animal Care and Used Committee guidelines.

The mice were randomly assigned into five groups with five mice per group as follows: group 1, normal control; group 2, shaving; group 3, shaving plus FHH-CZ 100 mg/kg; group 4, shaving plus FHH-CZ 200 mg/kg; group 5, shaving plus FHH-CZ 400 mg/kg. FHH-CZ was prepared in saline. Groups 1 and 2 were administered the saline, and groups 3–5 were administered FHH-CZ orally every day for four weeks. Two weeks after the start of oral administration, the back hair of the mouse was shaved using an electric animal cipper, and then the hair was completely removed with a depilatory cream. Two weeks later, the mice were sacrificed. After taking pictures, the back skin was collected for histological analysis. According to the degree of hair growth, 0~20%(1), 20~40%(2), 40~60%(3), 60~80%(4), 80~100%(5) Visual evaluation was performed.

2.7 H&E staining

Four percent paraformaldehyde was used to fix the tissues for 24 hr at 4 °C. The tissues were then washed in five changes of PBS for a total of 24 hr (2 hr: 2 hr: 2 hr: 2 hr: 16 hr). The tissues were then dehydrated in a graduated series of ethanol (ranging from 60%-100%) for 30 min each step. The tissues were put in two changes of xylene (2 hr each) and embedded in three changes of paraffin (1 hr each) and then finally blocked in new paraffin. Five micrometers of thin sections were cut with a microtome (Leica) and stained with Hematoxylin and eosin (H&E) stain kit (ab245880, Abcam) for evaluation of number of hair roots.

3 Result

3.1 Effect of FFH-CZ on cell viability and cell growth in HFDPC

WST experiment was performed to investigate the effect of CPG on cell viability of HFDPC. As a result, CPG treatment did not show toxicity up to a concentration of 500 μg/mL. However, at the concentration of 1000 μg/mL, the cell viability was 85% compared to the untreated group (Figure 1A). Therefore, in subsequent experiments, cells were treated with CPG up to a concentration of 500 μg/mL or less. The effect of CPG on cell growth in testosterone-treated HFDPC were observed through an optical microscope. As a result, testosterone treatment inhibited cell growth and led to cell death. On the other hand, when CPG was treated, cell growth was increased in a concentration-dependent manner.

3.2 Effect of CPG on changes in Bax/Bcl-2 ratio in testosterone-treated HFDPC

Western blot was performed to investigate the effect of CPG on changes in Bax/Bcl-2 ratio and Dkk-1 expression in testosterone-stimulated HFDPC. As a result, in the testosterone-treated cell group, the Bax/Bcl-2 ratio showed a significant increase up to 144% compared to the normal control group (Figure 2A). However, when CPG was treated at a concentration of 50-200 μg/mL, it decreased in a concentration-dependent manner to 94%, 36%, and 21%, respectively. The expression of Dkk-1 showed a significant decrease when CPG 100-200 μg/mL was treated (Figure 2B), but was not concentration-dependent. According to RT-PCR results, testosterone treatment significantly increased Dkk-1 gene expression. On the other hand, pretreatment with CPG 50-200 μg/mL significantly reduced the Dkk-1 gene compared to the testosterone alone group.

3.3 Effects of CPG on the expression of hair loss factors in testosterone-stimulated HFDPC

ELISA, western blot and RT-PCR were performed to investigate the effect of CPG on the expression of hair loss-related factors in HFDPC. Figure 3B shows the results of measuring the amount of 5αR type 2 production using an ELISA kit. The amount of 5αR type 2 production was increased by 136% in the control group compared to the negative control group. However, in the case of the CPG 200 μg/mL treatment group, it showed a significant decrease to 84%. There were no statistically significant changes in the CPG50-100 treatment group. According to Western blot results (Figure 3B), testosterone treatment significantly increased 5αR type 2 and AR expression. However, in the case of CPG

Table 1. Primer sequences used in this study.

Target	Primer sequence
Dkk-1	5’ tccgaggagaaattgaggaa 3’
	3’ cctgaggcacagtctgatga 5’
AR	5’ cggaagctgaagaaacttgg 3’
	3’ gctgtacatccgggacttgt 5’
5 α R	5’ ggaatctcagaaaaccaggaga 3’
	3’ atttccggaggtaccactca 5’
GAPDH	5’ gagtcaacggatttggtcgt 3’
	3’ ttgattttggagggatctcg 5’

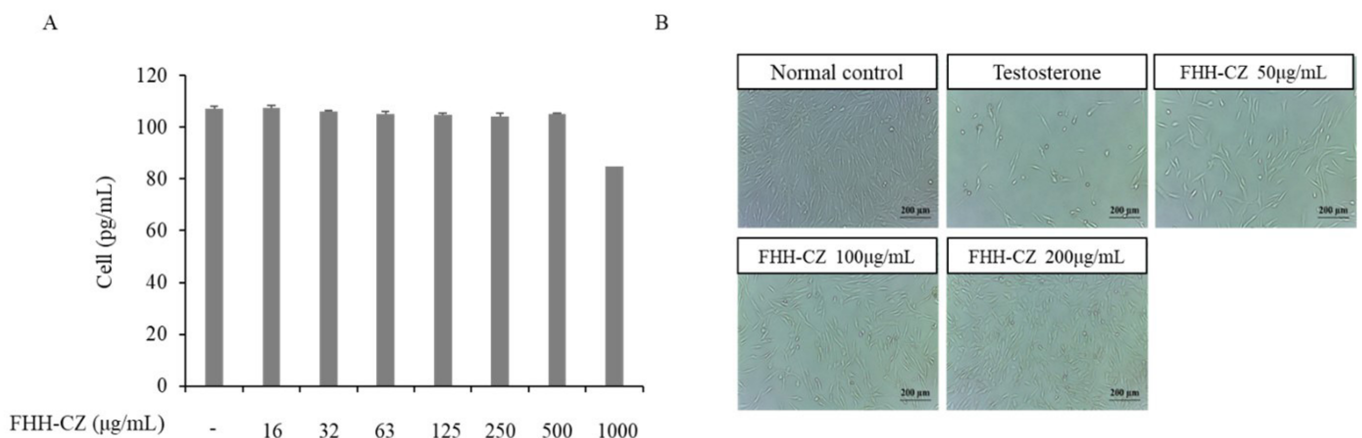

Figure 1. Effect of FHH-CZ on cell viability and cell growth in HFDPC. (A) Cells were treated with different concentration of FHH-CZ and cell viability was determined using Quanti-MAX™ WST-8. (B) Cells were preincubated with FHH-CZ at different concentrations for 1 h and then treated with testosterone for 24 h. Cell morphology was observed using an optical microscope (200X). Experiments were performed in triplicate. Each bar represents the mean ± SD. Different small case letters indicate significant differences at $p < 0.05$.

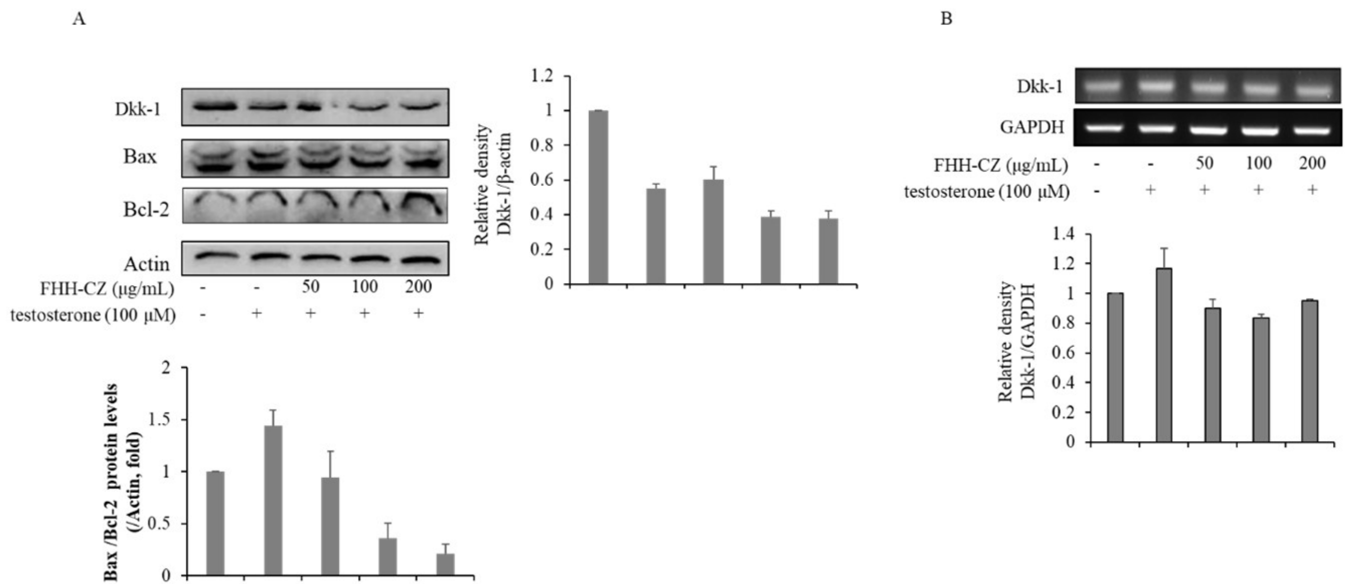

Figure 2. Effect of FHH-CZ on changes in Bax/Bcl-2 ratio and Dkk-1 expression in testosterone-treated HFDPC. (A) Dkk-1, Bax and Bcl-2 expression was determined by western blot analysis. (B) Dkk-1 gene expression was determined by RT-PCR analysis. Experiments were performed in triplicate. Each bar represents the mean ± SD. Different small case letters indicate significant differences at $p < 0.05$.

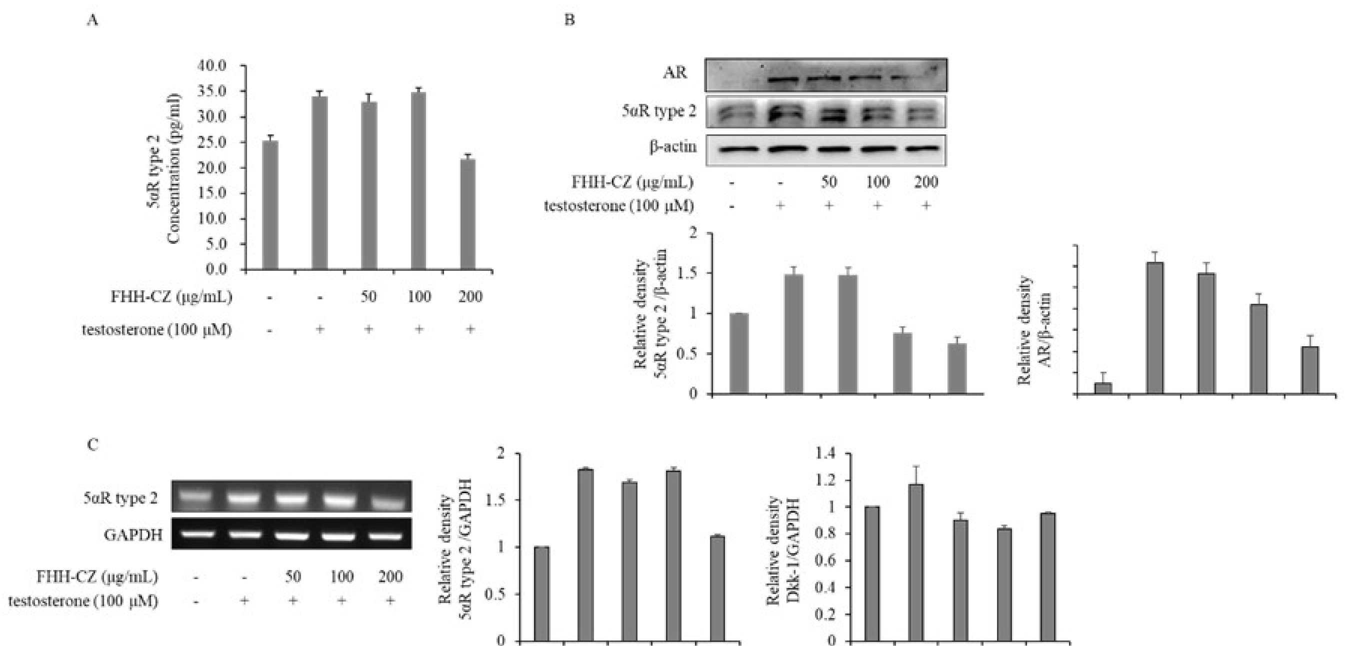

Figure 3. Effect of FHH-CZ on 5αR type 2 and AR expression in testosterone-treated HFDPC. (A) 5αR type 2 production was determined by ELISA. (B) 5αR type 2 and AR expression was determined by western blot analysis. (C) 5αR type gene expression was determined by RT-PCR analysis. Experiments were performed in triplicate. Each bar represents the mean ± SD. Different small case letters indicate significant differences at $p < 0.05$.

100-200 μg/mL treatment group, the expression of 5αR type 2 protein was decreased in a concentration-dependent manner. AR protein expression was also reduced in a concentration-dependent manner upon pretreatment with CPG concentrations of 100-200 μg/mL. 5αR type 2 mRNA expression was evaluated by RT-PCR (Figure 3C). Testosterone treatment significantly increased the expression of 5αR type 2 gene. CPG 200 μg/mL pretreatment reduced the expression of 5αR type 2 gene. There was no significant decrease in the CPG 50-100 μg/mL treatment group compared with the testosterone alone treatment group.

3.4 Effect of CPG on hair growth in C57BL/6 mice

We observed changes in hair after shaving C57Bl/6 mice to confirm the hair growth promoting and hair loss inhibitory effects of CPG shown in the cell experiment. As a result, there was no statistically significant change in CPG 100-200 mg/kg administration after inducing hair loss compared with the hair loss inducing group. On the other hand, in the 200 mg/kg administration group, it was confirmed that hair growth was significantly increased (Figure 4A and B). The results of observation of histological changes in mouse skin through H&E staining are shown in Figure 4C. Administration of CPG after shaving has been shown to increase the number of hair follicles.

4 Discussion

Our previous study demonstrated the antioxidant and anti-inflammatory synergistic effects of the complex FHH-CZ (Cho et al., 2021). Studies on the synergistic effect of bioactivity by mixing natural materials have been reported (Ozkan et al., 2021; Park et al., 2021; Lima et al., 2019).

In this study, the effects of FHH-CZ on promoting hair growth and inhibiting hair loss factors were investigated. At non-toxic concentrations, FHH-CZ promoted the growth of HFDCP reduced by testosterone. To elucidate the mechanism of action of these results, the effect of FHH-CZ on the expression of testosterone-related hair loss factors was investigated. 5αR plays a role in metabolizing testosterone to DHT, and DHT has a 5-fold increase in affinity for binding to androgen receptors (Ellis et al., 1998). Binding of DHT to AR induces follicle miniaturization and leads to telogen entry (Rathnayake & Sinclair, 2010). Therefore, inhibition of 5αR is emerging as an important strategy in preventing hair loss and alleviating symptoms. FHH-CZ inhibited the expression of 5αR from the mRNA level in testosterone-stimulated HFDPC. Moreover, the expression of AR was also shown to be reduced. The decreased AR expression is thought to be a result of the inhibition of 5αR by FHH-CZ. The effect of promoting hair growth by inhibiting 5a reductase of medicinal plant materials has been reported (Hirata et al., 2007; Kumar et al., 2012; Murata et al., 2013). Our results are consistent with those of these previous studies. However, it is considered that further studies on the mechanism of action on the inhibitory effect of FHH-CZ on 5αR expression are needed.

We also observed reduction of Bax/Bcl2 expression ratio and suppression of Dkk-1 expression by FHH-CZ treatment. In the catagen stage, hair goes through apoptosis process. In this process, the apoptosis protein Bcl-2 protein is decreased and the pro-apoptotic protein Bax is increased (Lindner et al., 1997). DKK-1 is known to induce apoptosis by increasing the Bax/Bcl2 expression ratio (Weng et al., 2009). Therefore, our results suggest that FHH-CZ can be used as a natural material that can delay apoptosis by reducing the Bax/Bcl2 expression ratio. These results are consistent with the results of previous studies in which cell treatment of medicinal plants suppressed the expression of Dkk-1 to suppress degenerative changes in hair follicles (Lee et al., 2017).

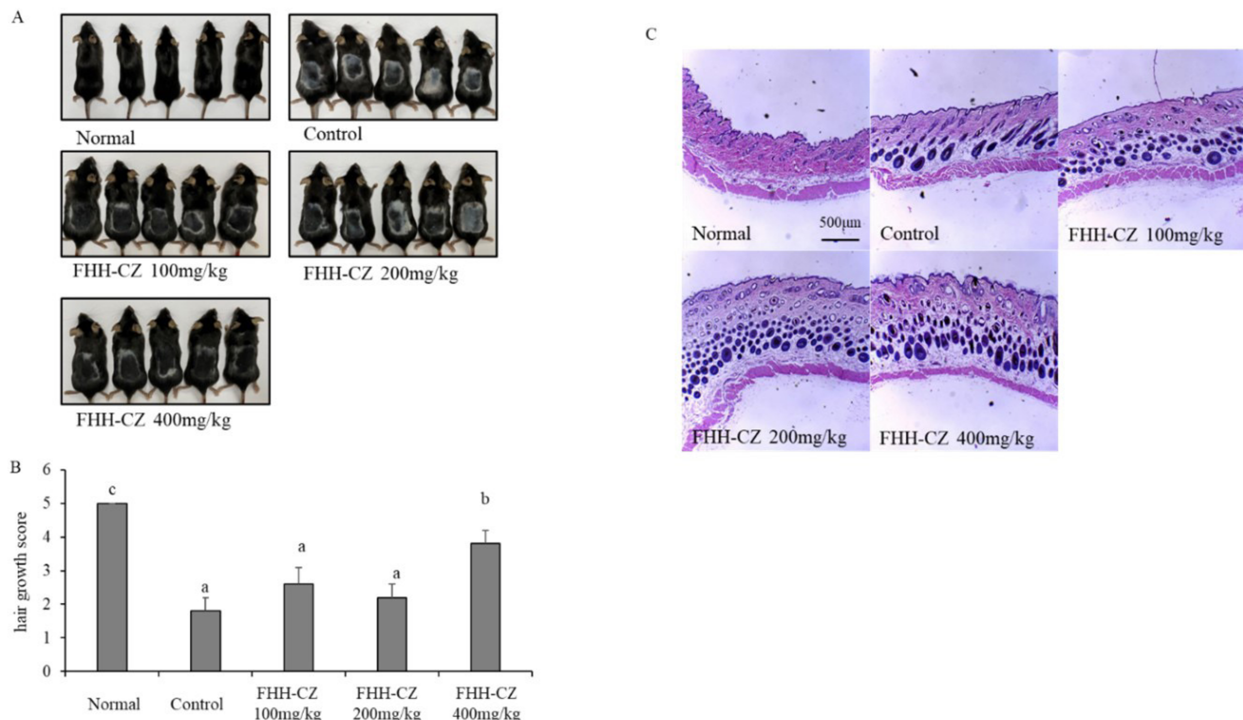

Figure 4. Effect of FHH-CZ on hair growth in C57BL/6 mice (A) The visual change of hair was photographed on the day of the end of the experiment. (B) The hair growth score was evaluated by 5 researchers in a double-blind manner. (C) Morphological changes were observed through H&E staining. Experiments were performed in triplicate. Each bar represents the mean ± SD. Different small case letters indicate significant differences at $p < 0.05$.

We confirmed that the FHH-CZ treated mouse skin grew faster than the untreated group, and the number of hair follicles increased from the animal test results. Although only visible changes were observed in the hair growth effect of FHH-CZ in animals, it is meaningful because it confirmed the same results as in vitro. It is thought that it is necessary to elucidate the mechanism of action of the hair growth promoting effect of FHH-CZ in animals through further studies.

In conclusion, FHH-CZ prevented the conversion of testosterone to DHT through inhibition of 5αR expression. In addition, FHH-CZ decreased the Bax/Bcl2 ratio through inhibition of Dkk-1 expression. Moreover, FHH-CZ promoted hair growth in mice in the same as in the cell experiments in animal experiments. Therefore, this study demonstrated that FHH-CZ is a potential material that can be used as a natural composite material that can prevent and treat hair loss by inhibiting the entry of hair follicles into catagen and telogen.

Conflict of interest

The authors declare no conflict of interest.

Funding

The Ministry of Trade, Industry, and Energy (MOTIE), Korea, under the 'Regional Specialized Industry Development Program (grant no. S2913418)' supervised by the Korea Institute for Advancement of Technology (KIAT) financially supported this research.

Acknowledgements

The authors thank FromBio Inc. for providing the FHH-CZ, especially Junho Lee for technical support and Young Sun Jang for administrative support.

References

Ayyanar, M., & Ignacimuthu, S. (2005). Medicinal plants used by the tribals of Tirunelveli hills, Tamil Nadu to treat poisonous bites and skin diseases. *Indian Journal of Traditional Knowledge*, 4(3), 229-236.

Botchkareva, N., Khlgatian, M., Jack Longley, B., Botchkarev, V. A., & Gilchrest, B. A.. (2001). SCF/c-kit signaling is required for cyclic regeneration of the hair pigmentation unit. *The FASEB Journal*, 15(3), 645-658. http://dx.doi.org/10.1096/fj.00-0368com. PMid:11259383.

Cho, B. O., Shin, J. Y., Kang, H. J., Park, J. H., Hao, S., Wang, F., & Jang, S. I. (2021). Anti-inflammatory effect of Chrysanthemum zawadskii, peppermint, Glycyrrhiza glabra herbal mixture in lipopolysaccharide-stimulated RAW264.7 macrophages. *Molecular Medicine Reports*, 24(1), 532. http://dx.doi.org/10.3892/mmr.2021.12171. PMid:34036392.

de Koning, E. B., Passchier, J., & Dekker, F. W. (1990). Psychological problems with hair loss in general practice and the treatment policies of general practitioners. *Psychological Reports*, 67(3 Pt 1), 775-778. http://dx.doi.org/10.2466/PR0.67.7.775-778. PMid:2287669.

Ellis, J. A., Stebbing, M., & Harrap, S. B. (1998). Genetic analysis of male pattern baldness and the 5α-reductase genes. *The Journal of Investigative Dermatology*, 110(6), 849-853. http://dx.doi.org/10.1046/j.1523-1747.1998.00224.x. PMid:9620288.

Georgala, S., Befon, A., Maniatopoulou, E., & Georgala, C. (2007). Topical use of minoxidil in children and systemic side effects. *Dermatology (Basel, Switzerland)*, 214(1), 101-102. http://dx.doi.org/10.1159/000096924. PMid:17191059.

Hines, R. S., Tho, S. P., Behzadian, M. A., & McDonough, P. G. (1999). Steroid 5alpha-reductase 2 gene melting polymorphisms in male subjects with azoospermia or oligospermia. *American Journal of Obstetrics and Gynecology*, 180(6 Pt 1), 1394-1398. http://dx.doi.org/10.1016/S0002-9378(99)70024-4. PMid:10368476.

Hirata, N., Tokunaga, M., Naruto, S., Iinuma, M., & Matsuda, H. (2007). Testosterone 5α-reductase inhibitory active constituents of Piper nigrum leaf. *Biological & Pharmaceutical Bulletin*, 30(12), 2402-2405. http://dx.doi.org/10.1248/bpb.30.2402. PMid:18057734.

Kiechle, F. L., & Zhang, X. (2002). Apoptosis: biochemical aspects and clinical implications. *Clinica chimica acta; international journal of clinical chemistry*, 326(1-2), 27-45. http://dx.doi.org/10.1016/S0009-8981(02)00297-8. PMid:12417095.

Kim, H. S. (2017). Extracts of Chrysanthemum zawadskii attenuate oxidative damage to vascular endothelial cells caused by a highly reducing sugar. *Cytotechnology*, 69(6), 915-924. http://dx.doi.org/10.1007/s10616-017-0110-7. PMid:28608258.

Kumar, N., Rungseevijitprapa, W., Narkkhong, N.-A., Suttajit, M., & Chaiyasut, C. (2012). 5α-reductase inhibition and hair growth promotion of some Thai plants traditionally used for hair treatment. *Journal of Ethnopharmacology*, 139(3), 765-771. http://dx.doi.org/10.1016/j.jep.2011.12.010. PMid:22178180.

Lee, Y., Kim, S. N., Hong, Y. D., Park, B. C., & Na, Y. (2017). Panax ginseng extract antagonizes the effect of DKK-1-induced catagen-like changes of hair follicles. *International Journal of Molecular Medicine*, 40(4), 1194-1200. http://dx.doi.org/10.3892/ijmm.2017.3107. PMid:28849028.

Li, Z., Li, J., Gu, L., Begum, S., Wang, Y., Sun, B., Lee, M., & Sung, C. (2014). Chrysanthemum zawadskii extract induces hair growth by stimulating the proliferation and differentiation of hair matrix. *International Journal of Molecular Medicine*, 34(1), 130-136. http://dx.doi.org/10.3892/ijmm.2014.1768. PMid:24807783.

Lima, A. B. S., Queiroga, I. M. B. N., Silva, G. M. S., Costa, J. S., Guedes, J. P. S., Dantas, C. O., & Cavalcanti, M. T. (2019). Characterization and application of Lippia alba (Mill) and Cymbopogon citratus D.C. Stapf. essential oils as natural sanitizers in coriander. *Food Science and Technology (Campinas)*, 39(4), 993-998. http://dx.doi.org/10.1590/fst.15718.

Lindner, G., Botchkarev, V. A., Botchkareva, N. V., Ling, G., van der Veen, C., & Paus, R. (1997). Analysis of apoptosis during hair follicle regression (catagen). *The American Journal of Pathology*, 151(6), 1601-1617. PMid:9403711.

Marberger, M. (2006). Drug Insight: 5alpha-reductase inhibitors for the treatment of benign prostatic hyperplasia. *Nature Clinical Practice. Urology*, 3(9), 495-503. http://dx.doi.org/10.1038/ncpuro0577. PMid:16964191.

McKay, D. L., & Blumberg, J. B. (2006). A review of the bioactivity and potential health benefits of peppermint tea (Mentha piperita L.). *Phytotherapy Research*, 20(8), 619-633. http://dx.doi.org/10.1002/ptr.1936. PMid:16767798.

Murata, K., Noguchi, K., Kondo, M., Onishi, M., Watanabe, N., Okamura, K., & Matsuda, H. (2013). Promotion of hair growth by Rosmarinus officinalis leaf extract. *Phytotherapy Research*, 27(2), 212-217. http://dx.doi.org/10.1002/ptr.4712. PMid:22517595.

Nasimi, M., Ghandi, N., Abedini, R., Mirshamsi, A., Shakoei, S., & Seirafi, H. (2019). Efficacy and safety of anthralin in combination with diphenylcyclopropenone in the treatment of alopecia areata: a retrospective case series. *Archives of Dermatological Research*,

311(8), 607-613. http://dx.doi.org/10.1007/s00403-019-01940-x. PMid:31165933.

Oh, J. Y., Park, M. A., & Kim, Y. C. (2014). Peppermint oil promotes hair growth without toxic signs. *Toxicological Research*, 30(4), 297-304. http://dx.doi.org/10.5487/TR.2014.30.4.297. PMid:25584150.

Ozkan, K., Bekiroglu, H., Bayram, Y., Sagdic, O. & Erbas, S. (2021). *In vitro* bioaccessibility, antioxidant and antibacterial activities of three different safflower (*Carthamus tinctorius* L.) genotypes. *Food Science and Technology*, In press. https://doi.org/10.1590/fst.08921.

Pal, R. S., Pa, Y., Singh, V., & Wal, P. (2016). Formulation and biological evaluation of some Herbal hair nourishing formulations. *International Journal of Pharmaceutics and Drug Analysis*, 4(7), 351-354.

Park, J. H., Shin, J. Y., Cho, B. O., Hao, S., Wang, F., Lim, Y. T., Shin, D. J., & Jang, S. I. (2021). Pectinase halophyte complex extract protects hairless mice skin damaged by UV-irradiation. *Food Science and Technology (Campinas)*, 22. http://dx.doi.org/10.1590/fst.72121.

Paus, R., & Cotsarelis, G. (1999). The biology of hair follicles. *The New England Journal of Medicine*, 341(7), 491-497. http://dx.doi.org/10.1056/NEJM199908123410706. PMid:10441606.

Rathi, V., Rathi, J., Tamizharasi, S., & Pathak, A. (2008). Plants used for hair growth promotion: a review. *Pharmacognosy Reviews*, 2(3), 185-187.

Rathnayake, D., & Sinclair, R. (2010). Male androgenetic alopecia. *Expert Opinion on Pharmacotherapy*, 11(8), 1295-1304. http://dx.doi.org/10.1517/14656561003752730. PMid:20426708.

Rossi, A., Cantisani, C., Melis, L., Iorio, A., Scali, E., & Calvieri, S. (2012). Minoxidil use in dermatology, side effects and recent patents. *Recent Patents on Inflammation & Allergy Drug Discovery*, 6(2), 130-136. http://dx.doi.org/10.2174/187221312800166859. PMid:22409453.

Roy, S. D., Karmakar, P. R., Dash, S., & Biswajit, D. (2013). Hair growth stimulating effect and phytochemical evaluation of hydro-alcoholic extract of Glycyrrhiza glabra. *Education*, 3(2), pp. 40-47.

Sinclair, R. (1998). Male pattern androgenetic alopecia. *BMJ (Clinical Research Ed.)*, 317(7162), 865-869. http://dx.doi.org/10.1136/bmj.317.7162.865. PMid:9748188.

Weng, L.-H., Wang, C.-J., Ko, J.-Y., Sun, Y.-C., Su, Y.-S., & Wang, F.-S. (2009). Inflammation induction of Dickkopf-1 mediates chondrocyte apoptosis in osteoarthritic joint. *Osteoarthritis and Cartilage*, 17(7), 933-943. http://dx.doi.org/10.1016/j.joca.2008.12.008. PMid:19217321.