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Bee Venom Promotes Hair Growth in Association with Inhibiting 5α-Reductase Expression

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Alopecia is an important issue that can occur in people of all ages. Recent studies show that bee venom can be used to treat certain diseases including rheumatoid arthritis, neuralgia, and multiple sclerosis. In this study, we investigated the preventive effect of bee venom on alopecia, which was measured by applying bee venom (0.001, 0.005, and 0.01%) or minoxidil (2%) as a positive control to the dorsal skin of female C57BL/6 mice for 19 days. Growth factors responsible for hair growth were analyzed by quantitative real-time PCR and western blot analysis using mice skins and human dermal papilla cells (hDPCs). Bee venom promoted hair growth and inhibited transition from the anagen to catagen phase. In both anagen phase mice and dexamethasone-induced catagen phase mice, hair growth was increased dose dependently compared with controls. Bee venom inhibited the expression of SRD5A2, which encodes a type II 5α-reductase that plays a major role in the conversion of testosterone into dihydrotestosterone. Moreover, bee venom stimulated proliferation of hDPCs and several growth factors (IGF-1R, VEGF, FGF2, and FGF7) in bee venom-treated hDPCs dose dependently compared with the control group. In conclusion, bee venom is a potentially potent 5α-reductase inhibitor and hair growth promoter.

Key words
Bee venom; alopecia; 5α-reductase; dihydrotestosterone; human dermal papilla cell
Hair is considered one of the most important features of appearance and hair loss can therefore negatively impact self-esteem and damage overall quality of life. The incidence of hair loss has rapidly increased worldwide. Hair loss is mostly induced by genetic factors but stress also plays an important role these days, especially in young people including women. Inflammation to the scalp is linked to hair loss. A recent comorbidity study among patients with diagnosed alopecia shows that 74.1% of the patients have inflammatory diseases like atopy and contact dermatitis and mental health problems are seen in 25.5%. 1) Autoimmune diseases such as thyroid disease, diabetes mellitus, and rheumatoid arthritis are comorbidities associated with alopecia areata.

The hair growth cycle consists of three phases namely anagen, catagen, and telogen, which represent the growing phase, involution phase, and resting phase, respectively. Approximately 90% of hair is in anagen phase. 2) Alopecia is induced by several factors such as decreased fibroblast follicle cell growth and renewal, increased dihydrotestosterone (DHT), and chemotherapy. 3) The three main types of alopecia are androgenetic, alopecia areata, and chemotherapy-induced. Androgenetic alopecia (AGA), the most common type of hair loss, affects both men and women. AGA is caused by two androgens, testosterone and dihydrotestosterone. Two synthetic drugs (topical minoxidil and oral finasteride) have been approved by the Food and Drug Administration to treat AGA, but these drugs have low cure rates (<50%) and side effects such as irritation, allergic contact dermatitis, and increasing growth or darkening of fine body hairs. 4, 5) Dexamethasone (DEX) has been used as a catagen-inducer agent. In mice, depilation induces anagen hair follicles (stages I to VI) within 9 days after depilation and DEX treatment triggers premature catagen development on day 13 after depilation. 6) Keratinocyte apoptosis is a major factor in the regulation of hair regression which induces catagen phase.
DEX is known as an apoptosis inducer, which means that DEX induces apoptosis of follicle keratinocytes resulted in premature termination of anagen phase and early entry into catagen phase.\(^7\)

5α-reductase converts testosterone to DHT, which has a stronger affinity for androgen receptors than testosterone and induces the expression of genes related to minimizing follicles, thus stimulating hair loss.\(^8\) Although 5α-reductase inhibitors such as finasteride and dutasteride have been approved for the treatment of hair loss, they can cause serious adverse effects such as sexual dysfunction, depression, and gynecomastia.\(^9,10\)

Ideally, a treatment for alopecia should prevent hair loss and promote hair growth. Indeed, a combination of 5α-reductase inhibitors and hair growth promoters has been shown to improve AGA.\(^11,12\) Alopecia may be treated topically and/or orally and by hair transplantation. However, hair transplantation is very expensive, oral medicines show hepatotoxicity, and topical medicines are not clearly effective in improving hair growth. Although minoxidil and finasteride show adverse effects, they are still commonly used to treat AGA. Identifying alternative treatments—especially herbs that exhibit relatively low toxicity compared with synthetic drugs—is therefore an active area of research. Several natural compounds have been reported to have hair growth-promoting properties.\(^13-16\)

Bee venom is an oriental medicine that has recently been developed to relieve pain and to treat diseases such as rheumatoid arthritis,\(^17\) Parkinson’s disease,\(^18\) cancer,\(^19,20\) asthma,\(^21\) and inflammatory disease.\(^22\) Bee venom contains many active peptides such as melittin, apamin, mast cell degranulating peptide, and adolapin, as well as a variety of proteins and amines including phospholipase A2, hyaluronidase, histamine, dopamine, and norepinephrine.\(^23\) In this study, we investigated the ability of bee venom to prevent hair loss.
and to promote hair growth.

MATERIALS AND METHODS

**Materials**  Bee venom containing 56.7% melittin was obtained from KUKJEON Pharmacy (Anyang, Korea). Minoxidil, melittin, and dexamethasone were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Polyethylene glycol and ethanol were obtained from J. T. Baker (Center Valley, PA, USA). Dulbecco’s Modified Eagle Medium (DMEM), fetal bovine serum, and penicillin/streptomycin for the cultivation of cells were obtained from HyClone (Logan, UT, USA).

**Hair Growth in C57BL/6 Mice**  Six-week-old female C57BL/6 mice were purchased from Koatech (Peongtak, Korea), and housed under constant environmental condition. Mice were housed in cages under strict standard conditions (22 ± 1°C; 55 ± 5% humidity; 12-h light and 12-h dark cycle). All animals had free access to water as well as a normal diet purchased from Feedlab (Gyeonggi do, Korea). All experimental procedures were approved by the Korea University Institutional Animal Care and Use Committee (Approval No. KUIACUC-2014-230) and were in accordance with the Guide for the Care and Use of Laboratory Animals (NIH Publication No. 85-23, 1996). After adaptation for 1 week, hairs on the dorsal skin were removed by a depilatory cream (Veet, Reckitt Benckiser, Berkshire, England) to investigate the hair growth effect of bee venom during the anagen phase. Animals were randomly divided into five groups (n=6): a negative control group (vehicle treated), a positive control group (2% minoxidil treated), and three test groups (0.001%, 0.005%, and 0.01% bee venom treated). The day after depilation, 100 μL of each test
compound was applied to the denuded area of each mouse once a day for 3 weeks. The vehicle was composed of distilled water, ethanol, and polyethylene glycol (in a 5:3:2 ratio).

To evaluate the inhibitory effect of bee venom on the induction of catagen phase, hairs on the dorsal skin were removed and dexamethasone was applied to induce catagen phase. Animals were randomly divided into six groups (n=6): the negative control group (vehicle treated), the dexamethasone (0.1%) only treatment group, the positive control group (2% minoxidil and 0.1% dexamethasone), and three bee venom treatment groups (0.001%, 0.005%, and 0.01% bee venom and 0.1% dexamethasone). On the 7th day after depilation, 100 μL of each test compound was applied on the depilated region once a day for 7 days and simultaneously on the 9th day after depilation, 1 mL of dexamethasone (0.1%) was applied to the depilated region once a day for 5 days. The lengths of 30 randomly plucked hairs were measured at 19 days after topical application of bee venom or minoxidil for each group.

Cell Culture and Cell Proliferation Assay  Human dermal papilla cells (hDPCs) were obtained from the Korean Cell Line Bank (Seoul, Korea). The cells were cultured in DMEM supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin. hDPCs were incubated at 37°C under 5% CO₂. Cells were assayed after reaching 80% confluency. WST-1 (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium, monosodium salt) reagent (Roche, Indianapolis, IN, USA) was used to measure the proliferation of hDPCs. Cells (1 × 10⁵ cells/well) were incubated overnight in a 96-well culture plate. Cells were then treated with bee venom (100 ng/mL, 200 ng/mL, and 500 ng/mL) or minoxidil (1 μM) for 24 h at 37°C under 5% CO₂. Next, 10 μL of WST reagent was added and viability was measured using a SpectraMax microplate reader (Molecular
Devices, Sunnyvale, CA, USA) at 450 nm. The relative cell viability (%) was expressed as a percentage relative to the untreated control cells.

Quantitative Real-Time Polymerase Chain Reaction (RT-PCR) After depilation, mice were sacrificed by exposure to CO₂ gas, and their dorsal skins were cut out. For each mouse, 50 mg of dorsal skin was homogenized in 1 mL of TRIzol reagent using a Taco™ Prep Bead beater (Taco, Taichung, Taiwan) according to the manufacturer’s protocol. hDPCs (2 × 10⁵ cells/well) were cultured in serum-free DMEM with bee venom (100 ng/mL, 200 ng/mL, and 500 ng/mL) or minoxidil (1 μM) in a 6-well plate for RNA extraction. After 24 h, tissue/cells were treated with 1 mL of TRIzol reagent (Invitrogen, Carlsbad, CA, USA) and total RNA was extracted according to the manufacturer’s protocol. Total RNA concentration was quantified with a NanoDrop ND-1000 Spectrophotometer (Thermo Scientific, Wilmington, DE, USA). Next, cDNA was prepared using a RevertAid First Strand cDNA kit (Fermentas, Burlington, Ontario, Canada), and RT-PCR was performed with a DyNamo™HS SYBR Green qPCR kit (FINNZYMES, Espoo, Finland) using a StepOnePlus™ Real-Time PCR System (Applied Biosystems, Foster City, CA, USA). PCR primers were purchased from Bioneer (Seoul, Korea): vascular endothelial growth factor (VEGF) (forward: 5′-GGGCAGAATCAGCAGAAGT, reverse: 5′-TGGTGTGGAACTCCTCA), insulin-like growth factor 1 receptor (IGF1R) (forward: 5′-CATTTCACCTCCACCAC, reverse: 5′-AGGCATCTGCCATTGCAT), fibroblast growth factor 2 (FGF2) (forward: 5′-AGAAGAGCGACCCTCACAT, reverse: 5′-GCCACTGTCTCTGATTTCC), fibroblast growth factor 7 (FGF7) (forward: 5′-CCTGGACACACACCAAGA, reverse: 5′-GCCACTGCTCGATTTCAT) for hDPCs; and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (forward: 5′-AAGGGTTCATCATCTGCCC, reverse: 5′-GTGATGGCATGGACTGTGGT), keratinocyte growth factor (KGF) (forward: 5′-
CGAAATGGATACTGACACG, reverse: 5′-CCCCTCCTCCATGTAGTCA), 5α-reductase 2 (SRD5A2) (forward: 5′- AATGTGCTGCTGGGTCTCTT, reverse: 5′-AGAAGGCAGTGGCTTTCAGA). PCR reactions were preheated at 95°C for 10 min followed by 40 cycles at 95°C for 20 s, 60°C for 20 s, and 72°C for 30 s. RT-PCR data were quantified based on the number of cycles needed for amplification-generated fluorescence to reach a specific detection threshold (the Ct value). Relative gene expression was quantified on the basis of equal amounts of RNA (1 µg) and the average Ct value for each gene, where delta Ct (ΔCt = Ct_target gene – Ct_reference gene) was calculated from the Ct values of genes in the same sample. GAPDH was used as the internal control reference gene. The ΔΔCt value was calculated with the equation ΔΔCt = (ΔCt_treated − ΔCt_untreated). The normalized expression change was expressed as 2^ΔΔCt (the GAPDH control was set to 1).24)

**Western Blot Analysis** Dorsal skin (50 mg) was homogenized in 1 mL of Pro-Prep protein extraction solution (Intron, Seoul, Korea) using a Taco™ Prep Bead beater (Taco, Taichung, Taiwan) according to the manufacturer’s protocol. After centrifugation at 10,000 × g for 5 min at 4°C, the supernatants were collected. hDPCs (2 × 10^5 cells/well) were cultured for 24 h with bee venom (100 ng/mL, 200 ng/mL, and 500 ng/mL) or minoxidil (1 µM) in 60-mm dishes. Cells were washed twice with PBS. Protein was extracted from cells using Pro-Prep protein extraction solution (Intron, Seoul, Korea). After centrifugation at 10,000 × g for 5 min at 4°C, the supernatants were collected. The concentration of protein was determined by the Bradford assay. Equal amount (30 µg) of protein from each sample was separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The separated proteins were transferred to a polyvinylidene difluoride membrane (Millipore, Bedford, MA, USA) with Trans-Blot semi-dry transfer cell (Bio-Rad, Hercules, CA, USA). The membrane was blocked with 5% non-fat skimmed milk in PBS with 0.05% Tween 20 (PBS-T) overnight at
4°C. The membrane was washed three times with PBS-T and then incubated with a 1:100 dilution of the anti-5α-reductase (Santa Cruz Biotechnology, Dallas, TX, USA) antibody, and a 1:500 dilution of the anti-VEGF antibody (BioLegend, San Diego, CA, USA) for 1 h at room temperature. GAPDH and β-actin, used as an endogenous control, were detected with a 1:5,000 dilution of anti-GAPDH antibody (Thermo Scientific, Rockford, IL, USA) and a 1:5,000 dilution of anti-β-actin antibody (Thermo Scientific), respectively. The membrane was washed three times with PBS-T, and incubated further with a secondary antibody. A 1:25,000 dilution of a goat anti-rabbit IgG antibody (Thermo Scientific) was used for secondary antibody detection of anti-VEGF and anti-5α-reductase. The 1:50,000 dilution of a goat anti-mouse IgG horseradish peroxidase conjugated antibody (Thermo Scientific) was used for secondary antibody detection of anti-β-actin and anti-GAPDH. Bound antibodies were detected by WesternBright Sirius western blotting detection kit (Advanta, Menlo Park, CA, USA). Blot images were obtained by imaging system FluorChem E (Proteinsimple, San Jose, CA, USA) and band intensity was measured using analysis tools. After normalization by the corresponding expression of GAPDH and β-actin, protein expression fold changes for the treatment groups were compared with that of the dexamethasone only-treated group and vehicle only-treated negative control group in mice and hDPCs, respectively. GAPDH and β-actin were used as internal controls.

**Histological Analysis** For histological analysis, dorsal skin sections were fixed in 10% formalin and embedded in paraffin. Sections were prepared at a thickness of 5 µm, stained with hematoxylin and eosin, and then mounted with Canada balsam. The slides were observed with an optical microscope at 100 x magnification.

**Statistical Analysis** All data are presented as means ± standard deviation (SD) of three
independent experiments that were performed in triplicate. The data were analysed using the SPSS statistical analysis program (Chicago, IL, USA). The statistical significance of the difference was determined using the Student’s $t$-test. A $p$-value of $< 0.05$ was considered significant. Statistical differences among groups were evaluated using an analysis of variance (ANOVA), followed by Duncan’s multiple range tests. Results were considered to be statistically significant at $p < 0.05$.

RESULTS

The Effect of Bee Venom on Hair Growth in C57BL/6 Mice Bee venom and minoxidil were topically applied to mouse dorsal skin for 19 days. Hair growth increased significantly and dose dependently in bee venom-treated mice compared with the control group (Fig. 1A). Mice treated with 0.01% bee venom showed the biggest increase in hair growth compared with the 2% minoxidil-treated group. The morphological structure of a longitudinal section of the dorsal skin is shown in Fig. 1B. Hair follicles were elongated in a dose-dependent manner in bee venom-treated mice compared with the control group. To investigate the inhibitory effect of bee venom on catagen phase development, the catagen phase was induced by treating with dexamethasone. Hair loss was clearly inhibited in dexamethasone-induced catagen phase mice by treatment of bee venom, especially at 0.005% and 0.01%, which was much more effective than 2% minoxidil (Fig. 2).
Hair length in the minoxidil- and bee venom-treated mice was increased compared with the control group (Table 1). Hair length increased dose dependently in bee venom-treated mice.

**The Effect of Bee Venom on Human Dermal Papilla Cell Growth** To investigate the proliferative effect of bee venom on hDPCs, cells were treated with bee venom (1–500 ng/mL) or 200 ng/mL melittin. hDPC proliferation increased 1.07-, 1.13-, 1.17-, and 1.09-fold in mice treated with 100 ng/mL, 200 ng/mL or 500 ng/mL bee venom, or 200 ng/mL melittin, respectively, compared with the control (Fig. 3).

**Bee Venom Inhibits 5α-Reductase and Keratinocyte Growth Factor Expression in C57BL/6 Mice** To investigate whether bee venom inhibits catagen development and stimulates hair growth in dexamethasone-induced catagen phase C57BL/6 mice by inhibiting 5α-reductase, SRD5A2 (encoding steroid 5α-reductase 2) expression was measured by RT-PCR and western blot analysis. SRD5A2 expression was significantly decreased ($p < 0.001$) in a dose-dependent manner (Fig. 4A) at the mRNA and protein level in both bee venom-treated mice and minoxidil-treated mice, compared with the control group (Fig. 4B). SRD5A2 mRNA levels decreased to 8.2%, 7.0%, 3.6%, and 1.1% in mice treated with 2% minoxidil, 0.001%, 0.005%, and 0.01% bee venom, respectively, compared with the dexamethasone only-treated group. SRD5A2 mRNA levels were 85.1%, 44.0%, and 12.9% of that of the minoxidil treated group, in mice treated with 0.001%, 0.005%, and 0.01% bee venom, respectively. At the protein level, 5α-reductase decreased 0.93-, 0.64-, 0.61-, and 0.37-fold in mice treated with 2% minoxidil, 0.001%, 0.005%, and 0.01% bee venom, respectively, compared with the dexamethasone only-treated group.
KGF stimulates follicular proliferation. KGF expression was significantly increased ($p < 0.05$) dose dependently in mice treated with bee venom compared with dexamethasone (with a 4.17-, 4.75-, and 5.66-fold increase in mice treated with 0.001%, 0.005%, and 0.01% bee venom, respectively, compared with dexamethasone only-treated group) (Fig. 5). In the minoxidil-treated group, KGF expression was increased 6.4- and 9.9-fold compared with the vehicle only-treated control and dexamethasone only-treated group, respectively.

**The Effect of Bee Venom on the Expression of Growth Factors in Human Dermal Papilla Cells** Hair growth is stimulated by various growth factors including fibroblast growth factor 2 (FGF-2), fibroblast growth factor 7 (FGF-7), insulin-like growth factor 1 receptor (IGF-1R), and vascular endothelial growth factor (VEGF). FGF2, FGF7, IGF-1R, and VEGF mRNA expression levels significantly increased in bee venom-treated groups ($p < 0.05$) compared with the control (Figs. 6 and 7). In the 1 μM minoxidil-treated group, VEGF mRNA was increased 1.9-fold compared with the control and 1.30-, 1.80-, and 1.85-fold in the 100, 200, and 500 ng/mL bee venom-treated groups, respectively, compared with the control. Both minoxidil and bee venom increased protein-level expression of VEGF (Fig. 7B) dose dependently compared with the control group, with a 1.95-, 2.95-, and 2.08-fold increase in mice treated with 100, 200, and 500 ng/mL bee venom, respectively, and a 1.47-fold increase in the 2% minoxidil group.

**DISCUSSION**

Alopecia is an important issue that can occur in people of all ages. Alopecia is defined as
abnormal hair loss and is mainly caused by genetic factors and aging. Alopecia is considered an autoimmune disease caused by lymphocytic attack of anagen hair bulbs. AGA results in decreased proliferation of dermal papilloma cells and a reduction in hair follicle size. Bee venom promotes proliferation of hDPCs and elongates hair follicles, thereby preventing AGA. Bee venom consists mainly of melittin (approximately 40–50%) as well as small quantities of polypeptides and low molecular compounds; the bee venom used in this study contained 56.7% melittin. Melittin has a variety of biological activities including proliferative effects on hDPCs and growth factor stimulation. Although we could not clearly explain that melittin is the active main component, melittin might play some role as a hair growth-promoting component in bee venom. Further studies are needed to elucidate the main active compound of bee venom to promote hair growth.

DHT is a sex steroid and androgen hormone that is produced from testosterone by 5α-reductase in hair follicles, prostate, and testes and, in the skin, occurs exclusively in dermal papilla cells. DHT is thought to shorten the growth phase of hair follicles and is a main determinant of hair loss in males. Hair loss in female individuals is more complex, but DHT still plays a role. We found that bee venom downregulates SRD5A2 gene and 5α-reductase protein expression, which suggests that bee venom could treat DHT-dependent hair loss by inhibiting 5α-reductase.

IGF-1 is a ubiquitous growth factor that is known to stimulate hair elongation and to regulate maintenance of the hair follicle in anagen phase. VEGF promotes angiogenesis thereby facilitating nutrient acquisition, which promotes hair follicle proliferation and hair growth. Fibroblast growth factors play a key role in hDPC proliferation and are involved in various endocrine signaling pathways. FGF7 (also known as keratinocyte growth factor) stimulates epithelial cell proliferation and affects tissue remodeling. Growth factors such as
FGF7, FGF2, VEGF, and IGF-1 are upregulated in hDPCs to maintain hair follicles in the anagen phase and may therefore promote hair growth. We found that bee venom stimulates the expression of several growth factors including FGF7, FGF2, VEGF, and IGF-1, which might promote hair growth and prevent hair loss through synergistic effects.

Hair length depends on the length of the anagen phase,32) and normally 90%, 1% and 9% of hair is in anagen, catagen, or telogen phase, respectively.21 In the bee venom-treated group hair length was increased compared with the control group, which suggests that bee venom may extend the anagen phase. Dexamethasone is used to induce the catagen phase, which results in hair follicle regression and massive hair loss. We show that bee venom clearly promotes hair growth in dexamethasone-treated mice compared with the dexamethasone-only treated group, which means that bee venom might inhibit the catagen phase, which is an important attribute for successful therapeutic agents of hair loss.

Minoxidil prevents hair loss by suppressing androgen receptor-related functions,33) and several mechanisms have been proposed for its hair growth-promoting effects including opening of potassium channels and stimulation of VEGF and cell proliferation.34) Although the underlying mechanisms are not fully understood, minoxidil is a well-known topical treatment for androgenic alopecia. Minoxidil is usually used as a 2% solution for women and 5% solution for men.5) In this study, we used female C57BL/6 mice and 2% minoxidil as a positive control. Importantly, we found that bee venom was better at promoting hair growth than minoxidil, especially at higher concentrations (0.005% and 0.01%).

Potential targets for the development of alternative hair growth-promoting agents are 5α-reductase, androgen receptors including 5α-reductase receptors, and growth factors.4) Alternative hair growth treatments commonly inhibit 5α-reductase35-37) or induce growth factors.38, 39) The ethanol extract of Asiasari radix root40) and methanol extract of Sophora...
flavescens root exhibit both activities. Compounds with multiple activities are potentially more promising hair growth promoting agents. Bee venom inhibits 5α-reductase expression, inhibits the catagen phase, and upregulates growth factors in hDPCs—bee venom might therefore be a promising hair growth-promoting agent.

Currently, approved hair growth agents have adverse effects; alternative treatments with improved safety and efficacy are therefore required. Although bee venom can cause an allergic response in humans, it is also known for its diverse effects as a traditional medicine for treating various diseases. Moreover, within the concentrations used in this study, bee venom did not cause erythema, edema, irritation, or cytotoxicity and instead stimulated proliferation of hDPCs. In addition, our results show that bee venom is more effective at promoting hair growth than minoxidil. In catagen phase-induced mice, bee venom reduced hair loss and stimulated new hair growth. In summary, bee venom might be developed as a therapeutic agent for hair loss given its ability to promote hair growth by stimulating the expression of dermal cell growth factors and inhibiting the development of the catagen phase, and its ability to prevent hair loss by inhibiting the expression of 5α-reductase.

CONCLUSION

Bee venom promotes hair growth in female C57BL/6 mice by reducing 5α-reductase expression; by stimulating the expression of growth factors such as VEGF, IGH-1, FGF7, and FGF2; and by possibly inhibiting the catagen phase. Bee venom also increases proliferation of hDPCs. We therefore demonstrated the potential value of bee venom as an alternative hair growth-promoting agent.
Acknowledgments  This research was supported by the Agricultural Biotechnology Development Program, Ministry of Agriculture, Food and Rural Affairs, Republic of Korea (114070-3).

Conflict of Interest  The authors declare no conflict of interest.
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Biological and Pharmaceutical Bulletin Advance Publication


Figures Captions

Fig. 1. The effect of bee venom on hair growth (A) and on the histological appearance of hair follicles (100×) (B) in C57BL/6 mice

Bee venom was topically applied on the dorsal skin for 19 days. Hair growth is shown after (a) 1 day, (b) 7 days, (c) 11 days, and (d) 19 days in (A). (a) NC, treated vehicle only; (b), 2% minoxidil; (c), 0.001% bee venom; (d), 0.005% bee venom; and (e), 0.01% bee venom in (B).

Fig. 2. The effect of bee venom on the prevention of hair loss in catagen-induced C57BL/6 female mice

(A), NC, treated vehicle only; (B), Dexamethasone (DM) treated; (C), DM+2% minoxidil; (D), DM+0.001% bee venom; (E), DM+0.005% bee venom; and (F), DM+0.01% bee venom.

Fig. 3. The effect of bee venom on the proliferation of human dermal papilla cells (hDPCs). Cell proliferation was measured using WST-1 reagent (p < 0.05).

Fig. 4. The effect of bee venom on SRD5A2 gene (A) and protein (B) expression in C57BL/6 mice
RT-PCR and western blot of 5α-reductase were performed after treatment with bee venom (0.001%, 0.005%, and 0.01%). NC, vehicle-treated control; DEX, dexamethasone; BV, bee venom; MIN, 2% minoxidil. Significantly different compared with NC (**p < 0.001).

Fig. 5. RT-PCR of KGF in C57BL/6 mice treated with bee venom. Significantly different compared with negative control (NC). NC, vehicle-treated control; DEX, dexamethasone; MIN, 2% minoxidil. (*p < 0.05, **p < 0.01).

Fig. 6. Effect of bee venom on the expression of FGF-2, FGF-7, and IGF-1R in hDPCs. MIN, 1 μM minoxidil. (*p < 0.05, **p < 0.01, ***p < 0.001).

Fig. 7. The effect of bee venom on VEGF gene (A) and protein (B) expression by RT-PCR and western blot in hDPCs, respectively. MIN, 1 μM minoxidil. (*p < 0.05, **p < 0.01, ***p < 0.001).
Table 1. Hair length after 19 days of treatment with bee venom

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<td>Length (mm)</td>
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Values are the mean ± standard deviation of six mice per group and 30 hairs were randomly plucked from each mouse. *p < 0.05.
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Fig. 1.
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(A) 

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</tr>
<tr>
<td>BV (%)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0.001</td>
<td>0.005</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>

5α-reductase 

| Fold | 1.09 | 1.0  | 0.93 | 0.64  | 0.61  | 0.37  |

GAPDH
Fig. 5.

![Normalized fold expression graph](image_url)
Fig. 6.

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Fig. 7.

(A) Normalized fold expression of VEGF and β-actin with different concentrations of bee venom.

(B) Western blot analysis showing the fold change in VEGF and β-actin expression under different concentrations of bee venom.