J. Soc. Cosmet. Scientists Korea
Vol. 38, No. 4, December 2012, 277-287

# 발모효과를 가지는 한방복합처방단

**이 준 영 · 임 경 란 · 정 택 규 · 이 명 희* · 윤 경 섭†**

(주)사임당화장품 기술연구소, *대전보건대학교 화장품과학과
(2012년 5월 25일 접수, 2012년 8월 9일 수정, 2012년 11월 14일 채택)

# Medicinal Herbal Complex Extract with Potential for Hair Growth-Promoting Activity

**Jun Young Lee, Kyung Ran Im, Taek Kyu Jung, Myoung-Hee Lee*, and Kyung-Sup Yoon†**

Saimdang Cosmetics Co. Ltd. R&D Center, 313-4 Songdae-ri, Ochang-eup, Cheongwon-gun, Chungbuk 363-886, Korea
*Department of Cosmetic Science, Daejeon Health Science College
(Received May 25, 2012; Revised August 9, 2012; Accepted November 14, 2012)

**요 약**: 탈모방지 및 발모효과를 가지는 소재를 개발하기 위해 한의학에서 전통적으로 사용되는 23가지 한방소재를 선정하여 한방복합처방단을 개발하였다. 한방복합처방단을 구성하는 한방소재들은 예로부터 전통적으로 발모 및 탈모방지, 흰머리방지, 염증 치료 및 혈액순환개선 효과를 가진 것으로 알려져 있는 당귀, 보골지, 측백엽, 한련초, 구기자, 복분자, 상백피, 숙지황, 여정실, 적하수오, 흑지마, 고삼, 백지, 익모초, 단삼, 도인, 몰약, 감국, 유향, 인삼, 천궁, 합환피, 현호색 등이다. 또한, 한방복합처방단의 발모효과를 확인하기 위해 *in vitro*와 *in vivo* 평가모델을 이용하여 모발성장 및 촉진에 미치는 영향을 실험하였다. *In vitro* 상에서는 모유두세포, 각질형성세포 및 섬유아세포의 증식을 확인하였다. 또한, 흰머리방지 효과와 관련하여 멜라노마 세포에서의 멜라닌 합성능력을 확인하였다. 한방복합처방단의 *in vitro* 상에서의 육모효과는 C57BL/6 마우스를 이용한 *in vivo* 상에서도 확인하였다. 연구 결과 한방복합처방단은 50 μg/mL의 농도에서 모유두 세포의 증식을 175 %까지, 섬유아세포인 NIH3T3 세포의 증식은 120 %까지 증가시켰으며, 20 μg/mL의 농도에서 각질형성세포인 HaCaT 세포의 증식을 133 %까지 증가시켰다. 멜라닌 합성의 경우, 50 μg/mL의 농도에서 154 %까지 증가시켰다. 또한, C57BL/6 마우스를 이용한 육모효과에 있어서는 한방복합처방단 처리 4주 후 98 % 이상의 육모효과를 나타내는 것을 확인하였다. 이상의 결과로 볼 때 본 연구에서 개발한 한방복합처방단은 모발의 성장 촉진에 유용하게 활용될 수 있는 처방으로 사료된다.

**Abstract**: To develop new therapeutic materials to prevent hair loss and enhance hair growth, we developed a medicinal herbal complex extract (MHCE) using 23 herbs traditionally used in oriental medicine. Medicinal Herbal complex extract was consist of *Angelica gigas* Nakai, *Psoralea corylifolia* Linne, *Biota orientalis* Endlicher, and *Eclipta prostrata* Linne, *Rehmannia glutinosa* Liboschitz var. purpurea Makino, *Ligustrum lucidum* Aiton, *Polygonum multiflorum* Thunberg, and *Sesamum indicum* Linne, *Sophora angustifolia* Sieboldet Zuccarini, *Angelica dahurica* Benthamet Hooker, and *Leonurus sibiricus* Linne, *Salvia miltiorrhiza* Bunge, *Prunus persica* Batsch, *Commiphora molmol* Engler, *Chrysanthemum indicum* Linne, *Boswellia carterii* Birdwood, *Panax ginseng* C. A. Meyer, *Cnidium officinale* Makino, *Albizia julibrissin* Durazzini, and *Corydalis ternata* Nakai that have traditionally been used for treating hair loss, preventing gray hair, anti-inflammation, and blood circulation in oriental medicine. In addition, we examined the hair growth effect of MHCE *in vitro* and *in vivo*. *In vitro*, we evaluated the effects of MHCE on cultured HFDPC, HaCaT cells, and murine embryonal fibroblasts (NIH3T3 cells). Also, we evaluated the ability of MHCE to prevent gray hair on murine melanoma cells (B16F1 cells). The hair

† 주 저자 (e-mail: ksyoonjh@hanmail.net)

growth-promoting effect of MHCE *in vitro* was also observed *in vivo* using C57BL/6 mice. Our results showed that MHCE significantly increased the proliferation of HFDPC (175 % proliferation at 50 μg/mL), HaCaT cells (133 % proliferation at 20 μg/mL), and NIH3T3 cells (120 % proliferation at 50 μg/mL). MHCE also showed consistent melanogenesis in B16F1 cells (154 % melanin synthesis at 50 μg/mL). Moreover, MHCE showed potential for hair growth stimulation in C57BL/6 mice experiments (98 % hair growth area on 4 weeks). These results indicate that MHCE may be a good candidate for promotion of hair growth.

***Keywords:*** *medicinal herbal complex extract, hair growth, human follicle dermal papilla cell, NIH3T3 cell, C57BL/6 mice*

## 1. Introduction

The incidence of hair loss and thinning in both men and women has increased in recent decades due to nutritional imbalances and lifestyle changes [1,2]. Many substances have been used to treat alopecia. However, only two drugs, finasteride and minoxidil [2,3], have been approved for treatment of hair loss by the US Food and Drug Administration (FDA, Silver Spring, MD). Finasteride is a type II 5α-reductase inhibitor and minoxidil is a potassium channel opener. However, the use of these drugs for hair loss treatment is limited because of potential adverse effects : chest pain, dizziness, fainting, fast heartbeat [4,5]. Therefore, it is important to develop new therapeutic materials for preventing hair loss and enhancing hair growth.

Alternative medicine is a promising area for further research. Although alternative medicine has not yet been incorporated into mainstream medical care because of limited scientific evidence and lack of mechanistic understanding, it is becoming an increasingly attractive approach worldwide [6]. In particular, natural herbs have now emerged as appropriate for use in cosmetics, and about 1,000 plant extracts have been examined for treating hair loss. Natural herbs comprise a fast-growing segment with vast scope for future manifold expansion [7].

To develop new therapeutic materials to prevent hair loss and enhance hair growth, we designed a medicinal herbal complex extract (MHCE) using herbs traditionally used in oriental medicine. Firstly, we chose 4 medicinal herbs including *Angelica gigas* Nakai, *Psoralea corylifolia* Linne, *Biota orientalis* Endlicher, and *Eclipta prostrata* Linne. They have hair growth-promoting effects according to the traditional Korean herbal decoctions (Gukhwasan, Yiseonhwan, Samulgamrihwan, Sinseonohundan, and Kamichangchulgo). These decoctions are originally listed in Donguibogam, that is Korean traditional Medical Book [8]. Secondly, we added 7 medicinal herbs including *Lycium chinense* Miller, *Rubus coreanus* Miquel, *Morus alba* Linne, *Rehmannia glutinosa* Liboschitz var. purpurea Makino, *Ligustrum lucidum* Aiton, *Polygonum multiflorum* Thunberg, and *Sesamum indicum* Linne that have anti-graying hair properties according to the traditional texts [8-10]. Thirdly, because hair loss is associated with inflammation [11-13], we added 3 medicinal herbs (*Sophora angustifolia* Sieboldet Zuccarini, *Angelica dahurica* Benthamet Hooker, and *Leonurus sibiricus* Linne) that have traditionally been used as antiinflammatory agents [8-10]. Finally, since hair loss is related to deteriorated blood status [8,14], we have added 9 medicinal herbs (*Salvia miltiorrhiza* Bunge, *Prunus persica* Batsch, *Commiphora molmol* Engler, *Chrysanthemum indicum* Linne, *Boswellia carterii* Birdwood, *Panax ginseng* C. A. Meyer, *Cnidium officinale* Makino, *Albizia julibrissin* Durazzini, and *Corydalis ternata* Nakai) that have traditionally been used to promote blood circulation [8-10].

Hair growth and loss in mammalian species is controlled by a follicular cell cycle that includes periods of growth (anagen phase), regression (catagen phase), and rest (telogen phase) [15]. In the early anagen phase, dermal papillae are re-stimulated to produce

new hair shafts. Matrix bulb cells die by apoptosis in the catagen phase, and follicles remain in a resting state in the telogen phase until signaling factors activate them. These cyclic changes involve rapid remodeling of both the epithelial and dermal components of the hair follicles.

Dermal papilla cells (DPCs), a group of specialized fibroblasts within the hair follicle bulb, are located at the base of the hair follicle and have an essential function in inducting new hair follicles and controlling hair growth, both in the normal hair cycle and also in the pathogenesis of certain conditions such as androgenetic alopecia [16-18]. Studies have shown that the number of DPCs is increased in the anagen phase, and DPCs have been strong implicated as the source of regulatory stimuli for hair growth and development [19,20]. Therefore, factors affecting DPCs function are important in potential hair loss therapies and human follicle dermal papilla cells (HFDPC), human keratinocytes (HaCaT cells) are often used for hair growth studies [21,22].

The hair follicle is an organ with high rates of both keratinocyte proliferation and differentiation, leading ultimately to the production of the hair fiber. Thus, the development of new hair follicles as well as the maintenance of hair growth requires strong interaction between dermal papilla fibroblasts and epithelial keratinocytes [23].

Several animal models, particularly the mice have been used experimentally to evaluate the degree of hair growth [24]. Pigmented C57BL/6 mice are the most commonly used strain, as their truncal pigmentation is entirely dependent on follicular melanocytes. The truncal epidermis in these species lacks melanin-producing melanocytes and melanin production is strictly coupled to the anagen phase of hair growth. This strict coupling leads to characteristic changes in skin pigmentation during the anagen phase development [25-27]. In this study, we examined the hair growth effect of MHCE *in vitro* and *in vivo*. First, we evaluated the effects of MHCE on cultured HFDPC, HaCaT cells, and murine embryonal fibroblasts (NIH3T3 cells). Inaddition, we evaluated the ability of MHCE to prevent gray hair on murine melanoma cells (B16F1 cells). We tested whether MHCE stimulates the telogen-to-anagen transformation in C57BL/6 mice. Finally, we observed the increase in the number of developed hair follicles through histological examination.

## 2. Materials and Methods

### 2.1. Chemicals and Reagents

Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from HyClone Laboratories, Inc. (USA). Follicle dermal papilla cell growth medium was obtained from Promo Cell (Germany). Viscozyme L was obtained from Novozymes (Denmark). All other reagents were analytical or high performance liquid chromatography grade.

### 2.2. Preparation of MHCE

The 23 herbs constituting MHCE were purchased from GUmediherb (Korea) that is the special herb shop in Korea. Among them, *Angelica gigas* Nakai, *Psoralea corylifolia* Linne, *Biota orientalis* Endlicher, *Eclipta prostrata* Linne. *Lycium chinense* Miller, *Rubus coreanus* Miquel, *Rehmannia glutinosa* Liboschitz var. purpurea Makino, *Sesamum indicum* Linne, *Angelica dahurica* Benthamet Hooker, and *Leonurus sibiricus* Linne, *Prunus persica* Batsch, *Panax ginseng* C. A. Meyer, *Cnidium officinale* Makino, and *Albizia julibrissin* Durazzini were domestic and *Morus alba* Linne, *Ligustrum lucidum* Aiton, *Polygonum multiflorum* Thunberg, *Sophora angustifolia* Sieboldet Zuccarini, *Salvia miltiorrhiza* Bunge, *Commiphora molmol* Engler, *Chrysanthemum indicum* Linne, *Boswellia carterii* Birdwood, and *Corydalis ternata* Nakai were imported from China.

The prescription of the MHCE is shown in Table 1. The contents of the 23 herbs that make up the MHCE was determined according to previous patent [28]. And the preparation of MHCE was carried out as previously described by Im *et. al.* [29]. The medicinal herbal complex (100 g) was extracted twice with 75 % aqueous ethanol under reflux at 60 ~ 90 ℃ for 4 h, filtered, and evaporated under reduced pressure.

**Table 1.** Composition of the Medicinal Herbal Complex Extract

| Herbal name | Amount (g) |
|---|---|
| *Angelica gigas* Nakai | 1.75 |
| *Psoralea corylifolia* Linne | 2.75 |
| *Biota orientalis* Endlicher | 2.75 |
| *Eclipta prostrate* Linne | 2.75 |
| *Lycium chinense* Miller | 4.0 |
| *Rubus coreanus* Miquel | 6.0 |
| *Morus alba* Linne | 6.0 |
| *Rehmannia glutinosa* Liboschitz *var.purpurea* Makino | 4.0 |
| *Ligustrum lucidum* Aiton | 3.0 |
| *Polygonum multiflorum* Thunberg | 4.0 |
| *Sesamum indicum* Linne | 3.0 |
| *Sophora angustifolia* Siebold et Zuccarini | 16.0 |
| *Angelica dahurica* Bentham et Hooker | 12.0 |
| *Leonurus sibiricus* Linne | 12.0 |
| *Salvia miltiorrhiza* Bunge | 3.0 |
| *Prunus persica* Batsch | 3.0 |
| *Commiphora molmol* Engler | 2.0 |
| *Chrysanthemum indicum* Linne | 2.0 |
| *Boswellia carterii* Birdwood | 2.0 |
| *Panax ginseng* C. A. Meyer | 2.0 |
| *Cnidium officinale* Makino | 2.0 |
| *Albizia julibrissin* Durazzini | 2.0 |
| *Corydalis ternata* Nakai | 2.0 |
| Total | 100 |

The 75 % aqueous ethanol extract (75 % EtOH ext.) (3.27 g) was redissolved in 10 % aqueous ethanol and then defatted with *n*-hexane. The extract was hydrolyzed by Viscozyme L at 50 ~ 60 ℃ for 20 h and fractionated by ethyl acetate. Finally, we obtained MHCE (0.9 g).

### 2.3. Cell Culture

HFDPC, HaCaT cells, NIH3T3 cells, and B16F1 cells were obtained from PromoCell, Cosmax Inc. (Korea), the Korean Collection for Type Cultures (Korea), and the American Type Culture Collection (USA), respectively. HFDPC was maintained in follicle dermal papilla cell growth medium. HaCaT, NIH3T3, and B16F1 cells were maintained in DMEM supplemented with 10 % fetal bovine serum at 37 ℃ in humidified 5 % $CO_2$ incubator.

### 2.4. Animals

Male C57BL/6 mice (5 weeks of age) were purchased from Koatech (Korea) and were acclimated for one week in a breeding room. Temperature and humidity were maintained at 22 ± 2 ℃ and 50 ± 20 % respectively, and the light cycle was automatically maintained (12 h light/darkness). Autoclaved water and food (Samyang Corp., Korea) were provided freely. The study was approved by the Laboratory Animal Research Center Ethics Committee of Chungbuk National University (Approval No.: CBNUA-301-11-01) and all procedures were conducted in accordance with the Korea Food and Drug Administration guidelines.

### 2.5. Proliferation Assay of HFDPC, HaCaT, and NIH3T3 Cells

HFDPC, HaCaT, and NIH3T3 cells were maintained in 96-well culture plates ($1 \times 10^4$ cells/well) in the respective media and incubated at 37 ℃ under humidified 5 % $CO_2$. On the following day, the cells were incubated for 24 h in the presence of 75 % EtOH ext. and MHCE. 3-(4,5-dimethyl-thiazole-2-yl)-2,5-diphenyltetrazolim bromide (MTT; Sigma-Aldrich, USA) solution was then added and the cells were further incubated for 4 h at 37 ℃. The medium was discarded and the formazan blue formed in the cells was dissolved in dimethyl sulfoxide (DMSO). The optical density at 540 nm was determined using a microplate reader (Bio-Tek, USA).

### 2.6. Melanin Synthesis Assay on B16F1 Cells

B16F1 cells were incubated for 72 h in the presence of 75 % EtOH ext. and MHCE. After culturing, the melanin content was measured using a minor modification of a previously reported method [30]. Cells were detached by incubation in trypsin/ethylene diamine tetraacetic acid and cell pellets were solubilized in 1 *N* NaOH containing 10 % DMSO. Spectrophotometric analysis of melanin content was performed by

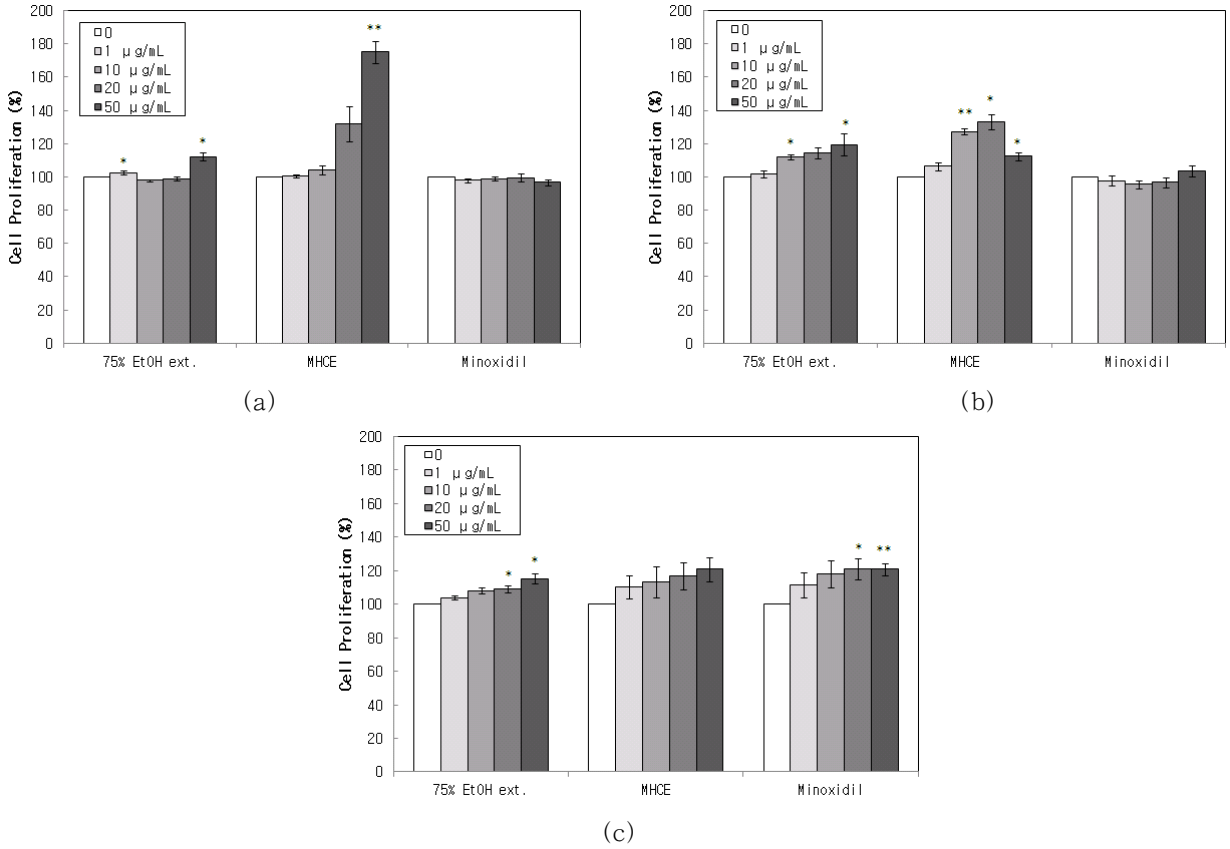


**Figure 1.** Effect of MHCE on proliferation of (A) HFDPC, (B) HaCaT cells, and (C) NIH3T3 cells. Results are means ± SE of triplicate measurements in separate experiments. Values are relative to the vehicle-treated control (0 $\mu$g/mL). *$p < 0.05$, **$p < 0.01$ indicate a significant difference from the vehicle-treated control.

quantifying absorbance at 490 nm. Three independent experiments were performed in triplicate.

### 2.7. Hair Growth Cctivity *in vivo*

The anagen phase was induced on the back skin of C57BL/6 mice that were in the telogen phase of the cycle by depilation, as described previously [31]. Briefly, five-week-old male C57BL/6 mice were allowed to adapt to their new environment for one week. The anagen phase was then induced in the back skin of the six-week-old male C57BL/6 mice by shaving, which led to synchronized development of the anagen hair follicles. Forty-eight animals in four randomized groups (n = 12) were used for the study. The animals in group 1 received an equal volume of the vehicle (75 % Ethanol) as a control group. Animals in group 2 received 3 % minoxidil in 75 % Ethanol as a positive control group. Animals in groups 3 and 4 received 0.1 % and 1 % MHCE in 75 % Ethanol, respectively. From the following day (day 1), 0.2 mL of the minoxidil, 0.1 % MHCE, 1 % MHCE and vehicle was topically applied to the back twice daily for 28 days. Hair growth promotion was evaluated by observing darkening of the skin color, indicating telogen-to-anagen conversion, and photographed with a digital camera at 0, 1, 2, 3, and 4 weeks after depilation.

### 2.8. Hair Growth Area

For quantitative comparison of hair growth, the image files for the photographs were used to calculate hair growth areas. Hair growth area was expressed as the percent area of hair growth of the total depilated area of the back using Image Analysis Software

(Image Partner; Saramsoft, Korea).

### 2.9. Histological Studies

One mouse from each group was euthanized at 2 and 4 weeks of the experiment. Skin biopsies were taken from the shaved area and fixed using 10 % neutral formalin solution and formatted with paraffin. The paraffin-formatted skin tissue was sliced to a thickness of 5 $\mu$m for hematoxylin and eosin (H&E) staining. The stained tissue was observed by optical microscopy.

### 2.10. Statistical Analysis

All values are expressed as the mean ± standard error (SE) from three independent experiments. The data were analyzed using a Student's t-test using SPSS (SPSS Inc., USA). The difference between the means was considered significant when $p < 0.05$.

## 3. Results

### 3.1. Hair Growth Activity *in vitro*

#### 3.1.1. Effects of MHCE on Cell Proliferation

MHCE significantly increased the proliferation of HFDPC compared with 75 % EtOH ext. and minoxidil, corresponding to 175 % increase at 50 $\mu$g/mL ($p < 0.01$). In aprevious study [22], minoxidil significantly induced proliferation of HFDPC at concentrations ranging from 0.01 ~ 1.0 $\mu$M, but minoxidil showed no proliferative effect in the present study (Figure 1A). MHCE significantly enhanced proliferation of HaCaT cells compared with the vehicle-treated control (133 % increase at 20 $\mu$g/mL); however, minoxidil showed no proliferative effect (Figure 1B). Boyera *et al.* [32] reported that minoxidil had a concentration-dependent biphasic effect on proliferation and differentiation of normal human keratinocytes; minoxidil stimulated keratinocytes proliferation at micromolar doses, while anti-proliferative, pro-differentiative, and partially cytotoxic effects were observed at millimolar concentration. MHCE and minoxidil increased cell proliferation of NIH3T3 cells in a dose-dependent manner (Figure 1C). The highest proliferative effect of MHCE was 120 % at 50 $\mu$g/mL.

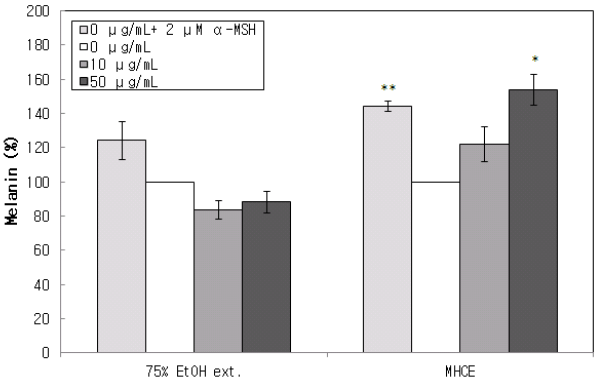


**Figure 2.** Effect of MHCE on melanogenesis in B16F1 cells. Results are means ± SE of triplicate measurements in separate experiments. Values are relative to the vehicle-treated control (0 $\mu$g/mL). *$p < 0.05$, **$p < 0.01$ are compared with the vehicle-treated control.

#### 3.1.2. Effects of MHCE on Melanogenesis

To determine the effect of MHCE on melanogenesis, B16F1 cells were treated for 3 days with 75 % EtOH ext. and MHCE. We also used $\alpha$-melanocyte stimulating hormone ($\alpha$-MSH) in the vehicle-treated group. Melanin production in the MHCE group was greater than that in the $\alpha$-MSH treated group, corresponding to 154 % increase at 50 $\mu$g/mL ($p < 0.01$). However, 75 % EtOH ext. had no effect on melanin production (Figure 2).

### 3.2. Effects of MHCE on the Anagen Phase Induction in C57BL/6 Mice

#### 3.2.1. Visible Changes in Hair Growth

To measure hair growth activity *in vivo*, we examined the effects of MHCE on C57BL/6 mice. The shaved skin of the telogen phase mice is pink and darkens with the anagen phase initiation. After being shaved, the skin color was observed to be pink. As shown in Figure 3, the depilated back skin was pink without any growth of hair for 1 week after depilation in all groups, and new hair began to appear at 2 weeks when the skin color was partially gray. The

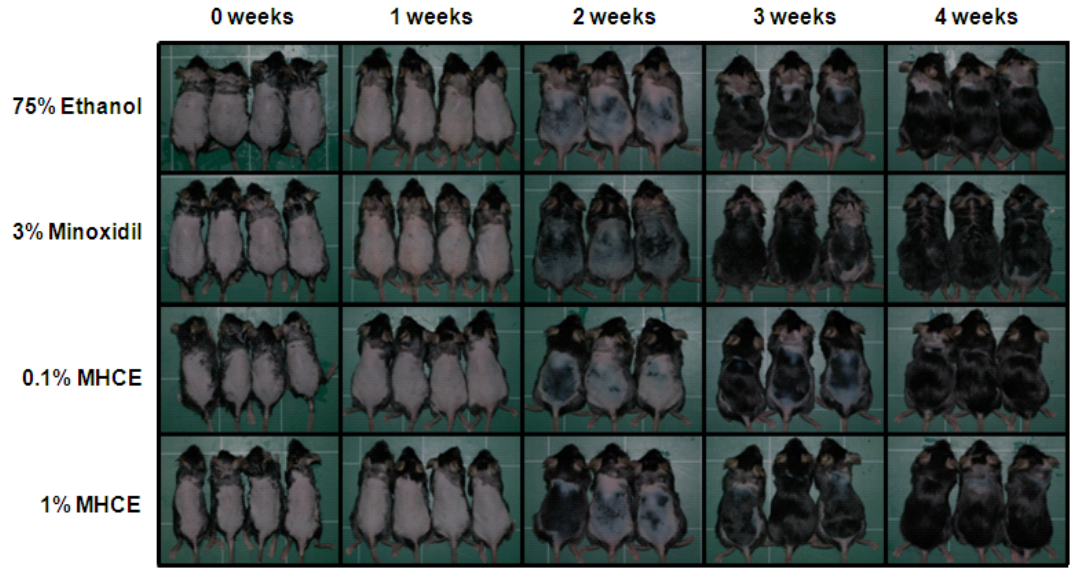


**Figure 3.** Effect of MHCE on the anagen phase induction in C57BL/6 mice. After shaving, the back skin was treated with MHCE every day for 4 weeks. The back skin was photographed at 0, 1, 2, 3, and 4 weeks after depilation. Hair growth area was increased in MHCE- and minoxidil-treated group compared to 75 % Ethanol-treated group.

skin areas with black hair in the 3 % minoxidil-treated group and the 1 % MHCE-treated group were larger than that of the 75 % Ethanol-treated group as a control. At 3 weeks, many new hairs from hair follicles were observed on the depilated back skin accompanied by a change in skin color to dark gray. Growth of hair was shown partially while some of the skin was still pink. At 4 weeks, the back skin was in the anagen phase in all mice. Most of the mouse in the control group did not grow hair at just below of the neck. On the other hand, that of other groups grew well. The skin area with black hair in the MHCE group was larger than that in the control group. Overall, these results indicate that MHCE induced early telogen-to-anagen conversion of hair follicles in the C57BL/6 mice.

### 3.2.2. Change in Hair Growth Area

In order to compare more quantitatively, the hair growth area was calculated using Image Analysis Software (Table 2). The hair growth area of the 1 % MHCE-treated group was 53.26 ± 15.33 %, similar to that of the 3 % minoxidil-treated positive control group (62.45 ± 8.29 %) and significantly higher than the 75 % Ethanol-treated control group (22.72 ± 9.44 %) at 2 weeks. At 4 weeks, the hair growth area of the 1 % MHCE-treated group was 97.82 ± 4.68 % compared to the control (78.16 ± 5.29 %), and that of the 3 % minoxidil-treated group was 98.93 ± 3.44 %. The hair growth area of the 1 % MHCE-treated group was markedly increased at 4 weeks compared to the control (Table 2, Figure 4). The hair growth area results for the 1 % MHCE-treated group and the 3 % minoxidil-treated group were similar.

**Table 2.** Hair Growth Area (%) in an Alopecia Model of C57BL/6 Male Mice Treated with of the 75 % Ethanol, 3 % Minoxidil, 0.1 % MHCE, and 1 % MHCE at 2 and 4 Weeks

| Group | Hair growth area (%) | | |
|---|---|---|---|
| | 0 Weeks | 2 Weeks | 4 Weeks |
| 75 % Ethanol | 0 | 22.72 ± 9.44 | 78.16 ± 5.29 |
| 3 % Minoxidil | 0 | 62.45 ± 8.29 | 98.93 ± 3.44 |
| 0.1 % MHCE | 0 | 32.46 ± 13.28 | 94.55 ± 8.27 |
| 1 % MHCE | 0 | 53.26 ± 15.33 | 97.82 ± 4.68 |

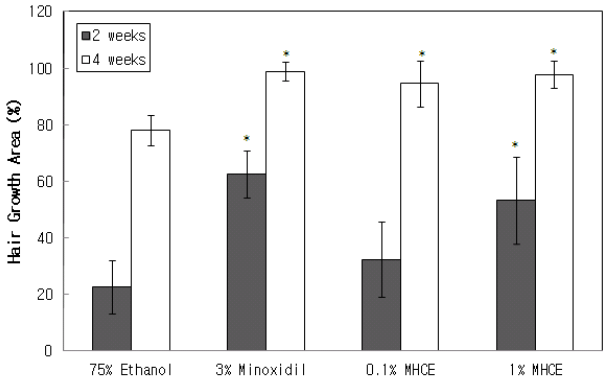


**Figure 4.** Hair growth area (%) in an alopecia model of C57BL/6 male mice treated with 75 % Ethanol, 3 % minoxidil, 0.1 % MHCE, and 1 % MHCE at 2 and 4 weeks. *$p < 0.001$ indicate a significant difference from the 75 % Ethanol.

### 3.2.3. Histological Examination of Hair Growth

H&E-stained skin tissues at 2 weeks and 4 weeks after depilation were used to observe the status of the hair follicles and hair growth by optical microscopy. At 2 weeks, the 3 % minoxidil- and 1 % MHCE-treated groups had well-developed hair follicles overall compared to the 75 % Ethanol as a control group (Figure 5). At 4 weeks after depilation, many fully grown hair follicles were observed in the 0.1 % and 1 % MHCE-treated group. On the other hand, the 3 % minoxidil-treated group did not show developed hair follicles. This result showed that the follicle activation activity of MHCE was greater than that of minoxidil. Histological studies showed that MHCE markedly increased the depth and size of the hair follicles compared to the 75 % Ethanol as a control treatment (Figure 5). This result supports the hypothesis that MHCE induces early onset of the anagen phase and stimulates hair growth.

## 4. Discussion

Hair loss or alopecia is a common patient complaint and a source of significant psychologic and physical distress [33]. Of the drugs currently approved for treatment, finasteride is a type II 5$\alpha$-reductase inhibitor originally used for treating prostatic hypertrophy [34], but later found to stimulate hair growth [35-37]. Minoxidil was originally synthesized as a potassium channel opener and was further developed as an anti-hypertensive. Moreover, it was found to stimulate growth of hair follicle cells *in vitro* [38] and to have hair cycle converting activity *in vivo* [39]. Recently, Han *et al.* [40] reported that minoxidil has proliferative and anti-apoptotic effects on dermal papilla cells. However, the uses of finasteride and minoxidil have been limited due to potentialad verse effects. Therefore, it is important to develop novel therapeutic materials using the natural traditional medicines.

In this study, we have developed the MHCE using herbs traditionally used in oriental medicine on the basis of traditional book. Moreover we have investigated the hair growth promoting effects of MHCE *in vitro* and *in vivo*. To investigate the effects of MHCE on cell growth in hair follicles, we examined

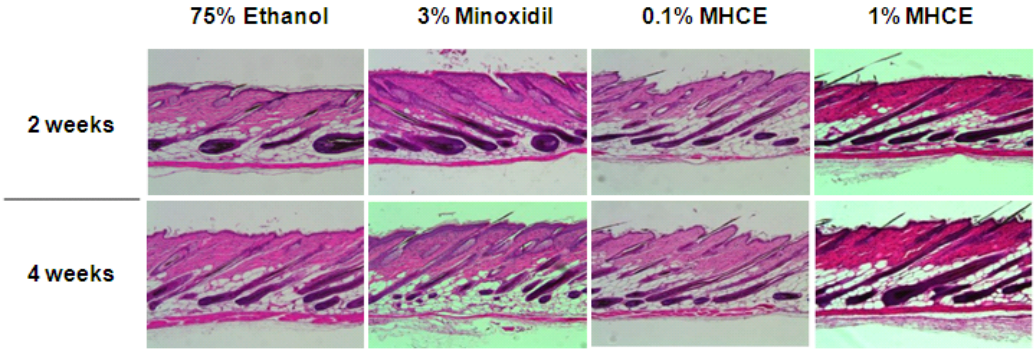


**Figure 5.** Representative histological observations of hair growth in an alopecia model of C57BL/6 male mice treated with 75 % Ethanol, 3 % minoxidil, 0.1 % MHCE, and 1 % MHCE at 2 and 4 weeks.

whether MHCE influenced the proliferation of HFDPC and HaCaT cells. MHCE significantly increased the growth of HFDPC and HaCaT cells. These results suggest that the hair growth promoting effect of MHCE may be mediated through mitogenic effects that occur in the dermal papilla region. Modulation of the balance of proliferation and apoptosis in the dermal papilla may be a key strategy for the control of hair growth and regression [40]. It has been reported that minoxidil stimulates hair growth as a result of the mitogenic effect on NIH3T3 fibroblasts by opening potassium channels *in vitro* [41-44]. To confirm this effect, we determined the proliferation effect of MHCE on NIH3T3 fibroblast. MHCE increased NIH3T3 cell proliferation as minoxidil in a dose-dependent manner. In addition, to determine whether MHCE has a preventative effect against gray hair, we tested its effect on melanin synthesis using B16F1 cells. MHCE increased melanin production significantly over the control and $\alpha$-MSH-treated cells.

The hair growth-promoting effect of MHCE *in vitro* was also observed *in vivo* using C57BL/6 mice. Through this animal test, we measured visible changes in hair growth status, hair growth area, and histological changes in the hair follicles. Experimental results indicated that MHCE induced early telogen-to-anagen conversion of hair follicles in the C57BL/6 mice. The hair growth area of the 1 % MHCE-treated group was slightly lower than that of the 3 % minoxidil-treated positive control group, but was significantly higher than that of the control group. On the other hand, the 3 % minoxidil-treated group experienced substantial skin cell death and inflammation. This inflammation appeared to affect the hair shine after hair growth. Histological changes in the hair follicles were observed by the H&E staining of skin tissues treated with MHCE after depilation. Many fully grown hair follicles were observed in the MHCE-treated group. Overall, MHCE markedly increased the depth and size of the hair follicles, compared to the control treatment. However, the 3 % minoxidil-treated group did not show the developed hair follicles and the cytotoxicity was observed. Thus, the follicle activation activity of MHCE appeared to be greater than that of minoxidil. Taken together, the results showed that the hair growth effect of MHCE is slightly lower than that of minoxidil; however, a comprehensive review of follicle cell growth, effects on enrichment of hair, and safety indicates some advantages of MHCE over minoxidil.

In conclusion, the results of this study demonstrate that MHCE promotes the hair growth *in vitro* via proliferation of dermal papilla cells, keratinocytes, and NIH3T3 cells. In addition, MHCE has a gray hair preventative effect via increase of melaninsynthesis in B16F1 melanoma cells. Moreover, MHCE promotes hair growth by inducing the telogen-to-anagen transformation *in vivo*. These results indicate that MHCE may be a good candidate for promotion of hair growth. However, the mechanisms by which MHCE promotes these effects remain to be elucidated, and are currently under further investigation in our laboratory.

## Acknowledgements

This work was financially supported by the Small and Medium Business Administration of Korea in 2010 (S1074059).

## References

1. K. D. Kaufman, E. A. Olsen, and D. Whiting, Finasteride in the treatment of men with androgenetic alopecia. Finasteride Male Pattern Hair Loss Study Group, *J. Am. Acad. Dermatol.*, **39**, 578 (1998).
2. M. Kerscher, S. Williams, and L. Dubertret, Cosmetic dermatology and skin care, *Eur. J. Dermatol.*, **17**, 180 (2007).
3. J. L. Burton and A. Marshall, Hypertrichosis due to minoxidil, *Br. J. Dermatol.*, **101**, 593 (1979).
4. S. Murad, L. C. Walker, S. Tajima, and S. R. Pinell, Minimal structural requirements for minoxidil inhibition of lysyl hydroxilase in cultured fibroblasts, *Arch. Biochem. Biophys.*, **308**(1), 42

(1994).
5. V. H. Price, Treatment of hair loss, *New Engl. J. Med.*, **341**, 964 (1999).
6. S. Bhaumik, M. D. Jyothi, and A. Khar, Differential modulation of nitric oxide production by curcumin in host macrophages and NK cells, *FEBS. Lett.*, **483**, 78 (2000).
7. V. Rathi, J. C. Rathi, S. Tamizharasi, and A. K. Pathak, Plants used for hair growth promotion: a review, *Phcog. Rev.*, **2**, 185 (2008).
8. J. Huh, Donguibogam, 78, 307, 449, 738, Namsandang, Seoul (1987).
9. D. K. An, Illustrated Book of Korean Medicinal Herbs, Kyohak Publishing Co. Ltd., Seoul (1998).
10. G. S. Moon, The Ingredients and Use of Medicinal Herb, Ilweolseogak, Seoul (1999).
11. R. L. DeVillez, Infectious, physical, and inflammatory causes of hair and scalp abnormalities, ed. E. A. Olsen, 71, McGraw-Hill Inc., New York (1994).
12. R. Ruckert, G. Lindner, S. Bulfone-Paus, and R. Paus, High-dose proinflammatory cytokines induce apoptosis of hair bulb keratocytes *in vivo*, *Br. J. Dermatol.*, **143**, 1036 (2000).
13. R. M. Trueb, Molecular mechanism of androgenetic alopecia, *Exp. Gerontol.*, **37**, 981 (2002).
14. B. Y. Chae, Oriental Medical Sergery, 285, Komoonsa, Seoul (1971).
15. E. Fuchs, Scratching the surface of skin development, *Nature*, **445**, 834 (2007).
16. S. Inui, Y. Fukuzato, T. Nakajima, K. Yoshkawa, and S. Itami, Identification of androgen-inducible TFG-beta 1 derived from dermal papilla cells as a key mediator in androgenetic alopecia, *J. Investig. Dermatol. Symp. Proc.*, **8**, 69 (2003).
17. C. A. B. Jahoda and A. J. Reynolds, Induction of follicle formation and hair growth by vibrissa dermal papillae implanted into rat ear wounds: vibrissae-type fibers are secified, *Development.*, **115**, 1103 (1992).
18. R. F. Oliver, The induction of hair follicle formation in the adult hooded rat by vibrissae dermal papillae, *J. Embryol. Exp. Morphol.*, **23**, 219 (1970).
19. K. Eliott, T. J. Stephenson, and A. G. Messenger, Differences in hair follicle dermal papilla volume are due to extracellular matrix volume and cell number: implications for the control of hair follicle size and androgen responses, *J. Invest. Dermatol.*, **13**, 873 (1999).
20. L. Ibrahim and E. A. Wright, A quantitative study of hair growth using mouse and rat vibrissal follicles. I. Dermal papilla volume determines hair volume, *J. Embryol. Exp. Morphol.*, **72**, 209 (1982).
21. S. S. Roh, C. D. Kim, M. H. Lee, S. L. Hwang, M. J. Rang, and Y. K. Yoon, The hair growth promoting effect of *Sophora flavescens* extract and its molecular regulation, *J. Dermatol. Sci.*, **30**(1), 43 (2002).
22. H. G. Yoo, I. Y. Chang, H. K. Pyo, Y. J. Kang, S. H. Lee, O. S. Kwon, K. H. Cho, H. C. Eun, and K. H. Kim, The additive effects of minoxidil and retinol on human hair growth *in vitro*, *Biol. Pharmaceut. Bull.*, **30**, 21 (2007).
23. K. S. Stenn and R. Paus, Control of hair follicle cycling, *Physiol. Rev.*, **81**, 449 (2001).
24. M. Hattori and H. Ogawa, Biochemical analysis of hair growth from the aspects of aging and enzyme activities. *J. Dermatol.*, **10**, 45 (1983).
25. A. Slominski, R. Paus, and R. Costantino, Differential expression and activity of melanogenesis related proteins during induced hair growth in mice, *J. Invest. Dermatol.*, **96**, 172 (1991).
26. A. Slominski and R. Paus, Melanogenesis is coupled to murine anagen:toward new concepts for the role of melanocytes and the regulation of melanogenesis in hair growth, *J. Invest. Dermatol.*, **101**, 90S (1993).
27. A. Slominski, R. Paus, P. Plonka, and A. Chakraborty, Melanogenesis during the anagen-catagen-telogen transformation of the murine hair cycle, *J. Invest. Dermatol.*, **102**, 862 (1994).
28. K. R. Patent 1008228350000 (2007).
29. K. R. Im, M. J. Kim, and K.-S. Yoon, Hair Growth Activity and Melanogenic Activity of Oriental Medical Prescription, *J. Soc. Cosmet. Scientists*

*Korea*, **37**(2), 161 (2011).
30. P. R. Gorden, C. P. Mansur, and B. A. Gilchrest, Regulation of human melanocyte growth, dendricity, and melanization by keratinocyte derived factors, *J. Invest. Dermatol.*, **92**, 565 (1989).
31. S. Müller-Röver and B van der Veen C. Handjiski, A comprehensive guide for the accurate classification of murine hair follicles in distinct hair cycle stages, *J. Invest. Dermatol.*, **117**, 3 (2001).
32. N. Boyera, I. Galey, and B. A. Bernard, Biphasic effects of minoxidil on the proliferation and differentiation of normal human keratinocytes, *Skin Pharmacol.*, **10**, 206 (1997).
33. A. Han and P. Mirmirani, Clinical approach to the patient with alopecia. *Semin. Cutan. Med. Surg.*, **25**, 11 (2006).
34. G. J. Gromley, Finasteride: a clinical review, *Biomed. Pharmacother.*, **49**, 319 (1995).
35. K. D. Kaufman, D. J. Girman, E. M. Round, A. O. Johnson-Levonas, A. K. Shah, and J. Rotonda, Progression of hair loss in men with androgenetic alopecia (male pattern hair loss): long-term (5-year) controlled observational data in placebo-treated patients, *Eur. J. Dermatol.*, **18**, 407 (2008).
36. K. D. Kaufman, J. Rotonda, A. K. Shah, and A. G. Meehan, Long-term treatment with finasteride 1 mg decreases the likelihood of developing further visible hair loss in men with androgenetic alopecia (male pattern hair loss), *Eur. J. Dermatol.*, **18**, 400 (2008).
37. K. J. McClellan and A. Markham, Finasteride: a review of its use in male pattern hair loss, *Drugs*, **57**, 111 (1999).
38. N. Tanigaki-Obana and M. Ito, Effects of cepharanthine and minoxidil on proliferation, differentiation and keratinization of cultured cells from the murine hair apparatus, *Arch. Dermatol. Res.*, **284**, 290 (1992).
39. H. Uno, A. Cappas, and C. Schlagel, Cyclic dynamics of hair follicles and the effect of minoxidil on the bald scalps of stumptailed macaques, *Am. J. Dermatopath.*, **7**(3), 283 (1985).
40. J. H. Han, O. S. Kwon, J. H. Chung, K. H. Cho, H. C. Eun, and K. H. Kim, Effect of minoxidil on proliferation and apoptosis in dermal papilla cells of human hair follicle, *J. Dermatol. Sci.*, **34**, 91 (2004).
41. A. E. Buhl, D. J. Waldon, S. J. Conrad, M. J. Mulholland, K. L. Shull, M. F. Kubicek, G. A. Johnson, M. N. Brunden, K. J. Stefanski, and R. G. Stehle, Potassium channel conductance: a mechanism affecting hair growth both *in vitro* and *in vivo*, *J. Invest. Dermatol.*, **98**, 315 (1992).
42. C. S. Harmon, D. Lutz, and J. Ducote, Potassium channel openers stimulate DNA synthesis in mouse epidermal keratinocyte and whole hair follicle cultures, *Skin Pharmacol.*, **6**, 170 (1993).
43. Y. Nakaya, H. Hamaoka, S. Kato, and S. Arase, Effect of minoxidil sulfate and pinacidil on single potassium channel current in cultured human outer root sheath cells and dermal papilla cells, *J. Dermatol. Sci.*, **7**(suppl), S104 (1994).
44. D. A. Sanders, I. Fiddes, D. M. Thompson, M. P. Philpott, G. E. Westgate, and T. Kealey, In the absence of streptomycin, minoxidil potentiates the mitogenic effects of fetal calf serum, insulin-like growth factor 1, and platelet-derived growth factor on NIH3T3 fibroblasts in a K+ channel-dependent fashion, *J. Invest. Dermatol.*, **107**, 229 (1996).