Introduction

Hair has the primary defensive functions of protecting and buffering against external physical shocks, sunlight and cold along with the functions of absorbing heavy metals to excrete outside the body\(^1\). There are various causes of hair loss, including genetic causes, endocrinologic causes (especially excessive male hormone), psychological stresses, hemodynamic disorders, nutritional unbalance, environmental contamination (atmospheric contamination, excessive use of chemical agents for scalp and so on), several diseases including autoimmune diseases and certain drugs. Some of these causes have become
more problematic with industrialization. Hair loss is considered to be an independent disease that can be accompanied with physical, psychological and social problems.\(^2\)\(^-\)\(^4\) For the current study, we designed a preparation with the aim of both protecting against hair loss and promoting hair growth. This preparation comprised 11 components: Polygoni Multiflori Radix, Lycii Radicis Cortex, Rehmanniae Radix, Rehmanniae Radix Preparat, Asparagi Radix, Liriopis Tuber, Lycii Fructus and Acori Graminei Rhizoma, that have been described in Yonryunggobon-dan\(^5\),\(^6\) and Yonnyuniksoobulro-dan\(^6\) and the agents of Angelicae Gigantis Radix\(^6\) and pine needle\(^6\), whose effects on hair loss protection have been reported. The preparation was named Yonnyuniksoogobon-dan and its stimulative effect on hair growth was examined.

The effects of Yonnyuniksoogobon-dan on hair growth were observed experimentally by histologically assessing the hair growth status of depilated C57BL/6 mice and by immunohistologically examining the expression levels of proteins involved in hair growth of these mice. These proteins were epidermal growth factor (EGF) and transforming growth factor-\(\beta\) (TGF-\(\beta\)), which are involved in the growth of hair follicles and hair, and vascular endothelial growth factor (VEGF) and inducible nitric oxide synthase (iNOS), which are involved in the supply of blood to the surrounding tissue of hair follicles. The significant results are reported here.

### Materials and Methods

1. **Experimental animals**

36 female C57BL/6 mice aged 5 weeks were purchased from Samtako Co. (Korea) and were used after one week of acclimation in a breeding room. Temperature and humidity were maintained at 21-23\(^\circ\)C and 40-60% respectively, and the light cycle was automatically maintained (12 hour light/darkness). Autoclaved water and food (Samyang Co., Korea) were provided freely. The study was approved by the Institutional Animal Care and Use Committee of Kyung Hee University and all procedures were conducted in accordance with the U.S. National Institutes of Health guidelines.

2. **Preparation of medication**

The contents of the Yonnyuniksoogobon-dan preparation are described in Table 1. Approximately

<table>
<thead>
<tr>
<th>Herbal medicine</th>
<th>Scientific name</th>
<th>Medical Use</th>
<th>Dosage (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polygoni Multiflori Radix</td>
<td>Pleuropterus multiflorus</td>
<td>lumpy root</td>
<td>16.0</td>
</tr>
<tr>
<td>Lycii Radicis Cortex</td>
<td>Lycium chinense MILL L.</td>
<td>dry bark vel·a·men·tous</td>
<td>10.0</td>
</tr>
<tr>
<td>Polia</td>
<td>Poria cocos Wolf.</td>
<td>sclerotium</td>
<td>10.0</td>
</tr>
<tr>
<td>Rehmanniae Radix</td>
<td>Rehmannia glutinosa (GAERTN.)</td>
<td>unripe root</td>
<td>6.0</td>
</tr>
<tr>
<td>Rehmanniae Radix Preparat</td>
<td>Rehmannia glutinosa Liboschitz var. purpurea Makino</td>
<td>ripe root</td>
<td>6.0</td>
</tr>
<tr>
<td>Asparagi Radix</td>
<td>Asparagus cochin chinensis (Lour.) Merr</td>
<td>lumpy root</td>
<td>6.0</td>
</tr>
<tr>
<td>Liriopis Tuber</td>
<td>Liriophe platyphylla Wang et Tang</td>
<td>lumpy root</td>
<td>6.0</td>
</tr>
<tr>
<td>Lycii Fructus</td>
<td>Liriophe platyphylla Wang et Tang</td>
<td>fruit</td>
<td>4.0</td>
</tr>
<tr>
<td>Acori Graminei Rhizoma</td>
<td>Acorus gramineus SOLAND.</td>
<td>lumpy root</td>
<td>4.0</td>
</tr>
<tr>
<td>Angelicae Acutiloba Radix</td>
<td>Angelica gigas Nakai</td>
<td>root</td>
<td>4.0</td>
</tr>
<tr>
<td>Pini Folium</td>
<td>Pinus densiflora Siebold. et Zuccarini</td>
<td>leaf</td>
<td>4.0</td>
</tr>
</tbody>
</table>

| Total | | | 76.0 |
760g, the equivalent of 10 packs of medication, was boiled for 2 hours with 4,000 ml of water in a decocting machine that was loaded with a condenser. The boiled solution was filtered before vacuum concentration with a rotary evaporator and freeze drying to acquire 94.8g of powered extract. The amount of medication administered per dose was 10 times the calculated body weight ratio amount of 15.8 mg/10g body weight. This amount was diluted in 0.2 ml drinking water and administered orally once a day for 12 days.

3. Depilation and Classification of experiment groups

To observe hair growth, the black hair on the back of 6 week old C57BL/6 mice was cut using an animal hair clipper, any remaining hair was completely removed using NICLEAN (Ildong Pharmaceutical, Korea), and the skin was cleaned. The experimental groups were Control - orally administered physiological saline solution, Group I - orally administered Yonnyuniksoogobon-dan, and Group II - orally administered Yonnyuniksoogobon-dan plus skin application of Yonnyuniksoogobon-dan. 12 mice were assigned to each group, and the oral administration of the medication or saline and skin application were performed at 10:00 every morning for 12 days starting on the day after the depilation.

4. Observation of hair growth

Hair growth status was examined by lightly anesthetizing the mice with 2% isoflurane on days 1, 5, 7, 9 and 12. The image of the back of each mouse was photographed with a digital camera (EOS-10D, Canon, Japan). The tail of each mouse was marked with different colored oil based pens to distinguish between them.

5. Measurement of hair regrowth index (HRG index)

For the quantitative comparison of hair growth status, the back of each mouse was photographed with a digital camera and the image was inputted to a computer for measurement using the “ImageJ” software of NIH (ver. 1.36). The HRG index was calculated using the melanin forming phenomenon of the C57BL/6 mice that correlates to the hair growth stage whereby the skin color changes from

![HRG Index](image)

Fig. 1. Representative sections of scores (A) and example of hair regrowth index (HRG index) calculated with area percentages and scores (B).
pink to gray, dark gray, and then black. Pink skin color of the depilated mouse was ranked as 4th grade and assigned a score of 0; gray skin color signifying the start of hair growth in hair follicles was ranked as 3rd grade with a score of 1; dark gray skin color when hair can first be seen outside of the skin with the naked eye was ranked as 2nd grade and assigned 2 points; and when the hair color became completely black as seen with the naked eye the ranking was 1st grade with a score of 3 points (Fig. 1-A). The ratio between the area with hair growth and total depilated area of the back was measured for each grade and expressed as a percentage, and this value was multiplied by the score assigned to that grade to give the HRG index. In this way, the HRG index had a minimum value of 0% and a maximum value of 300% (Fig. 1-B).

6. Treatment of skin tissue

On the 12th day of the experiment, the animals were sacrificed by anaesthetizing with an excessive amount of sodium pentobarbital, and some of back skin tissue was collected to freeze dry with dry ice-isopentane. 20 m sections were prepared to be used for immunohistochemical staining, and remaining tissue was fixed using 10% neutral formalin solution and dehydrated after washing to be formatted with paraffin. The paraffin-formatted skin tissue was sliced to a thickness of 8 m for hematoxyline and eosin (H&E) staining.

7. Immunohistochemical staining

After washing the freeze-dried skin tissue sections 3 times for 5 minutes with 0.05 M PBS, the sections were reacted with 1% H2O2 for 10-15 minutes and then washed 3 times before blocking with 10% normal horse serum ( Vectastain) and bovine serum albumin (Sigma) in PBS for 1 hour. After washing 3 times with PBS the sections were treated with the appropriate primary antibody. The primary antibodies used were anti-EGF (1:200, sc-1342, Santa Cruz, USA), anti-TGF-β1 (1:100, sc-146, Santa Cruz, USA), anti-iNOS (1:200, 610329, R&D Bioscience, USA), and anti-VEGF (1:100, sc-152, Santa Cruz, USA), diluted with PBS and Triton X-100 mixed solution and incubated for 12 hours at 4°C. After the reaction, each tissue section was washed with PBS and reacted for 1 hour with avidin-biotin immunoperoxidase according to the manufacturer’s instructions (ABC Vectastain Kit). EGF and iNOS expression in the tissue sections was visualized by chromogenic reaction with diaminobenzidine-tetrachloride (Sigma, USA) for 5-10 minutes. TGF-β1 and VEGF expressions was visualized by reacting with diaminobenzidine-tetrachloride mixed with NiCl2 (Sigma, USA) for 5-10 minutes. The reacted tissue sections were attached to poly-L-lysine coated slides and dried for 2-3 hours, then dehydrated and sealed to prepare the tissue samples. The immunohistochemical staining was observed by optical microscopy to evaluate the whole tissue. Unclear or weak staining was graded as ±, a weak positive reaction was graded as +, a medium positive reaction was graded as ++, and strong expression was graded as +++.

8. Statistical analysis

The experimental results were analyzed using SPSS® for Windows (version 10.0, SPSS, Inc., Chicago, U.S.A.) to perform one way ANOVA tests to compare the index value variation by the concentration of medication, and a P value <0.05 was considered to be significant. Scheffe’s test was performed as the post hoc test.

Results

1. Visible changes in hair growth status

In mice in the Control group, the depilated back skin was pink without any growth of hair until the 5th day after the depilation (Fig. 2, Control, 1st day), and new hair started to show on the 7th day when the skin color was partially gray, though parts of the skin were still pink (Fig. 2, Control, 7th day). On the 9th day, the growth of many new hairs from hair follicles was observed on the depilated back
skin overall accompanied with a change of skin color to dark gray. Growth of hair outside of the skin could be seen in parts while some of the skin was still pink (Fig. 2, Control, 9th day). On the 12th day, the full growth of black colored hair could be seen on part of the skin (Fig. 2, Control, 12th day). Group I (oral administration of *Yonnyuniksoogobon-dan*) showed similar hair growth as the Control up to and including the 9th day, though the area of skin changed to gray was larger than that in the Control group (Fig. 2, Sample I, 7th day, 9th day). On the 12th day, the area of skin with growth of black hair outside of the skin was larger than that of the Control group (Fig. 2, Sample I, 12th day). Group II (combined oral administration and skin application of *Yonnyuniksoogobon-dan*) showed a similar hair growth promotion pattern to that of Group I, with no significant difference between the two treatment groups (Fig. 2, Sample II).

### 2. Change of HRG index

The HRG index of the Control group was 0% on the 1st day, 61.1 ± 3.3% on the 7th day, 122.5 ± 13.0% on the 9th day, and 219.2 ± 15.0% on the 12th day. In comparison, Group I (orally administered *Yonnyuniksoogobon-dan*) had HRG indices

![Image](image_url)

**Fig. 2.** Representative sections of shaved C57BL/6 mice for 12 days after shaving. *Yonnyuniksoogobon-dan* administration (Group I) and *Yonnyuniksoogobon-dan* administration plus skin application (Group II) accelerated hair regrowth of the shaved C57BL/6 mice.

<table>
<thead>
<tr>
<th>Group</th>
<th>Days after shaving</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>1(n=12)</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
</tr>
<tr>
<td>Group I</td>
<td>0</td>
</tr>
<tr>
<td>Group II</td>
<td>0</td>
</tr>
</tbody>
</table>

Data presented as mean ± standard error.

Control: treated with saline after shaving

Group I: treated with *Yonnyuniksoogobon-dan* orally after shaving

Group II: treated with *Yonnyuniksoogobon-dan* orally plus skin application after shaving

Statistical significance was verified with repeated ANOVA among groups (*: P<0.05; **: P<0.01).
of 0% on the 1st day, 75.4 ± 5.5% on the 7th day, 180.0 ± 10.6% on the 9th day, and 268.3 ± 10.2% on the 12th day. Group I had significantly higher HRG indices than Control from the 7th day to 12th day (P<0.05, P<0.01). Group II (combined oral administration and skin application of Yonnyuniksoogobon-dan) had HRG indices of 0% on the 1st day, 69.6 ± 6.6% on the 7th day, 158.3 ± 18.7% on the 9th day, and 259.2 ±8.3% on the 12th day. The HRG index was only significantly higher compared to the Control (P<0.05) on the 12th day (Table 2, Fig. 2).

3. Histological examination of hair growth

H&E stained skin tissues from the 7th and 12th day after depilation were used to observe the status of hair follicle and hair growth by optical microscopy. On the 7th day, the Control group had partial weak development of hair roots with areas without enough inner root sheath development (Fig. 4-1). In comparison, Group I and Group II had well-developed hair follicles and inner root sheath overall (Fig. 3-A, 3-B) and an increased growth of hairs compared to the Control group (Fig. 3-B-1, 3, 5). On the 12th day after depilation, while many fully grown hair follicles were observed in all three groups, the

![Fig. 3. Representative skin sections of shaved C57BL/6 mice.](image)

(A) Longitudinal section of hair follicles. Group I (oral Yonnyuniksoogobon-dan administration) and Group II (oral Yonnyuniksoogobon-dan administration plus skin application) demonstrated histological improvement of the hair follicles. (B) Horizontal section of hair follicles. Group I and Group II demonstrated increases in number and diameter of the hair follicles. Sections are stained with H&E. Scale bar in section 6 is 200 μm.

### Table 3. Effect of Yonnyuniksoogobon-dan on EGF, TGF-β1, VEGF, and iNOS Expression of Shaved C57BL/6 Mice

<table>
<thead>
<tr>
<th>Groups</th>
<th>Immuno-reactivity</th>
<th>Control</th>
<th>Group I</th>
<th>Group II</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EGF</td>
<td>TGF-β1</td>
<td>VEGF</td>
<td>iNOS</td>
</tr>
<tr>
<td>Control</td>
<td>+</td>
<td>+</td>
<td>+(~5)</td>
<td>+++</td>
</tr>
<tr>
<td>Group I</td>
<td>+++/+/-</td>
<td>+/-</td>
<td>++/(10~)</td>
<td>+</td>
</tr>
<tr>
<td>Group II</td>
<td>+++/+/-</td>
<td>+/-</td>
<td>++/(10~)</td>
<td>+</td>
</tr>
</tbody>
</table>

Control: treated with saline after shaving
Group I: treated with Yonnyuniksoogobon-dan orally after shaving
Group II: treated with Yonnyuniksoogobon-dan orally plus skin application after shaving

Immuno-densities are demonstrated as ±, weak; +, mild; +++, moderate; and ++++, strong.
cross sectional area of hairs from Group I and Group II was thicker. There were a greater number of developed hair follicles with more inner root sheaths and outer root sheaths in the two treatment groups than in the Control group (Fig. 3-A-2, 4, 6; Fig. 3-B-2, 4, 6).

4. EGF expression

On the 12th day after depilation, the EGF response of skin tissue was observed by immunohistochemical staining. The Control group showed a weak (+ or +) positive response at the grown hairs of certain hair thickness and at the outer root sheath of thin vellus hairs, but a negative response was observed at the inner root sheath (Fig. 4-A-1, 2, Table 3). Group I showed a medium level (+++) of positive response at the outer root sheath of fully grown hair of a certain thickness, and a very strong (+++) EGF positive response was observed at the outer root sheath of newly growing fine hairs (Fig. 4-A-3, 4). Group II had a similar pattern of EGF expression as Group I (Fig. 4-A-5, 6, Table 3).

5. TGF-β1 expression

On the 12th day, the TGF-β1 response of skin tissue was observed by immunohistochemical staining. The Control group showed a weak (+) positive response at the inner root sheath of fully grown hair of a certain thickness, and the few hairroots had a very strong (+++) positive response. The inner out outer root sheaths at the hair roots of thin vellus hairs had weak (+ or +) positive responses (Fig. 4-B-1, 2, Table 3). Group I and Group II mice had similar expressions of TGF-β1 to the Control, though some cross sectional hair roots had decreased expression of TGF-β1 (Fig. 4-B-3, 4, 5, 6, Table 3).

6. VEGF expression

The immunohistochemical staining for VEGF of the skin on the 12th day was observed. The Control group had approximately 3-7 VEGF expressing cells in peripheral tissues of hair follicles and hair roots examined at 400 X magnification (Fig. 5-A-1, 2, Table 3). In contrast, Group I had 10-15 VEGF
expressing cells in peripheral tissues of hair follicles and hair roots, so had increased expression of VEGF compared to the Control (Fig. 5-A-3, 4, Table 3). Group II had more than 10 VEGF expressing cells, so also demonstrated an increase in VEGF expression compared to the Control group (Fig. 5-A-5, 6, Table 3).

7. iNOS expression

Immunohistochemical staining for iNOS in the skin on the 12th day demonstrated strong (+++) iNOS expression in the peripheral tissues of hair follicles and hair roots of the control group (Fig. 5-B-1, 2, Table 3). In contrast, Group I had medium level (+ or ++) iNOS expression at the peripheral tissues of hair follicles and hair roots so had decreased expression compared to Control (Fig. 5-B-3, 4, Table 3). Group II had a similar iNOS expression pattern to Group I (Fig. 5-B-5, 6, Table 3).

![Fig. 5. Representative sections of VEGF and iNOS immuno-reacted skin tissues of shaved C57BL/6 mice at 12 days. (A) VEGF, and Group I (oral Yonnyunikoóbón-dan administration) and Group II (oral Yonnyunikoóbón-dan administration plus skin application) demonstrated up-regulation of VEGF expression in the surrounding tissues of hair follicles. Red arrowheads indicate VEGF immuno-positive cells. (B) iNOS, Group I and Group II demonstrated down-regulation of iNOS expression in the surrounding tissues of hair follicles. Red arrowheads indicate iNOS expressions. Scale bar in section 6 is 100 μm.](image)

**Discussion**

Human hair grows in cycles, with periods of growth and shedding over several months or even 2-4 years in some cases. Generally, this hair growth cycle can be classified into the anagen period with the most active growth, the catagen period when the degeneration of hair starts and the telogen period when the growth of hair is stopped or inactivated. Hair loss is defined as the incidence of excessive hair shedding compared to normal due to hair growth abnormalities. Genetic causes, excessive secretion of male hormone, psychological stresses, hemodynamic disorders, nutritional disorders, aging, environmental contamination and dyeing have been shown to be causes of hair loss. Hair loss is also classified as an autoimmune disease. Since hair loss is due to complex causes, it is very difficult to suggest broadly applicable effective treatment approaches.

Hair growth-promoting agents or hair tonics that have been developed so far usually aim to either promote hair growth or prevent hair loss by correcting
the indirect causes of hemodynamic disorders, nutritional disorders, seborrheic dermatitis and stress that induce hair loss. Consequently the effects of such treatments have been incomplete. Minoxidil is well known as a hair growth promoter. It was originally developed to treat high blood pressure, but hypertrichosis was reported as a side effect, leading it to be developed as a hair growth promoting agent. The mechanism of its hair growth effect is considered to be an increase of nutrition supply through vasodilation and K+ channel opening. Another hair growth promoter is finasteride, which inhibits the activation of 5α-reductase, so affecting the metabolism of male hormone. Its disadvantage is that when its use is stopped, hair loss returns. Recently, studies have progressed on promoting the activation of dermal papilla cells, blood circulatory improvement around dermal papilla cells, inhibition of sebum, inhibition of male hormone transformation and inhibition of the activation of TGF-β, which promotes the transition of the hair growth cycle from the anagen phase to the catagen or telogen phases.

From the perspective of oriental medicine, hair is controlled by the kidney, and vigorous blood status is responsible for a rich hair condition while deteriorated blood status is responsible for poor hair condition. Thus, normal hair growth is closely related to normal kidney and blood functioning. On the other hand, blood heats, deficiency of both ki (vital energy) and blood, insufficiency of kidneys, insufficiency of lungs, and stresses have all been suggested as causes of hair loss.

When the studies of oriental herbal medicines for hair loss prevention or hair growth promotion were reviewed, the methanol extract of Mori Cortex, Sophorae Fructus, Polygoni Multiflori Radix and Chebulae Fructus have been reported to have effects on the promotion of hair growth, and the Mori Cortex, Chaenomelis Fructus, Aucklandiae Radix, Angelicae Gigantis Radix, Polygoni Multiflori Radix and Angelicae Dahuricae Radix have been reported to have effects on inhibiting 5α-reductase type II activation. The Sophorae Radix extract’s inhibiting effect on the activation of both 5α-reductase type I and type II has been reported to promote hair growth.

In a combined preparation, the Shineungyangjindan7, Dangguibohyultang-gamibang18, and Sangbaleum19 has been shown to significantly prevent hair loss and promote hair growth, and hair sprays18, hair essences20 and soaps21 containing oriental herbal medicine were reported to improve hair condition by increasing the hair density per unit area or by reducing the hair shedding ratio. In addition, Khil et al.22 described an antioxidant effect that reduced the content of thiobarbituric acid reactive substance of serum and liver in aging-induced white rats. Heo et al.23 reported the effects of Yonnyunggobon-dan on the sexual ability of aged rats, and found that it increased egg counts and the normal egg to fertilization ratio, and also effected fertility reduction by decreasing NOS gene expression at sexual glands. Park et al.24 reported that it delayed cellular aging by increasing the cell doubling frequency and by reducing cell doubling time in dermatic fibroblastic cells, cardiac vascular endothelial cells and glomerulus mesangial cells. Many studies have reported that the oriental herbal medicines in Yonnyuniksoogobon-dan prevent hair loss and promote hair growth.

Oral treatment with Yonnyuniksoogobon-dan resulted in a similar pattern of hair growth to that of the Control group until the 7th and 9th day, though the amount of gray skin was greater than in the Control. The enlargement of the hair growth area outside of the skin was observable at the 12th day. Treatment with Yonnyuniksoogobon-dan both orally and by skin application revealed a similar growth pattern to that of mice treated with oral Yonnyuniksoogobon-dan alone. The histological observation of hair follicles and hair growth status of H&E-stained skin tissue on the 7th and 12th days revealed thick cross-sectional areas of hair in Groups I and II, with hair follicles that had more inner and outer root sheath development and thicker hairs compared to the Control group, indicating an improvement in hair growth status.
Measurement of the HRG index from hair growth status and growth area revealed that the group that was orally administered *Yonnyuniksoogobon-dan* showed significantly higher HRG indices from the 7th day to the 12th day of the experiment. Mice that were treated both orally and topically with *Yonnyuniksoogobon-dan* showed significant HRG index increases only on the 12th day of the experiment. The relative inferiority of combined oral and topical treatment might be attributable to supplementary problems, such as skin deposition and retention of precipitant by the direct skin spreading of the extracts of the oriental herbal medicine. These problems could be tested by additional experiments using technologically developed skin spreading agents.

The hair follicle is an organ that contains very complex components. It is comprised of mesenchymal cells, called dermal papilla, and epithelial cells, and has 3 growth periods: anagen period of growth, the degenerating stage of the catagen period; and the inactivated stage of the telogen period. This growth cycle has been reported to be controlled by various factors, such as fibroblast growth factors, Sonic hedgehog, TGF-β, insulin-like growth factors, EGF, hepatocyte growth factors, platelet-derived growth factors, interferon and interleukins, thyroid hormone and Vitamin D, glial cell line-derived neurotrophic factor, and neurotrophins. Among these factors, EGF has been reported to be expressed at the outer root sheath of hair follicles to stimulate the cell propagation and the formation of follicles, while TGF-β1 has been known to be expressed at the both of inner root sheath and outer root sheath of fully grown hair follicles to inhibit the induction of the anagen period and promote the induction of the catagen period.

Therefore, in this study the expression of EGF and TGF-β1 was observed by immunohistochemical staining. On the 12th day, the Control group had a weak (± or +) EGF positive response or a (−) negative response. Group I had medium a (+++) positive response to EGF and part of skin showed very strong (++++) EGF positive response. Group II showed a similar EGF expression pattern to that observed for Group I. TGF-β1 expression in the Control group was observed with a weak (+) positive response, though the hair roots at parts of the skin had a very strong (++++) positive response. Neither oral-only treatment or oral plus topical treatment with *Yonnyuniksoogobon-dan* significantly effected TGF-β1 expression compared to Control, though parts of cross sectional hair roots had a reduction in TGF-β1 expression compared to Control. This result indicated that the hair growth effect of *Yonnyuniksoogobon-dan* was due to the promotion of EGF expression and the inhibition of the expression of TGF-β1 at hair follicles.

On the other hand, since the hair follicle itself and the epidermal tissue surrounding the hair roots do not have blood vessel distribution, the rapid cell division that occurs during the growth cycle of hair follicles increases oxygen and nutrition demand, which can lead to angiogenesis from the deep dermis. VEGF expression plays a major role at this process. Increased VEGF expression has been shown to promote the growth of hair follicles and increase the size of hair follicles and the thickness of hair. The formation of nitric oxide in the skin tissue by nitric oxide synthase (NOS) is known to be involved in melanogenesis, wound healing and vasodilation. In particular, since endothelial NOS (eNOS) is expressed in follicular papilla cells, it has been shown to have effects on the growth of hair follicles and on the hair growth cycle, and the expression of inducible NOS (iNOS) has been reported to be increased at the peripheral tissues of hair follicles by inflammatory stimulation and ultraviolet light.

For these reasons, the current study observed the expression of VEGF and iNOS at the peripheral tissues of hair follicles through immunohistochemical staining. On the 12th day, 3-7 VEGF expressing cells were observed at the peripheral tissues of hair follicles and hair roots in the Control group, whereas Group I had 10-15 VEGF expressing cells at the peripheral tissues of hair follicles and hair roots.
and Group II had more than 10 VEGF expressing cells, indicating that *Yonnyuniksoogobon-dan* treatment increased the number of VEGF expressing cells. Control mice had very strong (+++) iNOS expression at the peripheral tissues of hair follicles and hair roots, while Group I and Group II mice had weak or medium (+ or ++) levels of iNOS expression at the peripheral tissues of hair follicles and hair roots. These findings indicate that *Yonnyuniksoogobon-dan* treatment decreased iNOS expression. These results suggest that *Yonnyuniksoogobon-dan* promotes hair growth by improving the hemodynamic environment at the peripheral tissues of hair follicles and by reducing inflammatory responses.

Combining all the findings of this study, oral *Yonnyuniksoogobon-dan* promotes hair growth by the stimulating EGF expression at hair follicles and by inhibiting the expression of TGF-β1. Topical application of *Yonnyuniksoogobon-dan* was not effective, and the evaluation of *Yonnyuniksoogobon-dan* effects when topically administered to the skin will require the development of a prototype that will solve the supplementary problems of skin spreading.

**Acknowledgement**

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Immunohistochemical Study on the Hair Growth Promoting Effect of Yonnyuniksoogobon-dan


