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Effects of *Lycopersicon esculentum* extract on hair growth and alopecia prevention

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Synopsis

To evaluate the potential hair growth–promoting activity and the expression of cell growth factors of *Lycopersicon esculentum* extracts, each 3% (w/w) of ethyl acetate extract (EAE), and supercritical CO_2 extract (SCE) of *L. esculentum* and isolated lycopene Tween 80 solution (LTS) and test hair tonic (THT) containing LTS were applied on the dorsal skin of C57BL/6 mice, once a day for 4 weeks. At week 4, LTS and THT exhibited hair growth–promoting potential similar to that of 3% minoxidil as a positive control (PC). Further, in the LTS group, a significant increase of mRNA expression of vascular endothelial growth factor (VEGF), keratinocyte growth factor, and insulin-like growth factor-1 (IGF-1) was observed than PC, as well as the negative control (NC). In the THT group, increases in IGF-1 and decrease in VEGF and transforming growth factor-β expression were significant over the NC. In a histological examination in the THT group, the induction of anagen stage of hair follicles was faster than that of NC. In the Draize skin irritation study for THT, no observable edema or erythema was observed on all four sectors in the back skin after exposure for 24 or 72 h for any rabbit. Therefore, this study provides reasonable evidence that *L. esculentum* extracts promote hair growth and suggests that applications could be found in hair loss treatments without skin irritation at moderate doses.

INTRODUCTION

Normal hair loss from the scalp is about 50–60 hairs a day and does not have a noticeable effect on appearance, but an excess loss (>100 hairs) will result in baldness. The term alopecia is used to describe human hair loss, sometimes to the point of baldness. There are various forms of alopecia, the most common being androgenetic alopecia (AGA), which affects millions of men and women (1).

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AGA is hereditary and androgen-dependent, progressive thinning of the scalp hair that follows a defined pattern. The principal elements of the androgen metabolism involving androgen-dependent processes are predominantly due to the binding of dihydrotestosterone (DHT) to the androgen receptor (AR). DHT-dependent cell functions depend on the availability of weak androgens, their conversion to more potent androgens via the action of 5α-reductase, low enzymatic activity of androgen inactivating enzymes, and functionally active AR present in high numbers. The predisposed scalp exhibits high levels of DHT and increased expression of the AR. Conversion of testosterone to DHT within the dermal papilla plays a central role, whereas androgen-regulated factors deriving from the dermal papilla cells are believed to influence the growth of other components of the hair follicle (2,3).

It was known that normal hair growth occurs at the level of the hair follicle in a 3-phased cycle: anagen (active growth phase), catagen (transition and involution phase), and telogen (resting phase) (4,5). Various cytokines and growth factors are also involved in the regulation of hair morphogenesis and growth. Catagen has been suggested to occur as a consequence of decreased expression of an antigen-maintaining factor, such as insulin-like growth factor-1 (IGF-1), basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), and increased expression of cytokines such as transforming growth factor-β (TGF-β) and interleukin-1 (IL-1) promotes apoptosis (6,7).

Two U.S. Food and Drug Administration (FDA)-approved pharmacotherapies, minoxidil and finasteride, are available for the treatment of AGA. Topical minoxidil solution 2% and 5% (Rogaine for men and women; OTC, Pharmacia Corp., Peapack, NJ) has been shown to stimulate new hair growth and help prevent further hair loss in affected areas in both men and women with AGA (8,9), but the specific mechanism of the action is unknown.

Propecia (finasteride; Merck Co., Rahway, NJ) has recently been approved by the FDA in the United States for men with AGA. Propecia is a synthetic azosteroid and a potent and highly selective antagonist. Being a noncompetitive antagonist of 5α-reductase type 2, it binds irreversibly to the enzyme and inhibits the conversion of testosterone to DHT. In previous clinical trials in the treatment of men with male pattern hair loss, finasteride, 1 mg daily slowed the progression of hair loss and increased hair growth in treated men compared with those in the control group (10).

Generally, minoxidil is well tolerated with long-term daily use. The side effects of minoxidil such as skin irritation (11,12), dizziness, tachycardia (11), and contact dermatitis (13) are uncommon. Few adverse side effects of finasteride were reported in the 5-year data. In the finasteride group, loss of libido was reported in 1.9% and erectile dysfunction in 1.4% in the first year. The placebo groups reported these same events with frequencies of 1.3% and 0.6%, respectively. These events appeared to resolve on cessation of the treatment, and in some cases, during continued treatment (14).

Testosterone 5α-reductase catalyzes the conversion of testosterone to an active androgen, dihydrotetosterone, which binds to the ARs and shows various hormonal actions. An excessive accumulation of dihydrotetosterone is recognized as the leading cause of male pattern baldness. Therefore, treatment with testosterone 5α-reductase inhibitor would be expected to lead to a decrease of dihydrotetosterone concentration in tissues, and may be useful for the protection of depilation.

On the basis of this reasoning, much research has been performed for natural crude drugs having 5α-reductase inhibitory activities (15–17). However, there remains a demand for a highly effective 5α-reductase inhibitor or hair growth promoter with an excellent safety and efficacy profile.

L. esculentum is one of the most popular vegetables and lycopene is the principle carotenoid causing the characteristic red hue of *L. esculentum* (18). Several reports show that diet rich in lycopene has beneficial effects on human health (19,20). A possible role has been suggested for *L. esculentum* and its products in preventing cardiovascular disease (19,21) and protecting against some types of cancer (20,22).

Especially, epidemiological studies have consistently showed an association between high intakes of lycopene and reduced prostate cancer risk. The lycopene contributed to the reduction of prostate cancer by interfering with logical testosterone activation by down-regulating 5α-reductase, and consequently reduced steroid target genes expression (22).

On the basis of previous reports, our attention was drawn to the possibility of hair growth–promoting activity of *L. esculentum* extract, which contains high level of lycopene, regulating 5α-reductase activity. Therefore, in this study we evaluated the hair growth–promoting effect and the effect on several growth factors involved in hair growth of *L. esculentum* extract and isolated lycopene in C57BL/6 mice.

MATERIALS AND METHODS

SAMPLE PREPARATION AND MATERIALS

L. esculentum was provided by Chal-Tomato Co., Ltd. (Daejeo, Busan, Korea). Fresh *L. esculentum* was thoroughly washed with tap water. After washing, the seeds were removed and the *L. esculentum* was chopped into cubes. Then, the cubes were freeze-dried for 3 days until a moisture content of *ca.* 0.8% was reached. The freeze-dried *L. esculentum* were then ground in a mill to pass through a 500-mesh sieve and then stored at −20 °C until use (23).

PREPARATION OF THE *L. ESCULENTUM* EXTRACT

The ethyl acetate extract (EAE) of *L. esculentum* and the isolated (=semipurified) lycopene from *L. esculentum* was prepared according to the method of Roh *et al*. (23). In brief, EAE was extracted from 100 g of the powdered freeze-dried *L. esculentum* with 1 L of ethyl acetate on the orbital shaking machine (30 rpm) for 30 min. Then, the resulting *L. esculentum* EAE was evaporated at the reduced pressure of up to 10% of the initial volume. Isolated lycopene (0.14 g; >77.0%) from of *L. esculentum* EAE, using an antisolvent (methanol) salting-out method was prepared, and then dissolved in 100 ml Tween 80. The resulting lycopene Tween 80 solution was designated as LTS. Supercritical CO_2 extraction of *L. esculentum* powder (100 g) was performed at 45 °C in 300 bars for 4 h, according to the method of Cadoni *et al*. (24).

ANIMALS

Twenty-five female C57BL/6 mice (5 weeks old upon receipt; SLC, Shizuoka, Japan) were treated after acclimatizing to the laboratory conditions for 7 days. Animals were allocated

five per polycarbonate cage in a temperature (20°C) and humidity (40–45%)-controlled room. The light/dark cycle was 12:12 h and food (Samyang, Wonju, Korea) and water were supplied ad libitum.

DETERMINATION OF HAIR GROWTH–PROMOTING ACTIVITY

Hair growth–promoting activity of the *L. esculentum* extract was determined by the method reported by Roh *et al*. (25), with some modifications. Briefly, 6-week-old C57BL/6 mice were randomly divided into five groups for five treatments, as follows: the negative control (NC) group (10% ethanol as a vehicle), positive control (PC) group (3% minoxidil), *L. esculentum* extract 1 group [3% (w/w) of EAE], extract 2 group [3% (w/w) of supercritical CO_2 extract (SCE)], and extract 3 group [3% (w/w) of LTS]. Hair was removed from the 2 cm × 3 cm dorsal area of these mice by shaving carefully with an electric clipper. The substances and test materials were applied topically on the back skin of the mice, once a day for 4 weeks.

The hair growth–promoting activity of the substances was checked by the darkening of the dorsal skin, which indicated the anagen phase of the hair follicles. The hair growth scoring was performed by two independent dermatologists, who were unaware of treatment regimen and we used the average of the two scores. Hair growth was measured at every 1 week during 4 weeks by assigning a hair growth index, as follows: score 0 = no growth observed; 1 = up to 20% area of skin covered with hair; 2 = 20–40% area of skin covered with hair; 3 = 40–60% area of skin covered with hair; 4 = 60–80% area of skin covered with hair; and 5 = 80% to full area of skin covered with hair observed. Digital images of total hair growth on day 28 were obtained using Nikon Coolpix P100 (Nikon Co., Tokyo, Japan).

RNA EXTRACTION AND REAL-TIME RT-PCR

Total RNA extraction was performed with Trizol reagent (Life Technologies, Gaithersburg, MD), and the cDNA was synthesized by a reverse transcription reaction using the RNA PCR kit (Applied Biosystems; Roche Inc., Foster City, CA) in a 20 μl mixture containing 1 μg RNA, 50 mM KCl, 10 mM Tris/HCl, 5 mM $MgCl_2$, 1 mM of each dNTPs, oligo-(dT) primers, 20 units of RNAse inhibitor, and 50 units of MuLV reverse transcriptase. Nucleotide sequence of the primers used in this study is shown in Table I. The reaction mixture was incubated for 60 min at 42 °C, then heated at 90°C for 7 min in a thermocycler (GeneAmp PCR system 9600; PerkinElmer, Roche Molecular System, Branchburg, NJ). Real-time PCR was performed using a lightcycler instrument using FastStart DNA Master SYBR Green I PCR kit (Roche, Mannheim, Germany). Quantification of the VEGF, keratinocyte growth factor (KGF), IGF-I, and TGF-β mRNA expression was corrected by glyceraldehyde 3-phosphate dehydrogenase (GAPDH).

TEST HAIR TONIC WITH *L. ESCULENTUM* EXTRACT

For possible future application in hair growth–promoting agents and pharmaceutical products for hair growth and alopecia prevention, the test hair tonic solution with LTS was prepared and evaluated for hair growth–promoting activity. At first, 70 ml aliquots

Table I
Nucleotide Sequence of the Primers Used For PCR Amplifications in This Study

Growth Factor		Primer Sequence
VEGF	Forward	ACS CGG TGG TGG AAG AAG AG
	Reverse	CAA GTC TCC TGG GGA CAG AA
KGF	Forward	ACG AGG CAA AGT GAA AGG GA
	Reverse	TGC CAC AAT TCC AAC TGC CA
IGF-1	Forward	TCA TGT CGT CTT CAC ACC TCT TCT
	Reverse	CCA CAC ACG AAC TGA AGA GCA T
TGF-β	Forward	GCG GCA GCT GTA CAT TGA CT
	Reverse	ACT GTG TGT CCA GGC TCC AA
GAPDH	Forward	CAA TGA ATA CGG CTA CAG CAA C
	Reverse	AGG GAG ATG CTC AGT GTT GG

of distilled water were distributed into a glass beaker. A 0.3 g nicotine amide and 0.5 g salicylic acid were added and dissolved, and then the final volume was brought up to 100 ml immediately. The formulations studied were prepared in a PRIMIX RM homomixer (PRIMIX Co., Ltd., Osaka, Japan) at 500 rpm within 5 min and supplemented with 3% (w/w) of LTS. A placebo formulation was prepared without LTS.

HISTOLOGICAL ANALYSIS OF HAIR FOLLICLES

The substances and test materials were applied topically on the back skin of the mice, once a day for 4 weeks. After week 4, all of the mice were sacrificed. Their dorsal skins were removed and fixed in 4% formaldehyde solution and embedded in paraffin. The fragments were sectioned into two different patterns: transverse sections for determination of hair follicle count and longitudinal sections for the overall histological assessment. The 3-μm sections were stained with hematoxylin–eosin and toluidine blue and examined under a light microscope (Magnification: ×200) (Olympus, Melville, NY).

DRAIZE SKIN IRRITATION TEST

The irritation potentials of the test hair tonic solution with 3% LTS were evaluated according to the method of Draize (26) with slight modification (27). Briefly, two male New Zealand White (NZW) rabbits weighing 2.5–3.0 kg were acclimatized for 5 days before starting the study. The back of each rabbit was clipped free of hair and then divided into four sectors as shown in Fig. 8: after abrasion and application of hair tonic (upper left), after abrasion and no application of hair tonic (upper right), no abrasion and application of hair tonic (lower left), and no abrasion and no application of hair tonic as NC (lower right). The area of each sector was 6.25 cm^2 (2.5 cm ×2.5 cm). For abrasion, several layers of skin were removed with adhesive tape from one half (upper side) of the shaved backs. Adhesive tape stripping was done about five times. A 0.5 ml of the test hair tonic solution with 3% LTS was applied once, uniformly, on the left side only of the hair-free skin of each rabbit.

Skin was observed for erythema or edema as required by the Draize test (0: no erythema or no edema; 1: barely perceptible erythema or edema; 2: well-defined erythema or slight edema; 3: moderate to severe erythema or moderate edema; 4: severe erythema or edema) at 24 and 72 h after application (28,29). The Draize test was done and evaluated according to the Korea Food and Drug Administration guideline.

ANALYSIS BY HIGH-PERFORMANCE LIQUID CHROMATOGRAPHY FOR THE QUANTIFICATION OF LYCOPENE

The quantification analysis of the lycopene of the LTS was performed using a Waters 600E HPLC system (Waters Co., Milford, MA) equipped with Waters 486 UV detector, as described by Rho *et al.* (23). The chromatographic analysis was conducted using a reverse-phase ZORBAX Eclipse plus C18 column (4.6 mm × 250 mm; Agilent, Santa Clara, CA) with 5 μm particles. The characterization of the lycopene extracts was performed in isocratic mode and the mobile phase used was methanol/THF (90:10; v/v). Lycopene samples were dissolved in 20 μl of a methanol/hexane (1:2; v/v) solution. Chromatographic separation of extracts was performed at a constant flow rate of 1 ml/min. Lycopene was detected at 472 nm. For quantitative analysis, standard lycopene (Sigma L9879; Sigma-Aldrich, Ltd., St. Louis, MO) was also analyzed using the high-performance liquid chromatography (HPLC) system under the same conditions.

STATISTICAL ANALYSIS

Analysis of variance as a statistical analysis was performed using SPSS (version 12.00; SPSS Inc. Chicago, IL). A value of $p < 0.05$ was considered statistically significant.

RESULTS

YIELD OF *L. ESCULENTUM* EXTRACT

The yields of EAE, SCE, and LTS of *L. esculentum* (100 g) were 3.8 g, 0.75 g, and 0.014 g, respectively.

HAIR GROWTH–PROMOTING EFFECT OF *L. ESCULENTUM* EXTRACT

After 4 weeks of daily topical treatment on C57BL/6 female mice, 3% (w/w) formulations of EAE, SCE, and LTS all showed hair growth–promoting activity greater than the NC. The hair growth–promoting activity of LTS was also similar to that of the 3% Minoxidil control (PC) (Figs 1 and 2).

EFFECT OF *L. ESCULENTUM* EXTRACT ON THE MRNA LEVEL OF GROWTH FACTORS

According to Fig. 3, increases in VEGF and IGF-1 were significant only for SCE and LTS. Increases in KGF were significant only for LTS, and there were no significant increases in TGF-β over the NC.

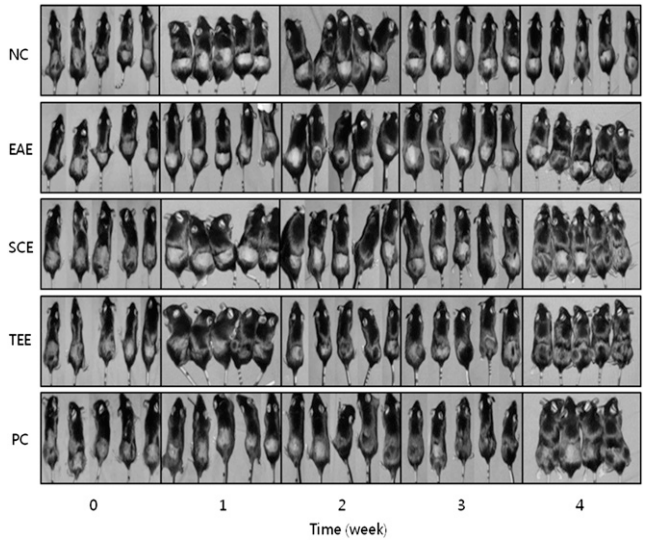

Figure 1. Hair growth change of C57BL/6 mice after topical application of *L. esculentum* extracts at 4 weeks after treatment. Group 1: NC (ethanol); group 2: 3% *L. esculentum* EAE; group 3: 3% *L. esculentum* SCE; group 4: 3% LTS; group 5: PC (3% minoxidil).

When the quantity of VEGF of mouse skin tissue treated with EAE, SCE, LTS, and minoxidil was evaluated, the amount of RT-PCR products was found higher by 8.09%, 10.29%, 10.29%, and 5.14 % than that in the negative group. In KGF, the amount of RT-PCR products was higher by 9.29%, 11.43%, 12.14%, and 5.00% than that in the negative group. In IGF, the amount of RT-PCR products was higher by 12.68%, 15.49%, 13.38%, and 8.45% than that in the negative group. In TGF-β, the amount of RT-PCR products was higher by 8.46%, 10.80%, 10.00%, and 5.38% than that in the negative group.

The mRNA expression of VEGF, KGF, and IGF-I in the 3% *L. esculentum* SCE group and 3% LTS group was found to be approximately two times more than those in the 3% minoxidil group. On the basis of these results, further tests were performed using LTS.

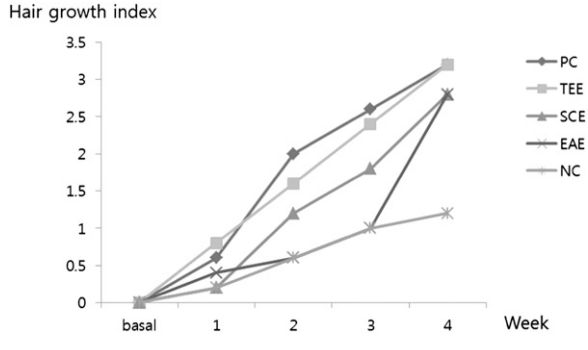

Figure 2. Hair growth index of C57BL/6 mice after topical application of *L. esculentum* extracts for 4 weeks. Group 1: NC (ethanol); group 2: 3% *L. esculentum* EAE; group 3: 3% *L. esculentum* SCE; group 4: 3% LTS; group 5: PC (3% minoxidil).

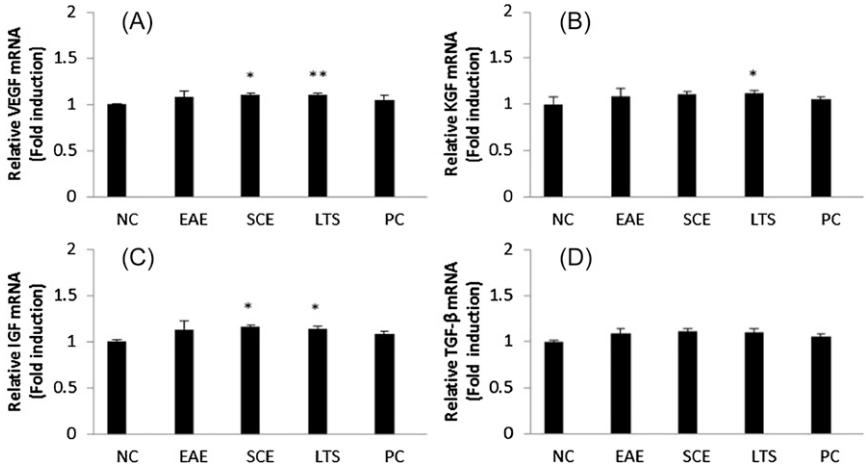

Figure 3. Comparison of (A) VEGF, (B) KGF, (C) IGF-1 and (D) TGF-β expression in C57BL/6 mice after topical application of experimental materials, NC: negative control (10% ethanol as a vehicle), EAE: 3% *L. esculentum* ethyl acetate extract, SCE: 3% *L. esculentum* supercritical CO_2 extract, LTS: 3% isolated lycopene Tween 80 solution, PC: positive control (3% minoxidil) for 4 weeks. $*p < 0.05$, $**p < 0.01$ compared with control.

HAIR GROWTH–PROMOTING EFFECT OF HAIR TONIC CONTAINING 3% LTS

After daily treatment for 4 weeks the C57BL/6 female mice treated with the hair tonic containing 3% LTS show increased hair growth compared to the NC group. The PC group treated with hair tonic containing 3% minoxidil showed hair growth levels similar to the 3% LTS product (Figs 4 and 5).

EFFECT OF TEST HAIR TONIC CONTAINING 3% LTS ON THE MRNA LEVEL OF GROWTH FACTORS

According to Fig. 6, increases in IGF-1 and a decrease in VEGF and TGF-β expression were significant for THT. No significant increases were observed in KGF over the NC.

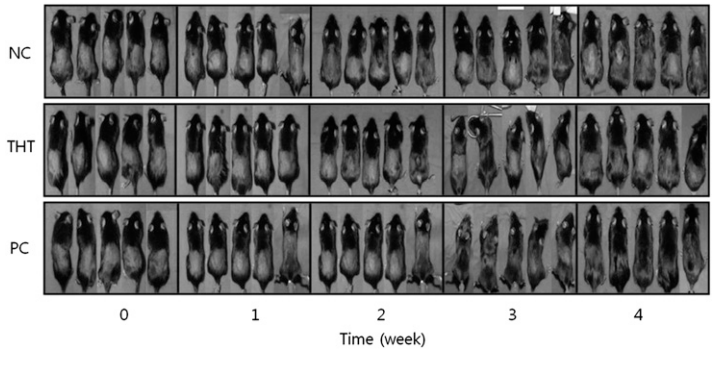

Figure 4. Hair growth change of C57BL/6 mice after topical application of hair tonic product with 3% LTS during 4 weeks. Group 1: NC (10% ethanol); group 2: THT (tonic product with 3% LTS); group 3: PC (3% minoxidil).

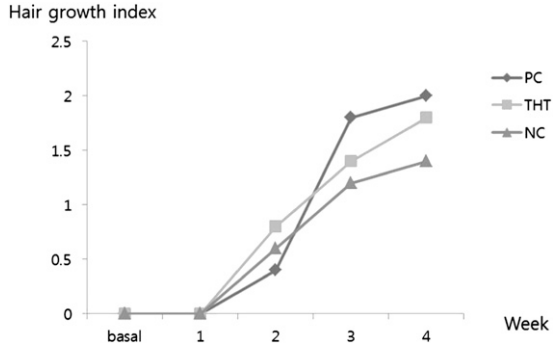

Figure 5. Hair growth index of C57BL/6 mice after topical application of hair tonic product with 3% LTS for 4 weeks. Group 1: NC (10% ethanol); group 2: THT (tonic product with 3% LTS); group 3: PC (3% minoxidil).

When the quantity of VEGF of mouse skin tissue treated with the test hair tonic product that contained 3% LTS or 3% minoxidil was evaluated, the amount of RT-PCR product-treated hair tonic with LTS was lower by 42.31% and 3.85% than that in the NC and PC, respectively. In KGF, the amount of RT-PCR products was higher by 41.67% and 133.33% than that in the NC and PC, respectively. In IGF-1, the amount of RT-PCR products was higher by 17.86% than that in the NC, and higher by 257.14% than that in the PC, respectively. In TGF-β, the amount of RT-PCR products was lower by 45.84% than that in the NC and lower by 15.28% than that in the PC, respectively (Fig. 6).

ANAGEN INDUCTION AND HAIR RESTORATION OF FEMALE C57BL/6 MICE BY TEST HAIR TONIC PRODUCT WITH 3% LTS

The morphological structure of the skin, obtained from a longitudinal section of the dorsal skin is shown in Fig. 7. In NC, early anagen was observed. Further, dyeing by toluidine blue was slightly observed in the epidermal cells of hair follicles undergoing anagen induction and

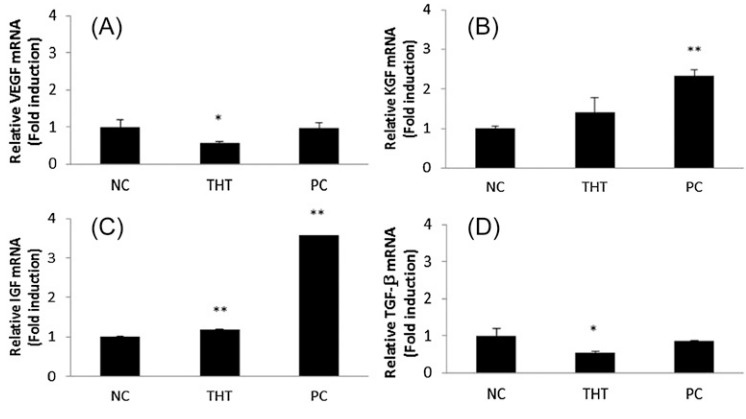

Figure 6. Comparison of (A) VEGF, (B) KGF, (C) IGF-1, and (D) TGF-β expression in C57BL/6 mice after topical application of experimental materials, NC: negative control (10% ethanol as a vehicle), THT (hair tonic with 3% LTS), PC: positive control (3% minoxidil) for 4 weeks. $*p < 0.05$, $**p < 0.01$ compared with control.

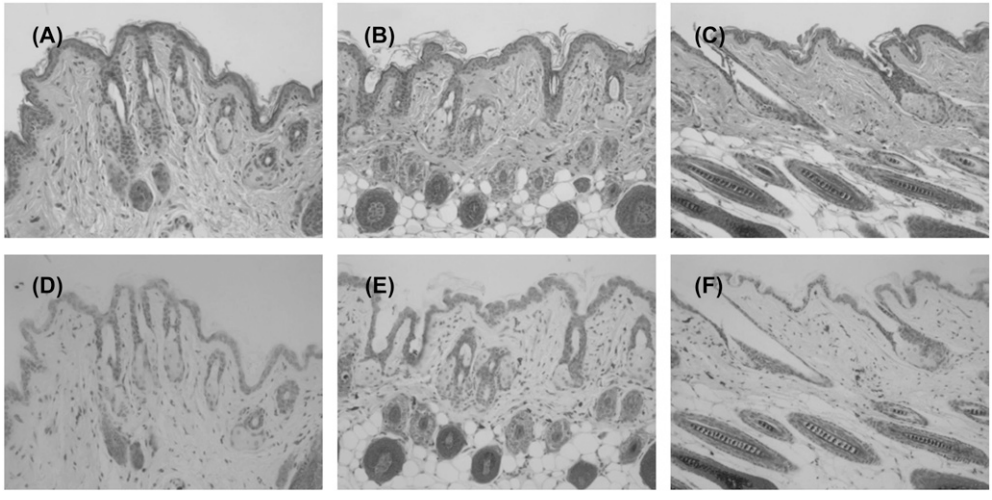

Figure 7. Hematoxylin–eosin (A–C) and toluidine blue (D–F) staining of the skin sections. The NC: negative control (10% ethanol as a vehicle; A, D), test hair tonic product contained 3% LTS (B, E) or 3% minoxidil (C, F) were applied topically on the back skin of the mice, once a day for 4 weeks. Magnification: ×200.

in growing hair follicles. Otherwise, 3% minoxidil (PC) showed most hair follicles were fully induced and growing into the subcutaneous region. In addition, the staining by toluidine blue was distinctly observed in the growing inner and outer root sheaths of the hair bulb. Findings with the 3% LTS-treated samples were very similar to the minoxidil control. However, in the test hair tonic experiment, the anagen induction rate for the 3% LTS product was slower than that of the minoxidil control, but greater than that of the NC (Fig. 7).

DRAIZE SKIN IRRITATION STUDY

The test hair tonic with 3% LTS showed no signs of irritation on abraded or intact back skin of two male NZW rabbits at 24 or 72 h after treatment (Fig. 8).

LYCOPENE QUANTIFICATION OF *L. ESCULENTUM* EXTRACT

We used HPLC to confirm the lycopene quantification of the 3% LTS. Lycopene was identified by comparing the retention times of the pigment in the extraction mixture with those of respective standard compounds (Sigma products). The HPLC chromatogram is shown in Fig. 9. When the area of the lycopene peak was calculated and compared with standard compounds, the all-*trans*-lycopene and *cis*-lycopene content of 3% LTS was 3.63 and 1.65 μg/ml, respectively.

DISCUSSION

There are various forms of alopecia, the most common being "AGA," which affects millions of men and women. For both men and women, AGA may begin as early as the teen

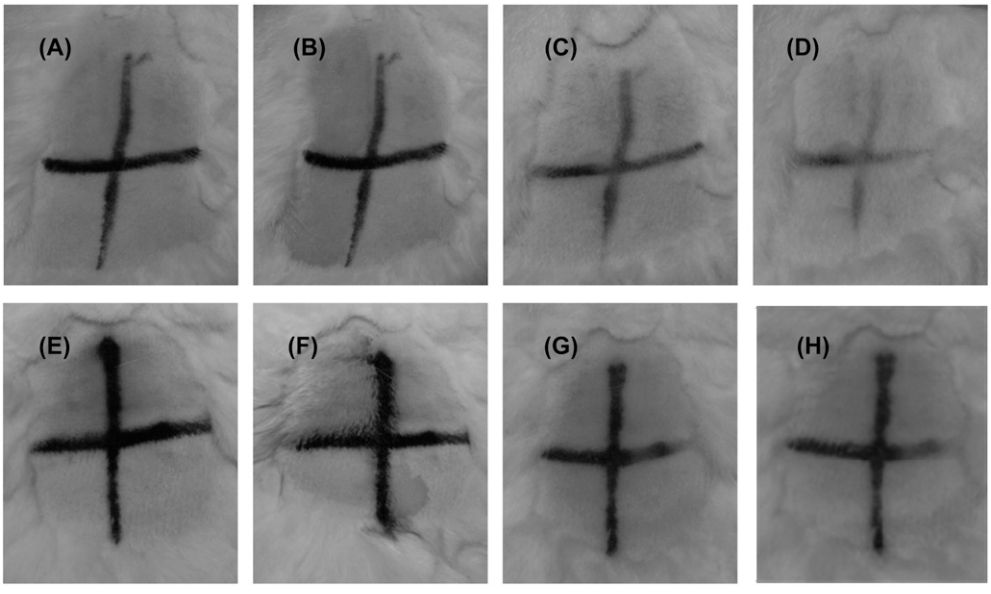

Figure 8. Draize skin irritation test on two male NZW rabbits; 1—(A) through (D), 2—(E) through (H). Before exposure (A, E), immediate after exposure (B, F), 1 day after exposure (C, G), 3 days after exposure (D, H). Back of each rabbit was clipped free of hair and then divided into four sectors as follow; after abrasion and application of hair tonic (upper left), after abrasion and no treatment as NC (upper right), no abrasion and application of hair tonic (lower left) and no abrasion and no treatment as NC (lower right), respectively.

years, but can even start in later decades of life. The severity of hair loss in women is usually much less than in men (30,31).

Two U.S. FDA-approved pharmacotherapies, minoxidil and finasteride, are available for treatment of male pattern baldness. Oral finasteride, a competitive inhibitor of type 2, 5α-reductase, and topical minoxidil, an adenosine triphosphate–sensitive potassium channel opener, have been reported to stimulate the production of VEGF in cultured dermal papilla cells. These drugs are most useful in men with thinning and many miniaturized hairs.

Generally, minoxidil is well tolerated with long-term daily use. Adverse events are primarily dermatologic and include irritant contact dermatitis, and less often, allergic contact dermatitis (12,13,30). Finasteride is also well tolerated with long-term daily use, except for slight adverse sexual effects (14). Therefore, there remains a demand for highly

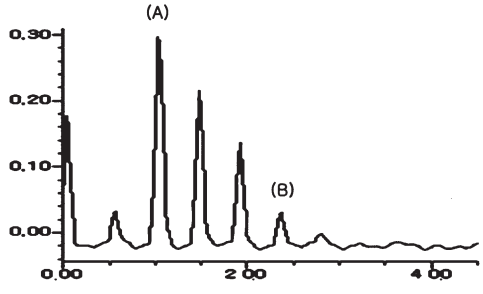

Figure 9. HPLC chromatograms of 3% LTS. (A) All-*trans*-lycopene and (B) *cis*-lycopene.

effective pharmacotherapies for the treatment of male pattern baldness with excellent safety and efficacy profiles. Thus, for many years, there have been numerous attempts to develop new agents capable of preventing and/or treating pattern baldness (25,31,32).

It has been reported that the mechanism of hair loss is similar to that of prostate disease. Namely, the cause of prostate disease and hair loss is known to be an excess amount of DHT from testosterone by 5α-reductase (33–35). Further, 1 or 5 mg finasteride per day was prescribed for both symptoms (36).

It has been recently reported that *L. esculentum* and lycopene is useful for the prevention and treatment of prostate disease (37,38). However, to our knowledge, it has not been determined if *L. esculentum* or lycopene is an effective treatment for hair loss. Therefore, in this study, we evaluated the potential hair growth–promoting activity and the expression of cell growth factors of crude *L. esculentum* extracts and isolated lycopene and test hair tonic containing LTS in C57BL/6 mice. Of the 3% (w/w) of EAE and SCE of *L. esculentum* and LTS, LTS exhibited hair growth–promoting potential similar to that of 3% minoxidil PC in our results (Figs 1 and 2).

It has been reported that the dermal papilla cell-derived growth factors, such as VEGF, KGF, and IGF-I, and the root-sheath cell-derived growth factors, such as TGF-β, have chemotactic effects on the surrounding cells, which in turn lead to hair growth (6,7).

To promote hair growth and to maintain anagen, it is essential that increased expression of anagen maintaining factor, such as IGF-1, bFGF, and VEGF, and decreased expression of cytokines promoting apoptosis, such as TGF-β and IL-1 (6,7).

In our results, the mRNA expression of VEGF, TGF-β, KGF, and IGF-I in both of *L. esculentum* extract and LTS as isolated lycopene was higher than those of the PC, as well as the NC. Especially, in the LTS group, significant increase in the expression of VEGF, KGF, and IGF-1 was observed (Fig. 3). However, as seen in Fig. 1, the treatment of both *L. esculentum* extracts and LTS obviously showed hair growth–promoting activity, but the expression of TGF-β genes in dorsal skin tissue of mice was not lower than PC and NC. Further research to elucidate the reason is needed.

To examine the possibility of commercial applicability, a hair tonic product containing 3% LTS showed hair growth–promoting potential similar to that of the same product containing 3% minoxidil (Fig. 4). In this case, TGF-β mRNA expression was lower than that of the PC and NC, and KGF and IGF-I mRNA expression was higher than the controls (Fig. 6). Further studies to elucidate the reason are needed.

After topical application onto the back skins of C57BL/6 mice daily up to 4 weeks, LTS induced earlier telogen-to-anagen conversion as compared to the vehicle-treated group. Histologic studies showed that LTS markedly increased the depth and size of hair follicles as compared with NC, showing rather slightly lower hair growth–promoting potential than that of PC. This result clearly supports that LTS induces early onset of anagen and stimulates hair growth (Fig. 7).

A Draize skin irritation test was performed to confirm the safety of the hair tonic product containing 3% LTS. No erythema, edema, or irritation was observed on abraded or intact back skin of male rabbits 24 or 72 h after treatment (Fig. 8). The results suggested that at moderate doses, humans can safely use the extracts.

When the lycopene quantification was performed (Fig. 9), the all-*trans*-lycopene and *cis*-lycopene content of 3% LTS was 3.63 μg/ml and 1.65 μg/ml, respectively, to elucidate

the main components of LTS. Until now, there is no research on hair growth–promoting activity of purified or authentic lycopene. In our results, although the purity of isolated lycopene is somewhat low (>77.0%), crude *L. esculentum* extracts and isolated lycopene showed considerable hair growth–promoting activity.

There are some limitations to this research study. The action mechanism of hair growth effect of minoxidil is not well known. In a previous study, Otomo (2002) proposed that minoxidil induces cell growth factors such as VEGF, IGF-1, and inhibits TGF-β-induced apoptosis of hair matrix cells (39). Therefore, we expected that quantities of growth factors except TGF-β of minoxidil-treated mouse skin tissues were significantly higher than that in the NC. But, in our study, quantities of VEGF and IGF-1 of minoxidil-treated mouse skin tissue were slightly higher, but not significantly higher than that in the NC. The quantity of TGF-β of minoxidil-treated mice was slightly lower, but not significant lower than that in the NC. In addition, quantities of KGF and IGF of test hair tonic–treated mouse skin tissue were higher than those in the NC. But quantity of VEGF of test hair tonic–treated mouse skin tissue was lower than that in the NC (Fig. 6). We need to further study the effect of test hair tonic containing 3% LTS on the mRNA level of growth factors.

In conclusion, this study provides potent evidence that *L. esculentum* extracts and isolated lycopene promote hair growth, and suggests that applications could be found in hair loss treatments without any adverse effect with moderate doses.

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